



Annals of Tropical Medicine and Public Health

ISSN: 1755-6783

International indexed journal

The journal is registered with the following abstracting partners: Baidu scholar, CNKI, EBSCO Publishing's Electronic database, Google Scholar, National Library, ProQuest, and African Index Medicus. It is indexed with DOAJ, ISI, EMASE, ESCI, Index Copernicus, Scimago Journal Ranking, and SCOPUS

Impact factor for 2018: 2.75

Title	Page
Expansion of General Practice Nurses' powers in primary health care organizations of the Republic of Kazakhstan	SP2033-19
Breast surgery, one of the causes of mastalgia	SP2034-19
Bactericidal activity of silver nanoparticles biosynthesized from Methicillin resistant Staphylococcus aureus against MRSA and MSSA isolated	SP2035-19
Synthesis of carbon nanotubes by using new route and using it as reduced carbon nanotube coating for corrosion protection enhancement of copper and aluminum in sea water	SP2036-19
Effect of educational exercises according to the technology of learning using glasses (3D) in learning some of the basic technical skills of female students	SP2037-19
The relation between chemotherapy, oxidative stress and apoptosis in females with breast cancer	SP2039-19
Aqueous extract of date palm fruit (Phoenixdactylifera) protect liver against ciproterone acetate toxicity in male mice	SP2040-19
Colorimetric determination of iron and zinc in people with gallstones in Diyala Governorate	SP2041-19
The impact of basketball education program according to the patterns of FARC on some of the kinetic characteristics of slow learners (6-8 years) years	SP2042-19
Detection of toxA gene in clinical isolates of Pseudomonas aeruginosa using real time PCR	SP2043-19
Molecular study of resistant microorganisms in post-operative patients on meropenem treatment	SP2044-19
Survivin targeting: An in silico approach for anticancer design	SP2045-19
Cytogenetic study of autism in Iraq	SP2046-19
Synthesized of thermally isolated antibacterial (Silicone/TiO ₂) Nano-composites paint for reservoirs	SP2048-19
Corneal epithelial and stromal thickness profile in keratoconus using anterior segment optical coherence tomography: A comparative study	SP2049-19

Expansion of General Practice Nurses' Powers in Primary Health Care Organizations of the Republic of Kazakhstan

*Zhazira M. UTEPPERGENOVA¹, Zhanna A. KALMATAYEVA²

1. Department of Health Policy and Organization, Higher School of Public Health; and Faculty of Medicine and Health care, Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan

2. Faculty of Medicine and Health care, Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan

*Corresponding Author: E-mail: Zh.Utepbergenova@gmail.com+77017953109

Abstract

CONTEXT: The problem of performing simple tasks that do not meet the qualifications of nurses of general practice (NGP) and the need to reduce the burden on doctors of general practice is acute in Kazakhstan.

AIMS: The aim of this research is to study the readiness of the professional environment for the introduction of the specialty of nursing extended practice.

METHODS AND MATERIAL: The analysis of the expenses of working time of 43NGP and an expert questionnaire with 129 healthcare specialists on functionality redistribution were conducted on the basis of 13 medical organizations of primary health care of Nur-Sultan city and Almaty city during April-May 2018.

RESULTS: The results of timekeeping show that the time spent on receiving one patient is on average 10.3 ± 0.56 minutes, which is below the regulated norm. The reduction in average indicators is primarily due to redistribution of functions among medical workers and increasing NGP independence and competence. The results of the survey have shown that changing the role of NGP, expanding their responsibilities, training in more complicated clinical skills, computerization of the paper work, monitoring the timeliness and quality of medical appointments, and redistribution of responsibilities among medical staff will allow to effectively implement the ongoing strategic initiatives in the the Republic of Kazakhstan.

CONCLUSIONS: The results of the study can be used in the process of further reformation of the medical sector of in Kazakhstan and other countries, in particular, primary health care, in order to optimize the medical service.

Keywords: Nurses of general practice, Standards of operating procedures, Timekeeping, Nursing, Primary Health Care, Republic of Kazakhstan.

KEY MESSAGES:

- delegation of a number of medical powers from specialists with a higher medical education to nurses of general practice to reduce the burden on doctors of general practice.
- changing the role of the nurse, new training programs, monitoring the work carried out.
- introduction of the notion "nurses of extended practice" and new staffing requirements.

How to cite this article: UTEPBERGENOVA ZM, KALMATAYEVA ZA (2019): Expansion of General Practice Nurses' Powers in Primary Health Care Organizations of the Republic of Kazakhstan, *Ann Trop Med & Pub Health*, 21: SP2033-19.

Introduction

Strategic initiatives of Kazakhstan in the field of improving the quality of medical care determine the modernization of primary medical care (PMC), designed to detect diseases in a timely manner and prevent the risk of their occurrence as a priority task^[1-6]. Realizing the need to form effective care at the primary care level, the Kazakh medical community, guided by the standards of world practice, is expanding the pool of nurses today through the introduction of extended practice nurses who have greater autonomy in the delivery of health services^[7-16].

In accordance with the new ideology, taken from the experience of the countries of the Organization for Economic Cooperation and Development (hereinafter — the OECD), primary role of healthcare should be given to the nurse who is the chief specialist of the first contact zone with the patient along with the general practitioner^[17].

As of 2018, nurses and midwives worldwide account for about 90% of contacts between health workers and patients^[19].

The World Health Organization today sees nursing staff as a real potential to meet the growing needs of the population for affordable health care, identifying them a leading role in providing safe, quality, effective and efficient primary care, prevention and rehabilitation services^[20-22].

According to a survey conducted among health personnel in public and private hospitals in Turkey, the majority of nurses consider the workload factor as one that strongly affects the efficiency of their work^[23]. Also, the negative impact of stress and increased work intensification on working capacity of nurses were reported in Iran^[24]. The Republic of Kazakhstan is not an exception in this regard. It is obvious that the rational allocation and efficient use of working time is one of the key aspects of the NGP performance.

It is worth noting that the problem of performing simple tasks that do not meet the qualifications of nurses is acute in other countries. Thus, according to the 2011-2012 survey, about 80% of nurses in 23 of OECD member countries noted that they consider themselves too experienced and qualified to perform some tasks (especially for medical workers with a master's degree or equivalent, to a lesser extent for workers with a bachelor's degree or below). This

^[25] problem can be addressed by providing nurses with additional support from lower-qualified health workers.

The best practices of foreign countries can serve as a good example in expanding NGP power. In the United States, practicing nurses are independent professionals who provide both primary and specialized care. They are engaged in diagnostics and laboratory testing (including interpreting results), making differential diagnoses, prescribing medications and nonpharmacological methods of treatment, disease prevention and patient counseling. According to the report of the expert working group of the Association of Nurses of Finland, there is a tendency in society to evolve the role of nurses, and in the future nurses will work even more independently, providing patients/clients with quality health care, in particular by monitoring cases of chronic diseases). Expanded practice nursing positions with a wide range of powers exist in more than 50 countries (notably Canada, Ireland, Sweden, Norway and other developed countries)^[25].

Materials and Methods

Data collection and procedure: At the first stage, the time measurement of the activities of nurses of general practice (43 people) was carried out, the second stage was the survey of specialists of practical health care about the rationality of redistribution functionality between doctors and nurses in PMC organizations (129 people).

Objects of study: Both stages of the study were carried out simultaneously on the basis of 13 medical organizations of PMC of Nur-Sultan city and Almaty city during April-May 2018. We observed the work of nurses of general practice and asked heads of departments, medical and nursing staff, representatives of non-governmental organizations etc. to answer questions about the organization of their work.

Testing of research tools: The research tools were tested on focus groups (timekeeping map – 4 respondents, expert questionnaire – 16 people).

Result and Discussion

Time costs in the structure of working hours of general practitioners: There is a dependence of indicators of time spent on reception and reasons of patients' referral to medical organizations. Given that the production process of primary health care workers depends also on the number of people served (number of patients), in the framework of this study we conducted an analysis of quantitative indicators of outpatient intake of general practitioners (Table 1). According to the regulations, the established capacity of the general practitioner's office is up to 25 patients served per day, provided that 15 minutes are allocated for each. The results showed that now it is on average 10.3 ± 0.56 minutes due to rationalization of the process (medical procedures by nurses, consultation and documentation by doctors). Most (1/3) cases in the office of a general practitioner is addressed because of general malaise (disease), less — for social and medical grounds, with the aim of medical examinations or for seasonal vaccinations. The least time during the medical and nursing reception is spent on the services of medical examination or vaccination, which is defined in the functional of medical professionals of this specialization, and is an average of 7.61 ± 0.33 minutes.

Time costs in the structure of working hours of the heads of medical organizations: According to the heads of medical organizations, it is impossible to limit the number of receptions of patients in the office of general medical practice, due to the violation of the purpose of PMC — patient-oriented focus of the provision of services. According to respondents, problems with receiving and providing medical services to patients, referring to the workload of medical staff, leads to multiple complaints from the population on unscrupulous and poor quality of medical care.

Time costs in the structure of working hours of nurses of general practice: The results of the chronological studies allowed to determine the structure of the expenses of working time of nurses at outpatient reception:

- A significant part of the working time of NGP is occupied by maintaining medical records (on average, $45.5 \pm 3.46\%$ of the total working day). As noted by nurses, the multiplicity of accounting forms leads to a reduction in time spent for interviews and manipulations with the patient. At the same time, an intensive automatization of medical records continues to develop and leads to the reduction of paper work. Over time, this should increase the time spent directly on medical care, dispensary and preventive work, as evidenced by studies on the example of computerization of the work of NGP in Russian medical organizations [29].
- The main activity, involving medical manipulation and conversation with patients on medical subjects, accounts for up to 45% of the total working time of a nurse, despite the fact that this activity should be one of the leading positions in NGP activities. This is due to the fact that during the delegation of authority from the doctor to the nursing staff, the nurse was transferred the most part of the documentation management functionality, while the transfer of responsibility for medical care (treatment and diagnostic procedures) is preferred. In addition to the implementation of the transferred functions and the technical execution of the assigned manipulations and procedures, the general practitioner must now put at the center of his/her activities the needs of treated patients.

Time spent by NGP on work with patients according to elements of labor operation: A significant part of manipulations consists of palpation, auscultation, pulse and respiratory frequency counting. NGP spends 10.8% of the entire working day giving advice and recommendations, as most of this activity is traditionally carried out by the doctor. The least time in the process of their practice nurses spend on manipulation to determine neurological status (1.3%) (Figure 1).

Time spent by NGP on organizational and preparatory activities: Unlike the cost of working time of the main activity of medical personnel with higher education on carrying out diagnostic, therapeutic and research manipulations and procedures, the work of the average medical personnel is required to carry out organizational and preparatory activities in the provision of medical services.

- Up to 11.54 ± 1.07 minutes of working time during a working day is to ***work with medications*** (up to 80.35 ± 4.11 minutes per week), in which the receipt and recording of medicines by the nurse, their packaging, storage and distribution to patients in accordance with the doctor's prescriptions are included.
- Up to 40 % of the total time spent working with medicines during the week is spent by nurses ***to write applications to obtain a list of all medicines*** and their coordination in the structural subdivisions of the medical organization. Despite the introduction of various information platforms and systems by some medical organizations, it is necessary to allocate up to 40 minutes a week for the processing of documents on obtaining medicines.
- Up to 75 minutes of basic activity per week, nurses allocate to ***the organization of sanitary and epidemiological conditions in the delivery of medical services***, in particular the preparation of disinfectant solutions for tool processing (up to 16.21 ± 0.31 min), their pre-sterilization treatment and disinfection of tools (up to 26.22 ± 2.57 min). Primary nurses in remote areas of the metropolis also indicated the need to take flushing from objects to bacteriological control (12.32 ± 0.28 min), decontamination and disposal of syringes and other medical products (23.51 ± 2.12 min), preparation of dressings (7.67 ± 2.09 min) and other similar procedures.

- **Ancillary activities** (up to 5% on average), which were not typical of the nurse's general practice and are not related to patient care (the need to bring a medical record, to include tests for emergency examination in diagnostic and laboratory units, etc.).

Required functionality of NGP of extended practice: The current situation with the organization of work of nurses has determined the need to review the functionality of nurses to optimize production process by introducing the specialty of nurses of general practice (hereinafter referred to as NGP) of extended practice. Expansion of the mandate of NGP will, first and foremost, reduce the burden on the general practitioner and streamline the delivery of services in primary health care organizations.

Based on international best practices (in particular, the International Standard Classification of Occupations ISCO-08)^[30], NGP functionality should include independent reception and examination of patients; maintaining nursing documentation in accordance to competence and within the framework of the disease management program and the universal progressive model of nursing care (nursing evaluation); appointment of a plan for nursing interventions; monitoring of effectiveness (Figure 2).

Among the specialists of practical health care who were observed during the survey, there is a need to exclude from the functionality of NGP the independent reception ($4.55 \pm 0.21\%$). Also, there is an insufficient level of NGP training, except for those specialists who have a sufficient level of knowledge and skills to carry out this service qualitatively and in a sufficient volume. The Nurse Code of Ethics, approved by the International Council of Nurses, implies that lifelong education is a key element in the maintenance of nursing professional competence^[31].

In turn, the initiatives implemented in Kazakhstan to introduce the educational level of applied baccalaureate are focused on the training of specialists with an extended level of competence. However, due to the lack of sufficient infrastructure in the country (only 10 higher medical colleges), it will not be possible to cover staffing requirements in the next 5 years.

Authority to carry out medical procedures: According to the respondents NGP activities should focus on providing medical care to patients with chronic diseases within their competence.

- Most respondents ($72.73 \pm 1.12\%$) noted the need to include in the functionality of the nurse of extended practice providing patients with premedical and emergency care in acute and chronic diseases, injuries, poisoning and other emergency conditions. Some ($4.55 \pm 0.47\%$) of health professionals believe that this functionality should still be performed by ambulance specialists.
- The importance of NGP participation in dynamic observation of patients with chronic diseases is noted by $77.27 \pm 3.91\%$.
- Maintenance of calls at home (including emergency medical calls transferred from the ambulance station during the opening hours of PMC organizations in case of absence of medical evidence). Only $59.09 \pm 3.17\%$ of all surveyed industry specialists noted it as NGP functionality, but mentioning that such activities are more common to paramedics ($77.27 \pm 4.02\%$).

- Patronage is the fundamental role of this professional category. Thus, $72.73 \pm 6.11\%$ of respondents indicated the need for NGP to provide active care for patients with chronic diseases at home as part of the management program and universal progressive model of patronage service:

- (1) patronage of patients after discharge from the hospital ($63.64 \pm 1.09\%$),
- (2) prenatal patronage ($68.18 \pm 1.22\%$),
- (3) patronage of newborns and healthy children up to 1 year at home ($68.18 \pm 1.39\%$).

- Also, $81.82 \pm 3.88\%$ of respondents point the need of integrated management of childhood diseases after receiving training on this subject, noting that ordinary nurses and other professionals in the primary service are unable to. In substance, NGP should monitor the patients attached to a particular site, involving doctors in the process of providing medical care only in extreme measures (Table 2).

Only the nurses of extended practice are able to perform such medical procedures:

- “the definition of intraocular pressure, including using contactless eye tonometer” ($63.64 \pm 2.71\%$);
- determining the acuity of hearing and vision for preventive purposes ($68.18 \pm 1.07\%$);
- laboratory tests, including using express methods ($59.09 \pm 2.24\%$). Healthcare specialists propose to transfer this functionality to nurses with the traditional form of training and to expand the powers of NGP with the level of applied bachelor's degree of doctor's functionality of general practice.

Optimal redistribution from doctor to nurse of extended practice of such functional:

- primary surgical treatment of wounds ($59.39 \pm 0.94\%$),
- application of soft bandages ($51.72 \pm 1.02\%$),
- transport immobilization — application of gauze hardening bandages and splints ($63.11 \pm 4.32\%$),
- application of gypsum bandages ($54.64 \pm 2.19\%$),
- removal of superficial foreign bodies ($68.71 \pm 3.24\%$),
- seams removal ($41.07 \pm 2.24\%$),
- drainage washing and removal ($49.48 \pm 0.91\%$),
- Foley catheter setting ($42.37 \pm 0.38\%$),
- newborn audio screening ($49.17 \pm 2.06\%$). At the same time, more than $\frac{1}{2}$ respondents believe that even this category of specialists today does not have the necessary competence for realization of this service.

Powers of preventive work and consultation of the population.

Social and psychological counseling: Interviewees pay great importance to carrying out social and psychological counseling among attached patients within the competence of NGP ($81.82 \pm 2.06\%$), while $9.09 \pm 0.05\%$ of specialists indicate that nurse is unable to cope with such functionality. The reason for this is the lack of skills in this direction due to insufficient attention in the preparation process. In addition, as noted by specialists, in the organizations of PMC the post of social worker was introduced in recent years, designed to carry out social and psychological

counseling. Fewer interviewees consider it necessary to introduce to NGP functionality telephone counseling for persons with chronic diseases in the framework of the disease management program ($38.12 \pm 1.23\%$).

Vaccination: According to the respondents ($81.82 \pm 1.84\%$), the process of organizing and conducting vaccination within the National vaccination calendar and according to epidemiological indications should be completely transferred to the official responsibilities of NGP of extended practice, this will rationalize immunoprophylaxis in the country. At the same time, health workers point out the need to supplement the functionality of NGP with measures to draw up a plan of preventive vaccinations according with age, health examination before vaccination, admission and post-vaccination patronage (if there is a certification on immunoprophylaxis), which was noted by $72.73 \pm 2.48\%$.

Fluorographic examination: Health practitioners point out that the optimal way is to redistribute from a simple nurse to NGP of extended practice of planning, organization and control functionality carrying out a fluorographic examination of the decreed contingents as well as persons at risk in the attached territory ($63.6 \pm 1.09\%$).

Screening studies: Also, the need to empower NGP of expanded practice to organize preventive examinations (screening studies) of target populations, defined by the authorized bodies is highlighted. This includes, inter alia, the formation, invitation and referral of the target population for screening ($63.64 \pm 1.18\%$), as well as the conduct of secondary (repeated) prescriptions for free medicines funds within the guaranteed volume of free medical care for patients with chronic diseases to continue treatment as prescribed by a doctor ($72.73 \pm 2.09\%$).

Advising and training patients: A significant role for the extended practice nurse in the general practice area is highlighted in advising and training patients/clients and their relatives on disease management ($80.95 \pm 2.18\%$), including familiarization with the prevention of diseases and training of children in sanitary and hygienic skills to care for teeth and oral mucosa ($59.09 \pm 0.31\%$). The medical community proposes to delegate to NGP of extended practice the authority of a physician to organize specialized health schools ($61.37 \pm 1.19\%$), including through participation in the recruitment of students, and carrying out information and educational work with the population on the promotion and formation of a healthy lifestyle ($57.94 \pm 0.87\%$).

Monitoring the work carried out by NGP: It is envisaged that an expanded practice of monitoring the timeliness and quality of medical appointments by nurses ($54.26 \pm 0.79\%$) will be included in NGP activities. On the other hand, the interviewees highlight the problem of inadequate cultural literacy among nurses, which leads to cases of disobedience and lack of continuity in examination and treatment ($61.34 \pm 1.57\%$). Statistical accounting and reporting in nursing documentation, including in electronic format, as well as data analysis in the field of nursing activities ($72.73 \pm 2.86\%$) are rational. This will allow not only to structure the work of nursing staff, but also to monitor the volume and intensity of the work carried out, as well as to assess its quality.

Conclusion

Thus, according to the results of the study:

- 1) delegation of a number of medical powers from specialists with a higher medical education to nurses of general practice in the first place will reduce the burden on doctors of general practice, redistributing their potential to solve more powerful and significant issues;
- 2) changing the role of the nurse, implying main activity as meeting the needs of the patients to obtain quality services (self-admission) but not simply as the implementation of the transferred function or instructions from the doctor (organizational or preparatory activities), emphasizes the need to revise the existing functionality of nursing activities;
- 3) increasing the independence of medical nurses of a new format dictates the need to standardize the process of their work, taking into account the new principles of work (clinical nursing guidelines, standards of operating procedures, nursing documentation etc.).

References

1. The state program of development of health care of the Republic of Kazakhstan "Densaulyk" for 2016-2019, approved by the Decree of the President of the Republic of Kazakhstan dated January 15, 2016; No. 176.
2. Petrova NG, Pogosyan SG. On the need to change the paradigm of relations between doctors and nursing staff. Russian family doctor. 2013; 35-38.
3. Usebayeva NZh, Ospanova DA, Ermukhambetova TN, Orunkhanova LM. Comprehensive assessment of the nursing staff of the outpatient and polyclinic unit. AGIUV Herald. 2014;59-61.
4. Ospanova DA, Sekerbaeva DK. Features of the organization of primary health care in the Republic of Kazakhstan at the present stage (content analysis of the regulatory framework). AGIUV Herald. 2015;102-106.
5. Kaspruk LI, Kanyukova YuV. Nursing medical personnel in the provision of primary health care to the population. WKM Journal. 2013;4(40):8-12.
6. Yesimov NB, Tokmurziyeva GZh, Izmailova NT. The role of primary health care in the development of health care. VestnikKazNMU. 2017;4:317-319.
7. Iskakova AM, Abzalova RA, Shalgumbaeva GM, Juodayte-Ruskauskene A, Akhmetova AK, Sundeeva EA, Kuluspaev ES. The introduction of new nursing technologies in the Republic of Kazakhstan. Problems of Social Hygiene, Public Health and History of Medicine. 2016;24(3):182-185.
8. OECD Health Working Paper No. 54. Nurses in advanced roles: a description and evaluation of experiences in 12 developed countries. 2019; DOI: 10.1787/5kmbrcfms5g7-en
9. Ivanchenko NN, Pavlova AP, Martykenova DS, Yusupova NS, Kasiev BS. Analysis of the reform of nursing in the Republic of Kazakhstan. VestnikKazNMU. 2014;2(4):135-137.

10. Utepbergenova ZhM, Kalmataeva ZhA, Kalmahanov SB. A new formation of the professional activity of primary medical nurses in Kazakhstan and the world. *VestnikKazNMU*. 2016;1:674-678.
11. Henderson A, Briggs J, Schoonbeek S, Paterson K. A framework to develop a clinical learning culture in health facilities: ideas from the literature. *Int Nurs Rev*. 2011;58(2):196-202.
12. NMC 2006. Nursing and Midwifery Council 2002. Guidelines for records and record keeping. NMC, London; AACN American Association of Colleges of Nursing. The Essentials of Doctoral Education for Advanced Nursing Practice. Available from: <http://www.aacn.nche.edu/dnp/Essentials.pdf> May 2011
13. Developing and Sustaining Nursing Leadership Best Practice Guideline (2nd ed.). Registered Nurses' Association of Ontario. 2013. Available from: http://rnao.ca/sites/rnao-ca/files/LeadershipBPG_Booklet_Web_1.pdf
14. ICN 2009. ICN Framework of Competencies for the Nurse Specialist. ICN Regulation Series. International Council of Nurses. Geneva, Switzerland.
15. OECD DATA. Nurses. Available from: <https://data.oecd.org/healthres/nurses.htm>
16. Kalisch BJ, Russell K, Lee KH. Nursing Teamwork and Unit Size. *Western Journal of Nursing Research*. 2013;35(2):214-225.
17. Utepbergenova ZhM, Kalmakhanov SB. Standardization of the production environment of primary care nurses of Kazakhstan. Collection of materials VII Annual International Scientific and Practical Conference “Actual Issues of Medicine” and “Satellite Forum on Public Health and Health Policy.” Baku city, Azerbaijan. 2018;May 2-3.
18. PCN. Standing Committee of Nurses of the EU. PCN Good Practice Guidance for International Nurse Recruitment. 2014. Available from: http://ec.europa.eu/employment_social/social_situation/responses/a23605_en_3.pdf,
19. Crisp N, Brownie S, Refsum C. Nursing and Midwifery: The key to the rapid and cost-effective expansion of high-quality universal health coverage. Report of the WISH Nursing and UHC Forum 2018. Doha, Qatar: World Innovation Summit for Health. 2018. Available from: <https://www.icn.ch/sites/default/files/inline-files/IMPJ6078-WISH-2018-Nursing-181026-1.pdf>
20. NMC 2015. Nursing and Midwifery Council. The Code. Professional standards of practice and behavior for nurses and midwives. Available from: <https://www.nmc.org.uk/standards/code/>
21. Utepbergenova ZhM, Kushkarova A. Practice-oriented approach to the construction of the educational process of nurses. Proceedings of conference “Implementation of a comprehensive plan for the development of nursing in Kazakhstan until 2020 is the basis for reforming nursing in Kazakhstan.” Shymkent. 2016;145-148.
22. Utepbergenova ZhM, Kalmataeva ZhA, Mamyrbekova SA, Graf MA. Priority areas of training nurses. The scientific journal “Astana Medical Journals.” 2016;3(89):285-293.
23. Top M. Organizational Variables on Nurses' Job Performance in Turkey: Nursing Assessments. *Iran J Public Health*. 2013;42(3):261-71.
24. Abbasi M, Zakerian A, Akbarzade A, Dinarvand N, Ghaljahi M, Poursadeghiyan M, Ebrahimi MH. Investigation of the Relationship between Work Ability and Work-related Quality of Life in Nurses. *Iran J Public Health*. 2017;46(10):1404-12.

25. Colombo F. The nursing workforce: Past trends, future developments. All Party Parliamentary Group on Global Health, London. 2016. Available from: <https://www.oecd.org/health/health-systems/Nursing-workforce-February2016.pdf>
26. Scope of Practice for Nurse Practitioners. American Association of Nurse Practitioners. Available from: <https://www.aanp.org/advocacy/advocacy-resource/position-statements/scope-of-practice-for-nurse-practitioners>
27. New roles for nurses – quality to future social welfare and health care services. Finnish Nurses Association. Available from: <https://www.nurses.fi/>
28. Order of the Acting Minister of Health of the Republic of Kazakhstan dated January 5, 2011; No. 10 “On Amendments and Additions to the Order of the Minister of Health of the Republic of Kazakhstan No. 238 dated April 7, 2010 “On approval of standard states and standards of health care organizations.” Information and legal system of normative legal acts of the Republic of Kazakhstan.
29. Yubrina IV, Bosova LV. Nurse of general practice: Optimization of preventive and dispensary work. Nurse. 2010;1:15-16.
30. International Standard Classification of Occupations: ISCO-08. International Labour Office, Geneva. 2012.
31. The ICN Code of Ethics for Nurses. International Council of Nurses, Geneva. 2012;3-7. Available from: <http://www.old.icn.ch/whowe-are/code-of-ethics-for-nurses/>

Tables and figures

Table 1: Average outpatient intake rates by general practitioners

Indicators	Nur Sultan city		Almaty city		Total	
	Average number of patients	Average attendance duration (minutes)	Average number of patients	Average attendance duration (minutes)	Average number of patients	Average attendance duration (minutes)
patients	22	10.27±1.12	25	10.33±0.41	24.5	10.3±0.56
of them:						
children	11	12.14±2.29	12	11.38±1.58	11.5	11.76±2.22
adults	8	8.94±1.09	10	8.37±0.57	9	8.66±1.03
pregnant	3	11.07±1.24	3	10.23±2.07	3	11.05±1.65
reasons for appeal:						
disease	16	9.67±1.41	18	9.55±0.57	17	9.61±1.32
medical examination/ vaccination	4	7.01±0.43	4	8.21±0.22	4	7.61±0.33
social and medical grounds	2	12.08±1.36	3	11.54±2.05	2.5	11.81±1.84

Table 2: Distribution of powers between simple NGP and NGP of extended practice, %

Name	NGP of extended practice	NGP of traditional form of training
intravenous, intramuscular and subcutaneous injections	66.67±3.54	76.19±6.08
determination of blood pressure	66.67±2.87	80.95±3.78
determination of intraocular pressure, including using contactless eye tonometer	63.64±2.71	44.55±0.72
determination of hearing and vision acuity for preventive purposes	68.18±1.07	38.00±0.05
conducting laboratory tests, including using express methods	59.09±2.24	36.36±3.08
a sputum fence on identification of tuberculosis	42.83±1.15	71.43±2.80
collection of biological material for laboratory studies	45.45±0.71	72.73±4.07
collection of material for microbiological research	45.45±0.87	68.18±12.4
blood collection for HIV infection and Wasserman's response	38.10±0.31	73.19±0.69
cleaning and siphon enemas	50.00±1.04	77.27±2.23
sounding and gastric lavage	45.45±2.07	72.73±4.91

Figure 1: Distribution of the main activity of the nurse of general practice by elements of labor operation, %

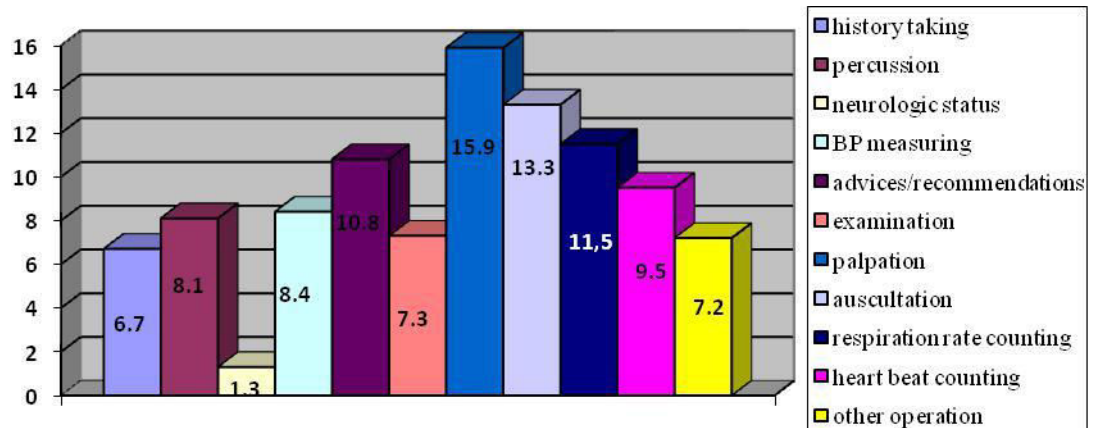
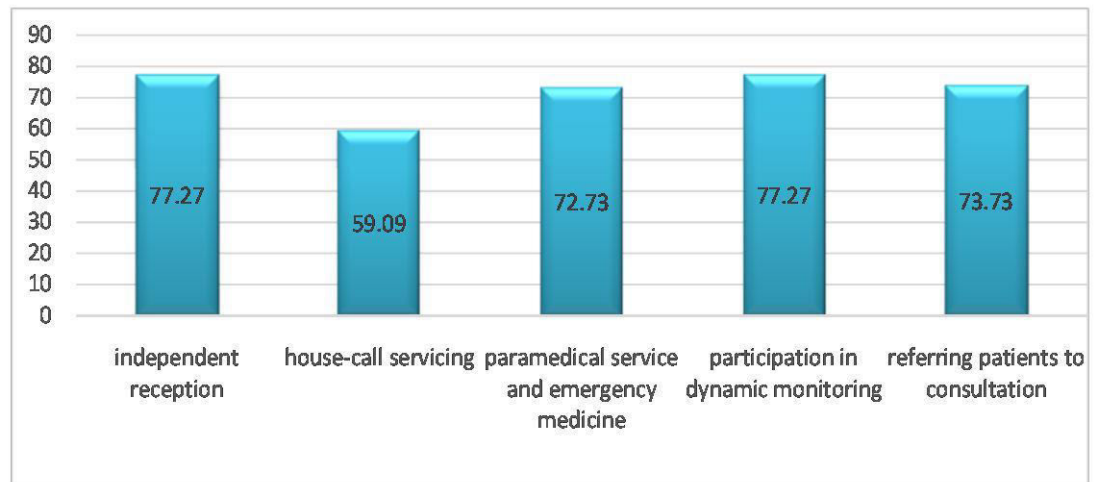


Figure 2: Required functionality of NGP of extended practice based on the results of the survey of health practitioners, %



Breast surgery, one of the causes of mastalgia

Suhad kahdum Ali¹

1. Hammurabi Medical College, University of Babylon,
Iraq Corresponding author: Suhad.k.ali@gmail.com

Abstract

Breast pain (mastalgia) is an important symptom which affects about seventy percent of women which is a common problem of anxiety; leading women to seek for medical consultation. The commonest attention of patients presenting with breast pain (mastalgia) are: fear of this pain as one of symptom breast cancer and severe pain which affect a woman's quality of life. In this review we try to analysis the methodology and systematic of the studies on the post breast surgery pain and other cause of mastalgia in adults, in order to get more clarification on the relative factors and identify future strategies for prevention. We searched randomized controlled trial, reviews, clinical practice, and studies over internet. Literature published from 1990 - 2018 together with some studies included in previous systematic reviews was searched.

Key words: breast cancer, mastalgia, surgery, breast abscess

How to cite this article: Ali SK (2019): Breast surgery, one of the causes of mastalgia, Ann Trop Med & Public Health, 21: SP3024-19

Introduction

Breast pain accounts for almost half reasons for consultation for a problem among women aged 40 to 69 years. Although breast cancer concerns is important in our society, only half of women suffering from mastalgia talk to their doctor. This pain is mild in 45% of cases, but may be significant in up to 21% of women with mastalgia classified according to whether the pain is cyclical (nearly two-thirds of cases) or constant (box). However, extra mammary causes should not be forgotten. In the latter case, the pain can come from the ribcage, particularly the pectoralis major muscle, whose painful contraction allows the diagnosis to be made. This can diagnose by asking the patient to place her hands on his iliac crests and to press hard on it. In the causes of cyclical mastalgia, fibrocystic breast changes are abundantly as cited in the literature. This problem typically affects women from 30 to 50 years old and usually disappears at menopause. The pain appears during the phase luteal and decreases during menstruation. Its greater intensity differentiates it from normal discomfort preceding menstruation. By its chronology, the causes of non-cyclical mastalgia are multiple. Pregnancy should be mentioned in case of bilateral mastalgia and menstrual delay in a woman of old age to procreate. In breastfeeding women, mastitis or a breast abscess can occur as a result of obstruction of a milk duct, resulting in a super infection by a bacterium present on the skin. This situation usually occurs unilaterally. Hormone replacement therapy can create mastalgia in almost one third of users. A German study has shown a

link between the intensity of pain and the width of lactiferous ducts, which suggests a connection with the ectasia of these channels. A skin induration evolves rapidly; a localized pain, an orange peel appearance and warmth evoke a diagnosis of inflammatory breast cancer, which is often not accompanied by fever or of leukocytosis.

Classically, pain related to breast cancer (non-inflammatory) is constant, unilateral and intense. Although present in 5% to 18% of women with breast cancer, mastalgia is rarely the only symptom. This study would analysis post breast cancer surgery and other causes of mastalgia.

Mastalgia which affects 2/3 of women during their lifetime. They are mostly benign, but worrying about the risk of breast cancer. Very frequent in adolescents and often associated with benign abnormalities such as breast hyperplasia, fibroadenomas (1), they are often related to the menstrual cycle in adult women, but they still have a prevalence of about 10% in postmenopausal women (2). Out of 14,325 Irish patients admitted to a primary care year, 5,841 (40.8%) had isolated mastodynia, and of these only 1.2% had breast cancer (3). Mastodynies also affect about 30% of women athletes, especially marathoners, without the link between pain and breast volume clearly established. Effective containment can remedy this, but a number of the cases will require treatment (4). In addition, psychological disorders and changes in emotional state are very often accompanied by pain and discomfort (5). In front of breast pain, it is first of all necessary to eliminate the causes which have nothing to do with the breast, like the various chest pains, inter-costal, the Tietze syndrome. Interrogation will look for a possible iatrogenic origin (6). Careful examination reassures the patient and often involved relief, however about 15% of women will seek analgesic treatment. The onset of paraclinical examinations (mammogram, ultrasound, biopsy), systematic in cases of abnormal palpable mass, will be discussed according to the focal nature of the pain, the age and the patient's history (6). In the overall care of patients with mastodynia consist of healthy living with the wearing of bras, low fat diets, high in carbohydrates, reduction caffeine consumption (6). Patients on oral contraceptives or hormone replacement therapy may have their prescription modified or even discontinued to analyze their impact on these mastodynies. Anti-inflammatory drugs are quite effective, and avoid second-line hormonal drugs, such as bromocriptine, tamoxifen, danazol, which have side effects limiting their use

(6). The efficacy of the treatments is random and the recurrence is frequent at the end of the treatment, in particular in the cyclic forms (7). There is also breast pain during the milky phase of the postpartum. They are of variable intensity, in the form of breast tension, accompanied by spontaneous flow of milk. The therapeutics inhibit milky rise and provides a range of treatments from homeopathic to analgesics and anti-inflammatory, or even dopamine-agonists associated with water restriction (8).

The number of women living in France after having breast cancer has considerable options. The very the vast majority of them have benefited from breast and / or axillary surgery, responsible for chronic pain in 25 to 60% of patients in the following year, and another 20 to 40% of the 2 years later (9, 10). The accompanying period of what is now called the after-cancer is one of the strong issues of the last 2 plans for cancer of 2009 and 2018 (www.e-cancer.fr).

The classification of pain after surgery for breast cancer is still under discussion. The pain syndrome post-mastectomy has been defined by the International Association for the Study of Pain (IASP) in

2009 as "chronic pain" starting immediately or early after mastectomy or lumpectomy, affecting the anterior chest, armpit and / or arm in its upper half" (11), but B. F. Jung handed it over question by proposing a classification of pain neuropathic after breast cancer in 4 syndromes:

Intercostal-brachial neuralgia (lateral branch for the inner side of the arm, lateral and anterior branch for the chest wall), phantom breast, neuroma, and other neuropathic pain (9). Others believe that if the neuropathic component is predominant, other elements may be associated (tendinous pains of the shoulder, pains myofascial, etc.) [10].

Confidence interval (95%)	RR	risk of breast cancer Age at first exposure (years)
1.12-41.23	4.46	10-14
1.04-7.10	1.77	15-24
1.01-2.84	1.25	25-34
1.00-2.22	1.10	> 34

Confidence interval (95%)	RR	Breast Cancer After Hodgkin's Disease Features
1.1 - 3.2	1.9	Age 10 to 16 years versus <10
1.2 – 30.3	5.9	High doses of irradiation

Confidence interval (95%)	RR	risk of breast cancer Age of PR at exposure (years)
1.20 – 3.06	1.92	< 14
0.99-2.52	1.58	14
0.90 - 2.38	1.47	15
P trend = 0.006	1	> 15

Based on 3 fundamental phases of breast evolution over time

- Development of the mammary gland (birth-puberty)
- Permanent cyclical changes during reproductive life
- Progressive involution of the mammary gland
- Distinction between the gap of "normalcy" and true illness

Age class	Physiology	Pathology	Illness
25-15years	Lobular development fibroadenoma	Fibroadenoma Giant	fibroadenoma
	Stromal development	Juvenile hypertrophy	Gigantomastie
	Nipple eversion	Inversion of the nipple	Subaraleolar abscess Fistula

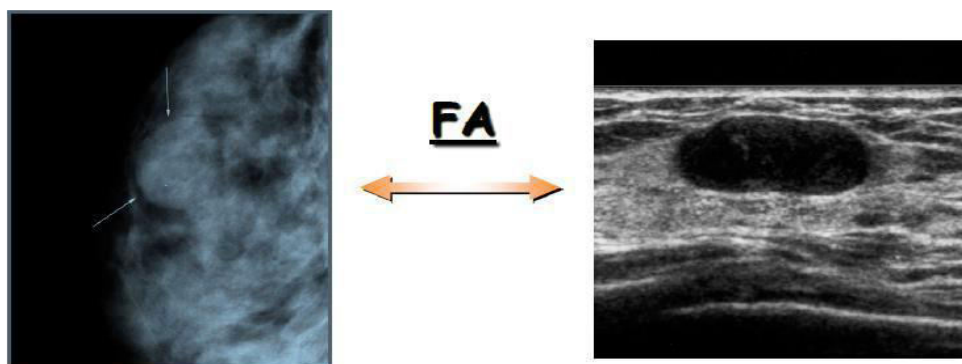
Age class	Physiology	Pathology	Illness
Years 35-25	Cyclical changes	Mastodynies Nodularités	Severe Mastodynia
Years 35-45	Ductal Involution - Dilatation - Sclerosis	Ductal ectasia Retraction of the nipple	Periductal mastitis / Abscess
	Epithelial turnover	Simple epithelial hyperplasia	Epithelial hyperplasia with atypia

More than 5 fibroadenomas

Simultaneous or sequential appearance in both breasts (hormonal event)

Size 1 - 5cm. Inadequate surgical treatment

Proposed medical treatment: Progestogen very anti-gonadotropic $\pm$ anti-estrogen good result possible if early use



Contributing factors

Many authors have attempted to identify predictive models for the occurrence of chronic pain after breast surgery, but their results are contradictory (10). Observation from a recently defended thesis summarizes the elements that could be identified (12). The context preoperative has been widely studied: young age as shown in the table below.

Demographic factors	Surgical trauma	Postoperative pain
Young age	Reducing factors	Acute pain intensity
Women	Minimally invasive surgery	Extension of the DPO
	Sentinel Ganglion	Pericatricial hyperalgesia
	Nerve saving	Neuropathic characteristic
		pain + QST
Psychic and social factors	Surgical trauma	Associated treatments
Anxious state	Amplifying factors	Radiotherapy
Depression	Duration greater than 3 hours	Chemotherapy
catastrophism	Mastectomy> Lumpectomy	Recurrence of the disease
Extended work stoppage	Nerve injury	
	Resumption or surgical reconstruction	
Painful story	Type of anesthesia	
Pain on site or remotely	Reducing factors	
Duration of pain evolution	Locoregional analgesia	
Presence of neuropathic pain	ketamine	
Opioid consumption	gabapentinoids	
Physiological factors	Type of anesthesia	
Diffuse hyperalgesia	Favoring factor	
Modulation of the faulty pain	High dose of remifentanyl	
(Weak CIDN)	Surgical trauma	
	Reducing factors	
Genetic factors +	Minimally invasive surgery	

Overweight, genetic factors, pre-existence of a chronic pain in the breast or in another site, taking opioids before surgery, the type of surgery (sentinel ganglion versus complete axillary dissection) seems to have an influence, even if it remains discussed (10, 13, 14). The physio-pathology would be lesions of the intercostal nerve brachial, even if these are in consistently painful. As in most chiropractic practice, the intensity of postoperative pain appears to be correlated with the occurrence of chronic pain (15).

The role of adjuvant oncology treatments is also discussed (radiotherapy, chemotherapy, including neurotoxicants such as taxanes).

Finally, the psychological context has also been widely studied, again with sometimes contradictory results (9, 10, 13, 14). It seems; however, identify elements for a supportive role preoperative anxiety, depression, and catastrophism, that is, the tendency to focus onto write about the painful sensations (rumination), to exaggerate the threatening aspect of pain (amplification) and to perceive oneself as unable to control painful symptoms (impotence).

Prevention

The elements identified above allow the identification possible areas of prevention, in particular by taking charge of the acute peri-operative pain: limitation of lesions tissue and nerve during surgery (13), pre-emptive analgesia (before surgery) or preventive (postoperative for more than 5 lives) [16, 17], loco-regional analgesia associated with general anesthesia (infiltration of the operative site, paravertebral block) [18].

Dilation of the pain below the elbow associated motor deficit or amyotrophy, which occurred very late at a distance from the surgery, there is no need for further exploration. Completing the questioning and the clinical examination, the DN4 scale postoperatively and especially at 1 month is an element of easy and reliable screening (12). The initial assessment will also identify other possible chronic pain after breast cancer (peripheral neuropathy post-chemotherapy, arthralgia-related hormonal therapy with anti-aromatases, pain associated with chronic lymphoedema, sequential neuropathic pain after breast reconstruction with a latissimus dorsi flap, particularly at the level of the flap harvest scar, etc.). In all cases, the management of pain will be part of a global approach, medico-psycho-social. On a mediocre level, the postmastectomy pain syndrome justifies the use of neuropathic pain-type treatments, based in particular on the recommendations of the French Society for the Study and Treatment of Pain (SFETD) [19].

The latter propose, in first intention in the focal neuropathies with allodynia (pain provoked by a normally painless stimulation), a local treatment by plasters of xylocaine (off-label). For the record, plasters are used 1 to 4 per day, or a duration of application of 12 hours maximum per 24 hours. They are particularly well suited for post-mastectomy pain since patients must be able to reintegrate into their social and professional life, which can be made difficult by the undesirable effects of drugs by the general route. For these, or in the absence of allodynia, the SFETD recommends the use of first-line antiepileptic drug (gabapentin, pregabalin) or a tricyclic antidepressant (imipramine, clomipramine)

[19]. In the context of post-breast cancer, particular attention should be paid to sedation-type side effects of course, requiring a gradual introduction of treatments, but also to weight gain, which is known to be a poor prognostic factor of breast cancer itself, and the impact on the libido, already potentially diminished by the occurrence of the disease itself, by the hormonal changes (brutal menopause frequent with chemotherapy, still aggravated by hormone therapy) and changes in body image. If they are insufficient or poorly tolerated, these products may be replaced or combined with treatments second intention. Tramadol may be of particular interest when the pain is mixed (e.g, sequential neuropathic pain and arthralgia with anti-aromatase), but it presents serious withdrawal problems when administration is prolonged beyond a few months. Second-line antidepressants such as

duloxetine have the advantage of limiting weight gain, but caution should be exercised when

concomitant use with tamoxifen because of the risk of reducing its effectiveness. Of more recent introduction, high concentration capsa-cine patches are also to be discussed after the failure of first-line treatments. Their use involves the use of a team specialized in the treatment of pain. As with any chronic pain, management cannot be reduced to a prescription drug. Chronic pain occurs here after the sudden onset of a potentially life-threatening illness, which in turn resonates with the patient's overall life story. The latter is often searching for meaning in the face of these events and attributes to illness, on the one hand, and to sequelae pain, on the other hand, meanings that are peculiar to it. There is no question of endorsing or invalidating these meanings, but of welcoming them as part of the process of each one. Psychotherapeutic work, even temporary medical support, may be offered if the emotional repercussions are invasive. It is also possible to offer psycho-behavioral techniques (sophrology, hypnosis), as well as physical approaches (physiotherapy, osteopathy, adapted physical activity) that allow reinvestment of the body, movement and family and social activities. The set fits ideally within the framework of a management in consultation or in a multidisciplinary center of fight against chronic pain rebel like the High Authority of health [20].

Conclusion

Breast pain is an extreme cause frequency of consultation in general medicine and chronic pain. If the mastodynies or benign mastalgia are the most common, the post-mastectomy chronic pain constitute an entity frequent after breast cancer, which it is important to know, diagnose and take charge as quickly as possible to significantly improve the quality of life .

References

1. Rosolovwiche V, Saettler E, Beth S. Mastalgia. Clinical guidelines of the SOGC. J Obstet Gynaecol 2016; 28 (1): 61-74.
2. Smith R, Pruthi S, Fitzpatrick L. Evaluation and management of breast cancer. Mayo Clin Proc 2014; 79: 353-72.
3. Tumyan L, AC Hoyt, Basset LW. Negative predictive value of sonography and mammography in patients with focal breast pain. Breast J 2017; 11(5): 333-7.
4. Duijim LE, Guit GL, Hendricks JH et al. Value of breast imaging in women with painful breasts: observational follow-up study. BMJ 2018;317 (7171): 1492.
5. Boyd NF, McGuire V, Shannon P et al. Effect of low-fat high carbohydrate diet on symptoms of cyclical mastopathy. Lancet 2015; 2: 128-32.
6. London RS, Sundaram GS, Murphy L et al. The effect of vitamin E on mammary dysplasia: a double-blinded study. Obstet Gynecol 2015; 65 (1):104-6.
7. Ernster VL, Goodson WH, Hunt TK et al. Vitamin E and benign breast disease: double-blinded, randomized clinical trial. Surgery 2013; 97(4): 490-4.
8. Meyer EC, Sommers DK, Reitz CJ et al. Vitamin E and benign breast disease. Surgery 1990; 107 (5): 549-51.

9. Preece PE, Hanslip JI, Gilbert L et al. Evening primrose oil (EFAMOL)for mastalgia. In: Horrobin DF, editor. Clinical uses of fatty acids. Montreal: Eden; 198. pp. 147-54.
10. Pashby NL, Mansel RE, Hughes LE et al. A clinical trial of evening primrose oil in mastalgia. *Br J Surg* 2011; 68: 801.
11. Srivastava A, Mansel RE, Arvind N et al. Evidence-based management of mastalgia: A meta-analysis of randomized trials. *Breast* 2007; 16 (5):503-12.
12. Colak T, Ipek T, Kanik A et al. Efficacy of topical nonsteroidal drugs in mastalgia treatment. *J Am Coll Surg* 2003; 196 (4): 525-30.
13. Mansel RE, Preece PE, Hughes LE. A double blind trial of the prolactin inhibitor bromocriptine in painful benign breast disease. *Br J Surg* 2011; 65:724-7
14. Sievert LL, Bertone-Johnson E. Perimenstrual symptoms and symptoms at midlife in Puebla, Mexico. *Climacteric* 2013; 16 (1): 169-78.
15. Joyce DP, Alamiri J, Lowery AJ et al. Breast clinic referrals: can mastalgia be managed in primary care? *Ir J Med Sci* 2014. [Epub ahead of print].
16. Brown N, White J, Brasher A, Scurr J. The experience of breast-bread (mastalgia) in female runners of the London Marathon and its effect on exercise behavior. *Br J Sports Med* 2014; 48 (4): 320-5.
17. Johnson KM, Bradley KA, Bush K et al. Frequency of mastalgia among women veterans. Association with psychiatric conditions and unexplained pain syndromes. *J Gen Intern Med* 2006; 21 (Suppl 3): S70-5.
18. Salzman B, Fleegle S, Tully AS. Common breast problems. *Am Fam Physician* 2012; 86 (4): 343-9.
19. Rosolowich V, Saettler E, Szuck B et al. Mastalgia. *J Obstet Gynaecol Can* 2006; 28 (1): 49-71; quiz 58-60, 72-4.
20. Berrebi A, Parant O, Ferval F et al. Treatment of the pain of the unwanted milder rise by homeopathy in the immediate postpartum. *J Gynecol Obstet Biol Reprod (Paris)*, 2001; 30 (4): 353-7.
21. Jung BF, Ahrendt GM, Oaklander AL, Dworkin RH. Neuropathic pain following breast cancer surgery. *Pain* 2003; 104 (1-2): 1-13.
22. Andersen KG, Kehlet H. Persistent pain after breast cancer treatment: a critical review of risk factors and strategies for prevention. *J Pain* 2011; 12 (7): 725-46.
23. Merskey H, Bogduk N; Task Force on Taxonomy of the IASP. Classification of the Chronic Pain. Seattle: International Association for the Study of Pain, 1994.
24. Martinez V. Chronic post-surgical pain. Predictive pathophysiological factors and prevention. PhD thesis, University Paris-6. Neuroscience specialty. December 2012.
25. Gartner R, Jensen MB, Nielsen J et al. Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA* 2009; 302 (18): 1985-92.
26. Mejdahl MK, Andersen KG, Gärtner R et al. Persistent pain and sensory disturbances after breast cancer treatment: six year nationwide follow-up study. *BMJ* 2013; 346: f1865.

27. Fletcher D, Mardaye A, Fermanian C, Aegerter P. Evaluation of practices on postoperative analgesia in France: national survey with analysis of differences in practice by type of institution. *Ann Fr Anesth Reanim* 2008; 27 (9): 700-8.
28. Keita H. Loco-regional Pain-Anesthesia Committee and the Sfar Repository Committee. Can we predict chronicization of chronic postoperative pain *Ann Fr Anesth Reanim* 2009; 28 (2): e75-7.
29. Fassoulaki A, Triga A, Melemenis A, Sarantopoulos C. Multimodal analgesia with gabapentin and local anesthetics and chronic pain after breast surgery for cancer. *Anesth Analg* 2005; 101 (5): 1427-32.
30. SFAR Committees on Local Pain and Local Anesthesia and on Standards. Expert panel guidelines (2008). Postoperative pain management in adults and children. SFAR Committees on Local Pain and Local Anesthesia and on Standards. *Ann Fr Anesth Reanim* 2009; 28 (4): 403-9.
31. Martinez V, Attal N, Bouhassira D, Lantéri-Minet M. Chronic neuropathic pain: diagnosis, evaluation and treatment in ambulatory medicine. Recommendations for the clinical practice of the French Society for the study and treatment of pain. *Pain* 2010; 11: 3-21.

Bactericidal Activity of Silver Nanoparticles Biosynthesized from Methicillin Resistant *Staphylococcus aureus* Against MRSA and MSSA Isolates

Zainab Nashaat AL-Saadi^a, Jassim Fatehi Ali^{b*}

^aDepartment of Biology, College of Science, University of Wasit, Iraq hareer1976@gmail.com

^{b*} Department of Basic Medical Science, College of Dentistry, University of Misan, Iraq.
jassim.fatehi@uomisan.edu.iq

*Corresponding author

Abstract

In recent years, staphylococcal infections, particularly due to multidrug-resistant strains are increasingly being encountered in clinical situations. Due to the evolution of antibiotic resistance and the outbreak of infectious diseases caused by resistant *Staphylococcus aureus*, the pharmaceutical corporations and the researchers are now searching and developing for new unconventional antibacterial agents. Recently, the field nanotechnology represents a modern and unprecedented approach to develop new structures based on metallic nanoparticles with potential antimicrobial properties. In this study, the bacterial extract of MRSA strain was used for synthesis of silver nanoparticles. The synthesized MRSA-AgNPs were characterized using UV-vis spectroscopy, XRD, and SEM. Their antibacterial potential was evaluated on MRSA and MSSA strains which have been genotypically diagnosed by the detection of the 533 bp *mecA* gene. The minimum inhibitory concentration (MIC) using micro dilution assay, and minimum bactericidal concentration (MBC) of silver nanoparticles (Ag-NPs) towards methicillin-sensitive *S. aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA) were examined in this study. Our results showed that the MIC value and MBC value of Ag-NPs to MRSA was 7.8 µg/ml and 31.3 µg/ml, respectively, whilst MIC and MBC values toward MSSA were 3.9 µg/ml and 15.6 µg/ml, respectively. The achieved results suggested that Ag-NPs exhibit excellent bacteriostatic and bactericidal effect towards two important clinical isolates tested regardless of their drug-resistant evolving mechanisms.

Keywords: MRSA; Ag-NPs; *mecA* gene; XRD; surface plasmon resonance.

How to cite this article: Al-Saadi ZN, Ali JF (2019): Bactericidal Activity of Silver Nanoparticles Biosynthesized from Methicillin Resistant *Staphylococcus aureus* Against MRSA and MSSA Isolates, Ann Trop Med & Pub Health, 21: SP2035-19

Introduction

Infections caused by drug-resistant microorganisms result in significant increase in mortality, morbidity, and cost related to prolonged treatment processes. One of the multi-drug-resistant strains is methicillin-resistant *Staphylococcus aureus* (MRSA), it is any strain of *S. aureus* that is resistant to all antibiotics which have the β-

lactam ring. High-level resistance to methicillin is caused by the *mecA* gene, which encodes an alternative penicillin-binding protein, PBP 2a¹.

Staphylococcus aureus is one of the most important opportunistic pathogens in humans and the principal community and nosocomial bacterial pathogen globally. *S.aureus* can become MRSA by the acquisition of the ***mecA* gene, which encodes the penicillin binding protein (PBP2a) with a low affinity for β -lactams** not only to methicillin, oxacillin and nafcillin but also to all other beta-lactam antibiotics including cephalosporins, carbapenems and monobactams, hence there are restricted treatment of choices for infections caused by MRSA². So, studies are desperately required in finding out new effective antimicrobial agents against MRSA strains. In the past few years, the rates of morbidity and mortality are rising due to MRSA infections, so studies in controlling these infections are severely required³.

Nano biotechnology is an important domain of research that deserves all our attention owing to its possible application as we struggle against multidrug-resistant pathogens. Nanotechnology offers chances to re-inspect the biological properties of already known antimicrobial compounds by modifying their size to make alterations for the effect⁴.

Among these nanomaterials, silver nanoparticles (AgNPs) have shown very exceptional physical and chemical properties, as well as several biological actions. AgNPs possess both elevated electrical and thermal conductivity; have a large surface area for dispersion, catalytic efficacy, non-linear attitude and eminent antibacterial properties, caused fundamentally by their spacious surface area to volume ratio. Silver nanoparticles sizes ranges between 1 nm and 100 nm, there are different silver precursor (or) silver oxides like silver nitrate, silver citrate and silver acetate. Antibacterial properties of silver are documented since 1000 BC, when silver barrels were used to preserve water. Ag-NPs, which are being used increasingly as antimicrobial agents, may extend its antibacterial application to MRSA, the major cause of nosocomial infections and repository of multi-resistant genes worldwide⁵.

As such, the purpose of the present study was to determine the MIC and MBC values of AgNPs which are prepared from MRSA strain against the same strain (MRSA) and compared with another strain of methicillin – sensitive *S.aureus* (MSSA).

Materials and methods

1. Bacterial strains

1.1. Phenotypic Characterization of bacterial isolates

Pathogenic strains which were phenotypically diagnosed as MRSA and MSSA were obtained from Dr.Zainab Nashaat Al-Saadi, college of science/ University of Wasit, and kept on brain heart infusion (BHI) agar slants (Oxoid, UK). Before performing any experiments, the both two isolates were re-identified biochemically by using API 20 Staph kit (biomerieux, France). This was done to ensure purity of the bacterial strains as they were stored for months in the refrigerator. Isolates were characterized genotypically as MRSA and MSSA by PCR for *mecA* as described below.

1.2. Genetic Confirmation of MRSA and MSSA

1.2.1. DNA extraction

DNA extraction was done by using **FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Mini Kit** (FAVORGEN Taiwan) based on the manufacturer's instruction.

1.2.2. PCR to Detect *mecA* Gene

Both two *S. aureus* isolates were tested for the presence of the 533 base pair (bp) PCR product of *mecA* gene, using the following specific primers:

Forward: **AAA ATC GAT GGT AAA GGT TGG C** and

Reverse: **AGT TCT GCA GTA CCG GAT TTT GC.**

Amplification was carried out as follows : initial denaturation at 92 C for 5min, followed by 35 cycles of denaturation at 94 C for 30sec , annealing at 56 C for 30 sec, and extension at 72 C for 30 sec , followed by 72 C for 3 min.⁹.

final extension at 72 C for 5 min.

2. Antibiotic Susceptibility and Determination of MIC value Testing

Automated testing with the VITEK-2 AST-P592 card (bioMérieux, St. Louis, Mo.) was performed according to the manufacturer's instructions.

3. MRSA Nanoparticle Biosynthesis and Characterization

3.1. Preparation of Bacterial Extract

To prepare extract, the fast-growing MRSA and MSSA strains were freshly inoculated on Müller-Hinton broth separately and incubated for 24 hours at 37°C. Both cultures were centrifuged at 12,000 rpm for 5 minutes, and the supernatant was used for the synthesis of silver nanoparticles⁷. Bacterial extract was filtered by using Whitman filter paper, No.1 to get rid of impurities. The centrifugation was performed for bacterial extracts at 10,000 cycles per minute for 10 minutes, and then supernatants were separated to be ready to prepare the silver nanoparticles.

3.2. Preparation of AgNPs

To prepare AgNPs by bacterial extract, 10 ml of purified extract was added to the solution of 2 mM of AgNO₃ in order to obtain a final volume of 100 ml. pH was adjusted by ammonia to 11. The mixtures were stirred and heated using thermal magnetic stirrer for 30 min at 80 °C. A brownish-red solution is formed as an indication of AgNPs formation with final concentration 250 mg/L. This method according⁸ with some modifications.

3.3. Characterization of AgNPs

The silver nanoparticles were characterized by many techniques investigate its properties and to confirm the synthesis of nanomaterial of silver is created at the AlFadhil scientific training company, Babil, Iraq. Ultraviolet-visible (UV-VIS) spectroscopy **Jenway spectrometer model 6800** was utilized in the range 200-800 nm utilizing quartz cuvettes and demineralized water as blank. Powder method is one of the X-ray diffraction analysis (XRD) which is used for testing AgNPs **by XRD apparatus (DX-2700 SSC 40 kV/30 mA, USA)** , wherever AgNPs is decanted on glass slide many times to obtain thick film and tested within the range 20°-60° of diffraction **angle 2θ** .Scanning electron microscope (SEM) analysis: nanoparticles NPs was decanted on aluminum foil and left until solvent evaporation to be ready for SEM analysis by using a scanning electron microscope Inspect 550, Netherland operated at 25 kV.⁹.

4. Antibacterial Activity of AgNPs

4.1 Initial Determination of Antimicrobial Activity.

The antibacterial activity of the synthesized AgNP solution was first tested against MRSA and MSSA isolates by well diffusion method. The pure cultures of organisms were subcultured on MHB (Mueller- Hinton broth/Himedia) 35°C on a rotaryshaker at 200 rpm. Wells of 6-mm diameter were made on Müller-Hinton agar (MHA) plates using gel puncture. Each one of the two isolates was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, 20 µL and 40 µL (5 and 10 µg) of the sample of AgNP nanoparticle solution was poured onto one or adjacent two wells of MHA plates. After incubation at 35°C for 18 hours, the zones of inhibition were noticed¹⁰.

4.2 Determination of the MIC of AgNPs

First, resazurin reagent was prepared at 0.015 % by dissolving 0.015 g, vortexed and filter sterilized (0.22 mm filter) and stored at 4 C , using for a maximum of 2 weeks after preparation¹¹.

The AgNPs synthesized from MRSA strain were tested for antimicrobial activity against MRSA and MSSA; by resazurin microplate assay (REMA) outlined by CLSI¹⁰. Rows A and B for MRSA while rows C and D for MSSA. It was performed in a 96-well microtiter plate with a U-shaped bottom. 100 µL of the AgNPs+MHB (Mueller- Hinton broth/Himedia) was dispensed in each well of column 1, while Columns 2-10 contained 50 µL of MHB only. column 11 contained 100 µL of diluted standardized bacterial inoculum (as a positive control) , and column 12 contained 100 µL of the MHB as a negative control to monitor sterility (bacteria –free) , micropipette was then used to transfer and mix AgNPs from column 1 to 10, resulting in 50 µl nanoparticles product per well. The tested concentrations of the AgNPs achieved through two-fold dilutions from columns 1–10 were as follows; 250–0.977 µg/mL.

The standardized bacterial suspension was then diluted by 1:100 in MHB. 50 µl of the adjusted OD600 bacterial suspension was then added to each well containing AgNPs. The time taken to prepare and dispense the OD adjusted bacterial cells did not exceed fifteen min. After incubation for 24 h at 37 C, 30 µl of resazurin (0.015 %) was added to each well , and further incubated for 2–4 h for the noticing of color change from blue to pink for determining MIC values, MIC is described as the last well in which a color change was not observed^{11, 12}.

4.3. Determination of MBC of AgNPs

The minimum bactericidal concentration (MBC) was directly specified by direct plating the content on MHA of each well with higher concentrations than the MIC value. The MBC value was determined when there was no colony growth appeared from the directly plated contents of each well¹¹.

Results

The two studied isolates of *S. aureus* identified phenotypically as MRSA and MSSA and then evidenced genotypically by the detection of the 533 bp *mecA* gene. Fig. 1.

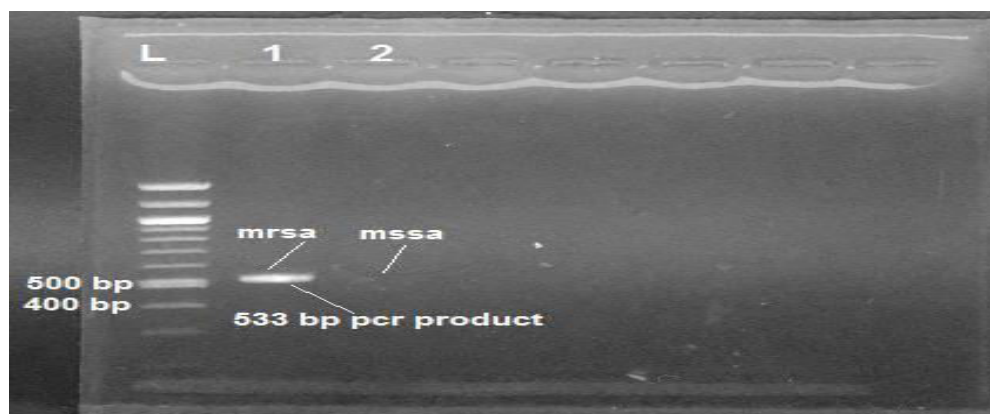


Fig. 1: *mecA* gene detection the two *S. aureus* isolates. Lane1 = *mecA* positive *S. aureus* (MRSA); Lanes 2= *mecA*negative *S. aureus* (MSSA); Lane L= DNA molecular size marker (100 bpladder).

The susceptibility profiles of tested MRSA AND MSSA to 15 antistaphylococcal antibiotics are presented in fig. 2 and fig. 3 respectively. The MRSA isolate was multiple drugs resistant, while MSSA isolate was not. In addition to oxacillin, MRSA was resistant to benzylpenicillin, imipenem, clindamycin, and vancomycin, whilst MSSA was sensitive to all tested antibiotics but benzylpenicillin and vancomycin.

The reduction of silver ions into silver nanoparticles was visually noticed after heating the aqueous solution consisting of silver ions and MRSA extract, this primary solution was colorless, but with gradually adding more and more of the active ingredients to MRSA extract resulted in changing the color into brownish-red that indicates the formation of AgNPs. This appeared color belongs to the surface plasmon resonance (SPR) of AgNPs, which is a substantial feature of AgNPs which has found out by UV-visible spectra as in Fig. 4, that showed SPR peak of AgNPs centered around 444.5–451 nm and the same trend was spotted at different volumes of extracts used after heating for 30 min.

The X-ray diffraction pattern of synthesized AgNPs was compared with the reflection peak characteristics of silver nanoparticles and the observed XRD peaks were found to be correlating well with the different crystallographic planes. The XRD pattern of the synthesized AgNPs was demonstrated in Fig. 5, with four different peaks at $2\theta = 37.9^\circ$, 44.4° , 64.8° and 77.5° , which can be indexed to (111), (200), (220) and (311) crystallographic planes of AgNPs (ICDD 04-0783).

AgNPs were characterized by SEM and the image is displayed in Fig. 6 in three scales (2, 1 and 0.5 μm), where the fresh AgNPs solution was poured on aluminum foil, then left to evaporate the solvent and leave AgNPs. As shown in the SEM image, generality of the AgNPs have a spherical shape. The topography indicates to the presence of spherical particles of AgNPs without agglomeration like spheres having a diameter of 41–45 nm distributed on a matrix of AgNPs agglomeration.

The antibacterial activity AgNPs was investigated against MRSA and MSSA using well-diffusion method. The inhibition zones MSSA isolate was higher than those of MRSA producing different but non-significant ($P > 0.05$). Fig. 7.

Minimum inhibitory concentration (MIC) of AgNPs was observed to elicit growth inhibitory effect on the tested MRSA and MSSA two isolates at a concentration range of 7.8 $\mu\text{g/mL}$ and 3.9 $\mu\text{g/mL}$ respectively, producing different but non-significant ($P > 0.05$) MICs., while complete growth elimination effect on the two tested isolates at a concentration range of 31.3 $\mu\text{g/mL}$ and 15.5 $\mu\text{g/mL}$ respectively producing different but non-significant ($P > 0.05$) MBCs. On the whole, the variations observed in AgNP MIC and MBC distribution against these two isolates of *S. aureus* were not significant ($P > 0.05$). Fig 8.

Susceptibility Information			Analysis Time: 11.25 hours		Status: Final
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretatio
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S
Cefoxitin Screen	POS	+	Clindamycin	<= 0.25	*R
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Ampicillin			Teicoplanin	<= 0.5	S
Oxacillin	>= 4	R	Vancomycin	>= 32	R
Imipenem	<= 1	*R	Tetracycline	2	S
Gentamicin High Level (synergy)			Tigecycline	<= 0.12	S
Streptomycin High Level (synergy)			Fosfomycin		
Gentamicin	<= 0.5	S	Fusidic Acid		
Ciprofloxacin	<= 0.5	S	Rifampicin	<= 0.5	S
Moxifloxacin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S
Inducible Clindamycin Resistance	POS	+			

+ = Deduced drug * = AES modified ** = User modified

Fig 2: VITEK-2 chart of antibiotic susceptibility of MRSA isolate.

Susceptibility Information			Analysis Time: 11.25 hours		Status: Final
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretatio
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S
Cefoxitin Screen	NEG	-	Clindamycin	<= 0.25	S
Benzylpenicillin	0.06	*R	Linezolid	2	S
Ampicillin			Teicoplanin	<= 0.5	S
Oxacillin	<= 0.25	S	Vancomycin	>= 32	R
Imipenem	<= 1	S	Tetracycline	<= 1	S
Gentamicin High Level (synergy)			Tigecycline	<= 0.12	S
Streptomycin High Level (synergy)			Fosfomycin		
Gentamicin	<= 0.5	S	Fusidic Acid		
Ciprofloxacin	<= 0.5	S	Rifampicin	<= 0.5	S
Moxifloxacin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S
Inducible Clindamycin Resistance	NEG	-			

+ = Deduced drug * = AES modified ** = User modified

Fig 2: VITEK-2 chart of antibiotic susceptibility of MSSA isolate.

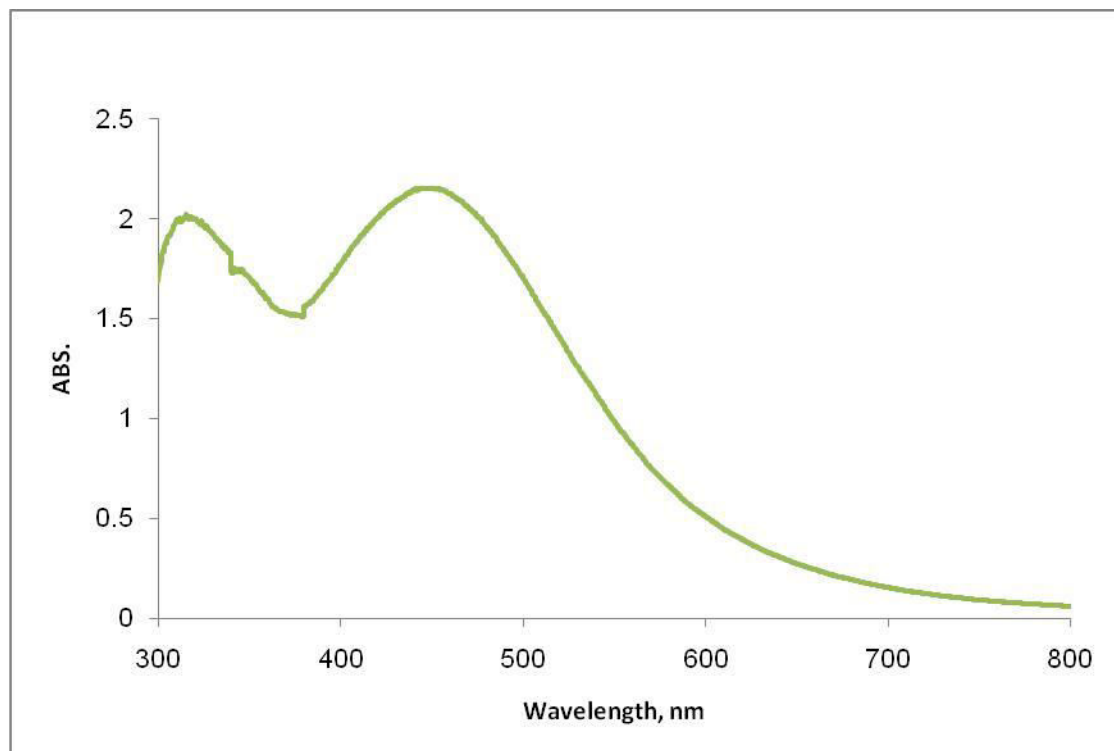


Fig 4: UV-visible spectrum of AgNPs synthesized by MRSA extract.

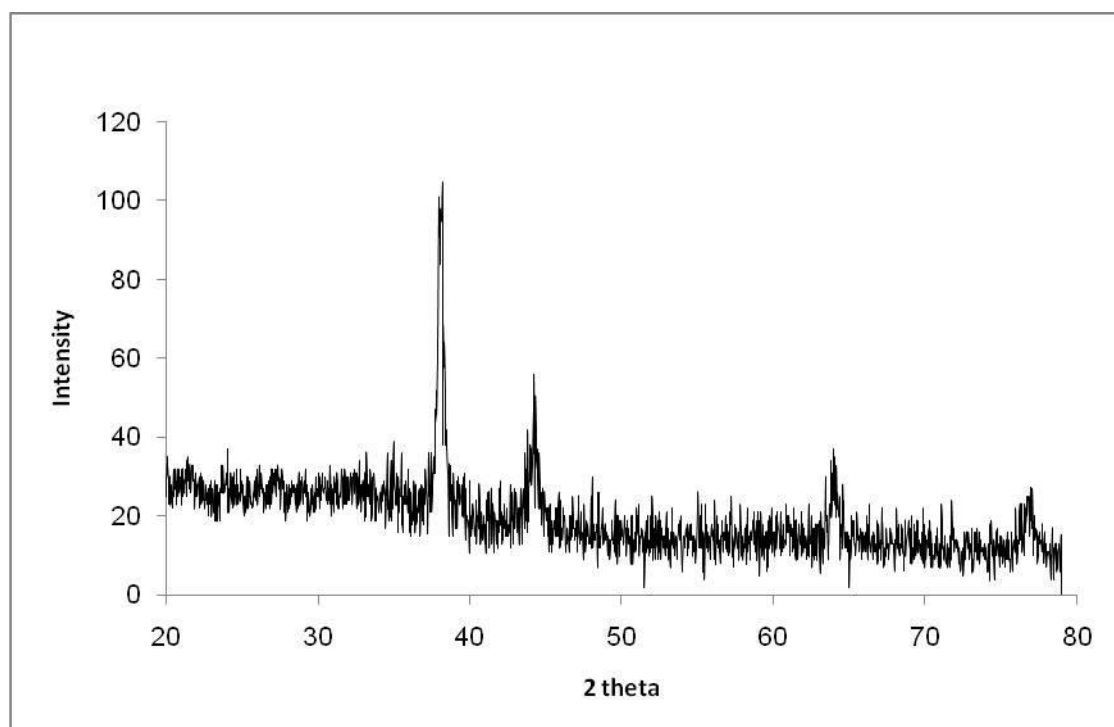


Fig 5: Powder XRD pattern of AgNPs.

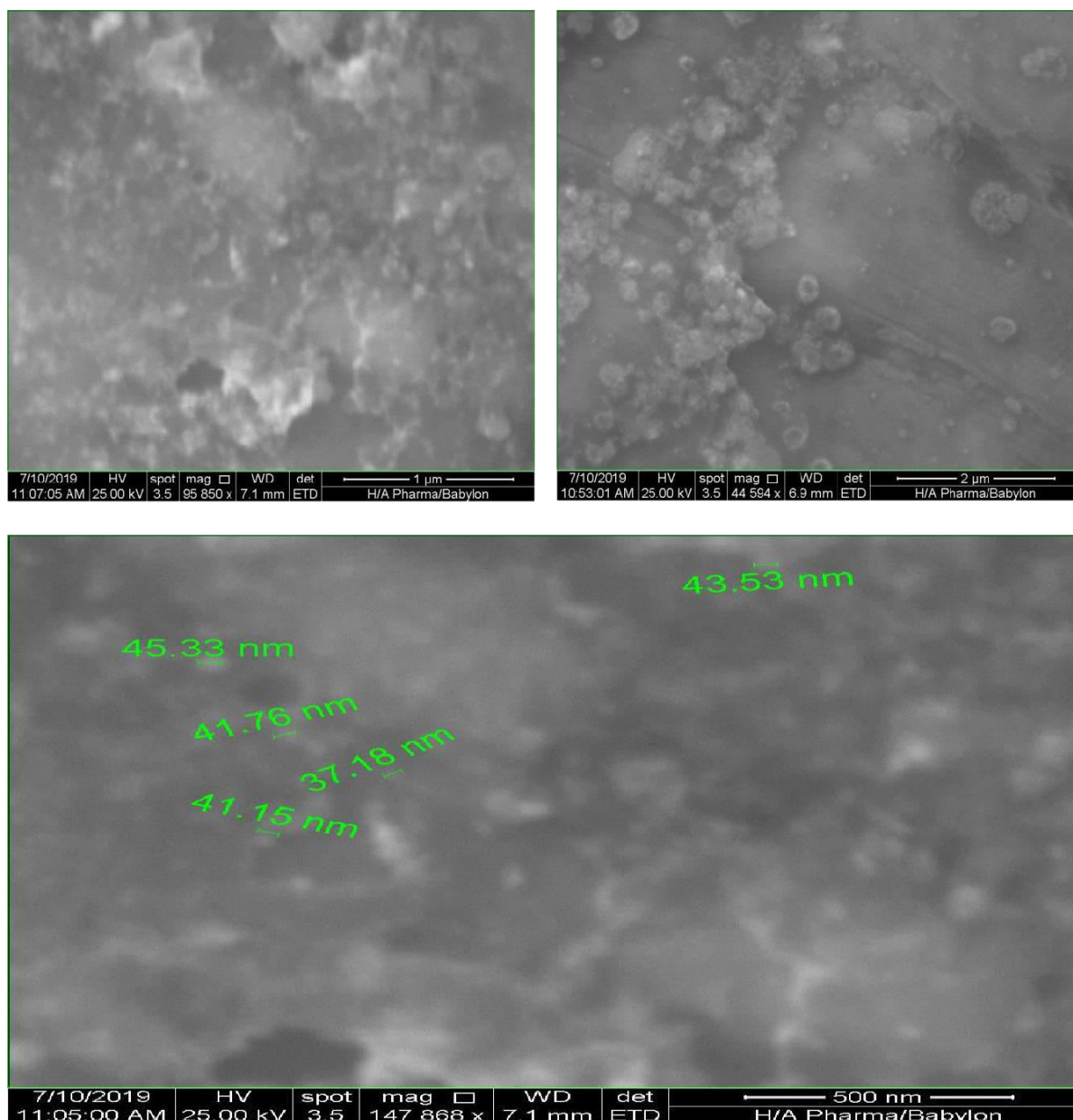


Fig 6: SEM image of fresh synthesized AgNPs in three scales (1, 2 and 0.5 µm) that are synthesized by adding 4 ml of extract to 0.001 M of Ag ion at 80°C.

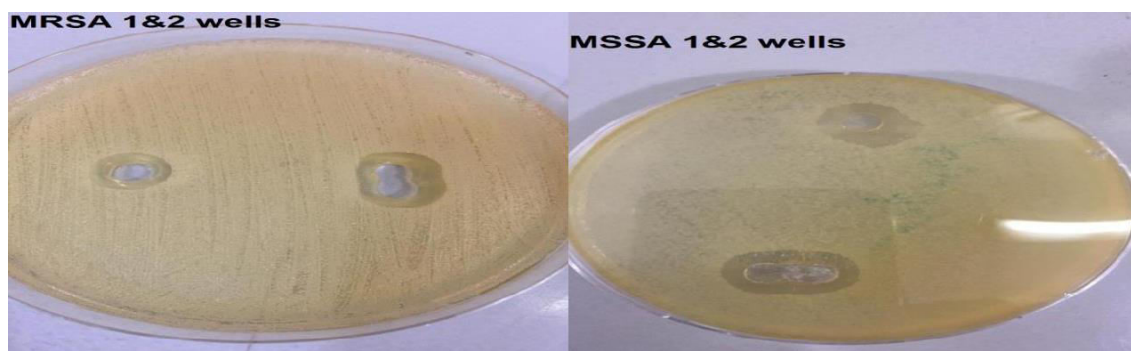


Fig 7 :Inhibition zones of MRSA and MSSA isolates bywell-diffusion method.

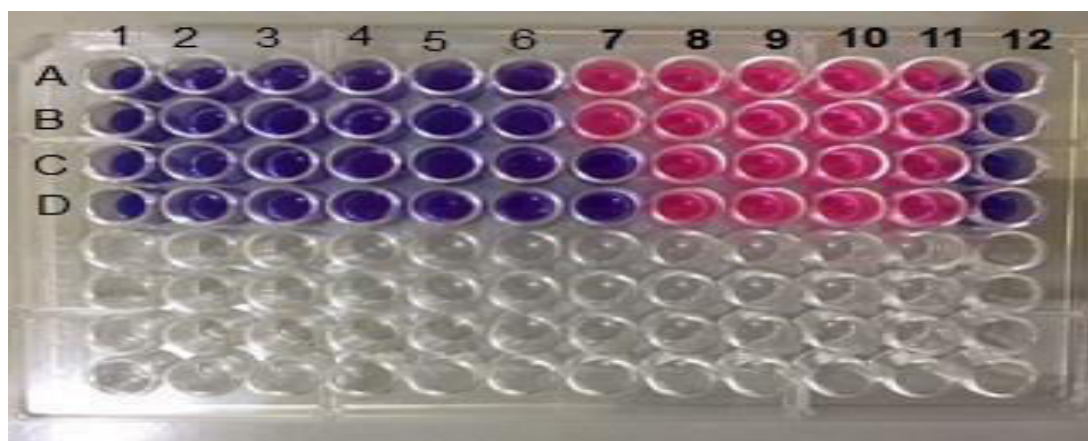


Fig 8: Microtiter plate showing MIC detection by Microbroth dilution method.

Discussion

The current major concern about *S. aureus* is its preponderance as multidrug resistant MRSA and highly virulent MSSA isolates in the hospital and community settings in many countries of the world, including Iraq¹³⁻¹⁷. Due to the carriage of the *mecA* gene, MRSA isolates are naturally resistant to beta-lactam drugs^{18, 19}. In this study, the MRSA also elicited 100% in vitro resistance to all the beta lactam antibiotics tested namely: benzylpenicillin, oxacillin, and imipenem.

Our study represents as the first try to test the antibacterial effect of AgNPs derived from MRSA extracts against the same viable strain as well as the MSSA one. From the UV-Vis spectra recorded for the aqueous silver nitrate-MRSA reaction medium as a function of time (Figure 4) it can be observed that the silver surface plasmon band of AgNPs fastened around 444.5-451 nm and the same trend was spotted at various volumes of extracts used after heating for 30 min. The surface plasmon band in the silver nanoparticles solution remains high to around 444.5-451 nm throughout the reaction period, proposing that the particles are dispersed in the aqueous solution with no evidence for aggregation. After consummation of the reaction, the silver nanoparticles solution was examined for stability. It was noticed that the nanoparticle solution of silver was extremely stabilized for more than six months with no signs of aggregation even at the end of this duration. The particles are thus stabilized in solution by the coating agent that is likely to be proteins excreted by the biomass. Similar findings resulted in studies^{20, 21}.

The XRD pattern of the AgNPs, reveals the face centered cubic structure with the calculated lattice parameters, $a = b = c = 4.071 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$, which agree well with the previous reported values²².

The particles of AgNPs looked like a cloud, and this cloud was as a result of tiny AgNPs merged together after solvent being evaporated because of its high energy, fine size, and big surface area. Thus, there was a variant distribution of size between 20 and 50 nm, where the smaller amassed to form a cloud or a bunch and another shape is independent and not merged with other particles, thus, they seemed clearly like a sphere. Our result was similar to studies done by^{9, 23}, where AgNPs are described as clusters and bunches [39].

The antibacterial activity AgNPs was investigated against MRSA and MSSA using well-diffusion method. The inhibition zones MSSA isolate was higher than those of MRSA producing different but non-significant ($P > 0.05$). Fig 6.

The present study focused on the biosynthesis of AgNPs with the development of an extracellular process by using MRSA not only to make it simple, suitable, and cheap but also to generate stable nanoparticles^{24,25}. Though MRSA produces a few toxins and sufficient exoenzymes to be dangerous, we used the fresh overnight culture throughout the synthesis of nanoparticles to avert the interaction with silver. Duran et al, have demonstrated the biosynthesis of AgNPs from *Fusarium oxysporum* strains by extracellular process. The method of synthesizing metallic nanoparticles by downstream mode is simple and eco-friendly²⁶.

The reduction of Ag ions was distinctly visible when the supernatant of MRSA was added to the AgNO₃, and the color changed from clear yellow to brownish-red. The appearance of brownish-red color denotes the formation of AgNPs²⁶. The brown color of the medium could be consequent of the excitation of surface plasmon vibration of AgNPs²⁷. Silver has been used for its well-recognized antimicrobial properties since Roman times. However, the improvements in generating Ag-NPs have made possible a rebirth of the use of silver as a powerful bactericide. The present research work mostly emphasized MRSA and MSSA, because they are found to be resistant to a wide range of broad-spectrum antibiotics. Sondi and Salopeak-Sondi used *Escherichia (E) coli* as a model for gram-negative bacteria and evidenced that AgNPs may be used as an antimicrobial agent²⁸. Shahverdi et al also mentioned that the AgNPs have an antimicrobial effect on *S. aureus* and *E. coli*²⁹.

In our study, the values of MIC and MBC of Ag-NPS against MSSA and MRSA two isolates were found in the range of 1.9-3.9 **µg/ml and 15.5-31.3 µg/ml, respectively**. The strongest evidence of the sensitivity of MRSA and MSSA to AgNPs may be as a result of their cell wall plasmolysis or the segregation of cytoplasm from their cell wall¹⁷. The antimicrobial mechanisms of bio nano silver particles may vary from species to species of bacteria and size of the nanoparticles. The susceptibility of MRSA to AgNPs is advanced with the earlier work done by Songet al, who suggested that the susceptibility of MRSA is due to the inhibition of bacterial cell wall synthesis³⁰.

Our finding can be unique since the mean of MIC and MBC concentrations observed in our study were never observed by any other study till now. The nearest MIC and MBC values to our study was results reported by

Iwalokunet al, who found that AgNP elicited growth inhibitory effects against the *S. aureus* over a concentration range of 2.5–10 **µg/mL with average MICs of 4.7 µg/mL and 4.9 µg/mL, respectively, against both MRSA and MSSA**³¹. While Fernandez et al showed that MIC and MBC values of Ag-NPs of **12.5 µg/ml and 25 µg/ml**,

respectively for *S. aureus*³². Martinez et al reported that Ag-NPs were inhibitory at concentration of 16.67 **µg/ml against S. aureus**³³. On the other hand our results showed very high antibacterial activity as compared to earlier work of Ayala-Nunez et al have reported MIC and MBC values of Ag-NPs **1800 µg/ml and 2700 µg/ml, respectively**³⁴.

Conclusions

We record here that the extracellular synthesis of bioactive AgNPs has been intensively studied during the course of this research. It is clear that MRSA can be used to synthesize bioactive nanoparticles efficiently using cheap substances in an eco-friendly and nontoxic environment. The synthesis of bioactive nanoparticles was seen within few hours of inoculation of the bacterial strain MRSA in AgNO₃.

The description of Ag ions exposed to this bacterial supernatant by UV-vis spectrophotometry proved the reduction of Ag ions to Ag-NPs. Synthesis of AgNPs was carried out by using MRSA isolate extract after

heating to obtain the brownish-red solution of colloidal AgNPs. AgNPs display SPR and a maximum absorption at around 444.5-451 nm.

The Ag-NPs prepared from MRSA has demonstrated full effectiveness against the same isolate. The inhibition zone appeared in the screening test indicated the antibacterial activity against MRSA, MSSA two isolates. The work shows that the silver nanostructures synthesized by this process have a ready application in the field of nanomedicine.

In future, Nanobiotechnology owing to its ability to fight against multidrug-resistant microbes is an important area of research and hope. To evaluate the efficacy of Ag-NPs as a bactericidal agent to be used in-vivo further studies are requisite to assess the toxic effects and properties in human cells and microorganisms.

Conflict of Interests

The authors advertise that there is no real or potential conflict of interests in relation to this article.

References

1. AL-Saadi Z.N. In vitro Antagonistic Activity of *Bifidobacterium breve* Isolated from Breast-fed Infants Human Gastrointestinal Microflora Against Two Clinical Strains of *Staphylococcus aureus* (MRSA and MSSA). *J Adv Bio & Biotech*, 2016; 10(2): 2394-1081.
2. AL-Saadi, Z.N. Estimation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Cell-Free Extracts of *Bifidobacterium* Species Against Methicillin-Resistant *Staphylococcus aureus* in vitro. *AmJBiomedLife Sci*, 2016; 75-80.
3. Al-Saadi Z. N., Al- Jorani K.R.H. Synthesis, characterization and anti-Methicillin Resistant *Staphylococcus aureus* (MRSA) evaluation of 4-bromo-2(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI] and its complexes with CoII, CuII metal. *J Pharm Sci Res*, 2018; 10 (11): 2866-2871.
4. Rangari A.A., Sharma I.N.K., Goyal M.R. et al. Evaluation and Efficacy of In-Vitro Antibacterial Activity of Silver Nano Particles Against Multidrug Resistant Bacterial Isolates from Skin Infections of Patients at a Tertiary Care Hospital in Western Uttar Pradesh of India. *Int J Curr Microbiol App Sci*, 2015; 4(10): 764-773.
5. Parades D., Ortiz C., Torres R. et al. Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against *Escherichia coli* O157:H7 and methicillin-resistant *Staphylococcus aureus* (MRSA). *Int. J Nanomed*, 2014; 9:1717-1729.
6. Omar N.Y., Ali H.A.S., Reem Abdel Hameed Harfoush R.A., El Khayat E.H. Molecular Typing of Methicillin Resistant *Staphylococcus aureus* Clinical Isolates on the Basis of Protein A and Coagulase Gene Polymorphisms. *Int J Microbiol*, 2014; 650328.
7. Nanda A., Saravanan M. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. *Nanomed Nanotech BioMed*, 2009; 5(4):452-456
8. Hashim R., Oda A.M. and Fadhill. Green synthesis of Silver Nanoparticles by Beta vulgaris (Chard) Extract: Characterization and Antibacterial Activity. *AsJ Chem*, 2019; 31(8):1881-1884
9. Oda A.M., Abdulkadhim H., Jabuk S.I.A., Hashim R. et al. Green synthesis of silver nanoparticle by cauliflower extract: characterization and antibacterial activity against storage. *IET Nanobiotechnol*, 2019; 13(5): 530-535.
10. Clinical and laboratory standard institute. Performance standards for antimicrobial susceptibility. Twenty-fourth information supplement, 2016; CLSI document M100-S26. CLSI, Wayne, PA.
11. Elshikh M., Ahmed S., Funston S. et al. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnol Lett*, 2016., 38:1015-1019.
12. Coban A.Y. Rapid Determination of Methicillin Resistance among *Staphylococcus aureus* Clinical Isolates by Colorimetric Methods. *J Clin Micro*, 2012; 50(7): 2191-2193.

13. Tong S.Y.C., Davis J.S., Eichenberger E., Holland T.L., Fowler V.G. *S. aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clin Microbiol. Rev*, 2015;2: 603–661.
14. Diekema D.J., Pfaller M.A., Schmitz F. J., Smayevsky J., Bell J., Jones R.N., Beach, M., SENTRY Participants Group. Survey of Infections Due to Staphylococcus Species: Frequency of Occurrence and Antimicrobial Susceptibility of Isolates Collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin. Infect. Dis*, 2001; 32(2): S114–32.
15. Laupland K.B., Church D. L. Population-Based Epidemiology and Microbiology of Community-Onset Bloodstream Infections. *Clin. Microbiol. Rev*, 2014; 27: 647–664.
16. Ali M.A., Al-Mathkhury H.J.F. Incidence of Ciprofloxacin-Resistant of Methicillin Resistant *Staphylococcus aureus* isolated from Iraqi patients. *Iraqi J Sci*, 2018; 59(3A): 1225-1230.
17. Jassim F.A. Bacteriological Evaluation of Diabetic Foot Infections of Patients in Al- Salam Hospital in Mosul City/ Iraq and Antibiotic Sensitivity Pattern. *IJAR*, 2014; 2(5): 614-623.
18. Abdulgader S.M., Shittu A.O., Nicol M.P., Kaba M. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus Aureus* in Africa: A Systematic Review. *Front Microbiol*, 2015; 6: 348.
19. Liu Y., Zhang J., Ji Y. PCR-based Approaches for the Detection of Clinical Methicillin-Resistant *Staphylococcus Aureus*.
20. Kolya H., Maiti P., Pandey A., Tripathy T. Green synthesis of silver nanoparticles with antimicrobial and azo dye (Congo red) degradation properties using *Amaranthus gangeticus* Linn leaf extract. *J Anal Sci Technol*, 2015; 6(33): 1-7.
21. Dubey S.P., Lahtinen M., Särkkä H., Sillanpää M. Bioprospective of *Sorbus aucuparia* leaf extract in development of silver and gold nanocolloids. *Colloids Surf. B: Biointerfaces*, 2010; 80(1): 26-33.
22. Anuratha M., Jawahar A., Umadevi M., Sathe V.G., Milton Franklin Benial A. Adsorption of N-(1-(2-bromophenyl)-2-(2-nitrophenyl)ethyl)-4-methylbenzenesulfonamide on silver nanoparticles: SERS investigation. *Acta A*, 2015; 138: 234-240.
23. Muhammad A.R., Zakia K., Anum R., et al. Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes, *Nanomat*, 2016; 6: 74.
24. Mukherjee P., Senapati S., Mandal D., Ahmad A., Khan M.I., Kumar R., et al. Extra cellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. *Chem Biochem*, 2002;3:461-3.
25. Ahmad A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R., et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B*, 2003;28:313-8.
26. Sastry M., Ahmad A., Khan M.I., Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycetes. *Curr Sci*, 2003;85:162-70.
27. Ahmad A., Senapati S., Khan M.I., Kumar R., Sastry M. Extra-/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, *Trichothecium sp.* *J Biomed Nanotechnol*, 2005;1:47-53.
28. Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J Colloids Interface Sci*, 2004;275:177-82.
29. Shahverdi A.R., Fakhimi A., Shahverdi H.R., Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine*, 2007;3:168-71.
30. Song H.Y., Ko K.K., Oh I.H., Lee B.T. Fabrication of silver nanoparticles and their antimicrobial mechanisms. *Eur Cells Mater*, 2006;11:58.
31. Iwalokun B.A., Akinloye O., Udoh B.E., Akinyemi K. O.. Efficacy of silver nanoparticles against multidrug resistant clinical *Staphylococcus aureus* isolates from Nigeria. *J Immuno Immunoch*, 2019; 40(2): 1-23.
32. Fernandez E.J., Garcia-Barrasa J., Laguna A., Lopez de Luzuriaga J.M., Monge M., Torres C. The preparation of highly active antimicrobial silver nanoparticles by an organometallic approach. *Nanotech*, 2008; 19:1-6.
33. Martinez-Castanon G.A., Nino-Martinez N., Martinez-Gutierrez F., Martinez-Mendoza J.R., Ruiz F. Synthesis and antibacterial activity of silver nanoparticles with different sizes. *J Nano Res*, 2008; 10:1343-1348.

34. Ayala-Nunez N.V., Lara H.H., Turrent Liliana del C.I., Padilla C.R. Silver nanoparticles toxicity and bactericidal effect against Methicillin-resistant *Staphylococcus aureus*: Nanoscale does matter. *Nanobiotech*, 2009; 5(1): 2-9.

Synthesis of carbon nanotubes by using new route and using it as reduced carbon nanotube coating for corrosion protection enhancement of Copper and Aluminum in sea water

Mazin Hasan Raheema¹

1. Al-Turath University College, Baghdad, Iraq

Abstract

In this research, carbon nanotubes (CNT) were produced from graphite flakes by Hammers method after some minor change in the procedure. We develop a new chemical route to prepare carbon nanotubes . After that reduced carbon nanotube was electrodeposited on copper and aluminum for corrosion protection• implementation in artificial seawater (3.5% NaCl) at four temperatures, namely, 20, 30, 40, and 50 C were studied using three electrodes potentiosta. All corrosion calculations, kinetics, and thermodynamics parameters were nominated from Tafel plots. The synthesized carbon nanotubes (CNT) was diagnosed by a scanning electron microscopy(SEM), SEM images demonstrated the formation of carbon nanotubes and carbon nanofibers.

Keywords: carbon nanotubes, corrosion protection, Copper and Aluminum

How to cite this article: Raheema MH (2019): Synthesis of carbon nanotubes by using new route and using it as reduced carbon nanotube coating for corrosion protection enhancement of Copper and Aluminum in sea water, *Ann Trop Med & Pub Health*; 21: 2036-19

1. Introduction

Carbon nanotubes, first discovered by Sumio Iijima in a year in 1991[1], are a single sheets of graphene furled into a cylinder. Carbon nanotubes such as fullerene, graphene and nanotubes are now products attention in current research as well as future industrial applications. This is because the gap in the range of single-walled carbon nanotubes (SWNTs) can vary from zero to about 2 volts so their electrical conductivity can be one of metal or semiconductors [2]. Carbon nanotubes can be prepared by different routes such as arc discharge (AD), laser ablation, chemical vapour deposition (CVD), growth mechanism, HiPco and CoMoCat, new direction in the chemical vapour deposition [3-17]. Most of these methods as well as some of the more new routes working with high pressure of the carbon monoxide or some unique catalytic mixture and need high energy requirement with low production yield. Theoretically, it is possible to build carbon nanotubes by winding the hexagonal graphene sheet, as shown in Figure 1 [18].

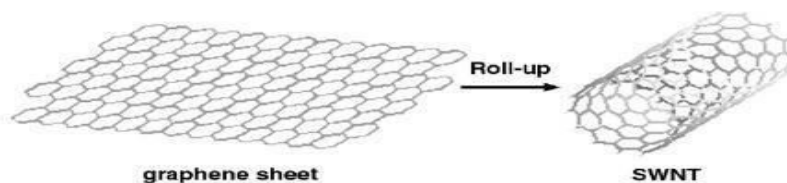


Fig. 1: Shows the fashioning of single-wall carbon nanotube by winding – up a graphene sheet [18].

We have benefited from this in the production of carbon nanotubes by converting graphene oxide to carbon nanotube using the method of Hammers [19], which is the oxidation of the graphite and convert it to the graphene oxide. Corrosion is the result of the electrochemical reactions that occur between the metal and the surrounding, which leads to the destruction of metals. Therefore, a number of researches have been carried out to address this problem, the most important of which is the coating of metals with nanomaterials that protect metals from corrosion [20-23].

Carbon nanotube has no wide application in the protection of metals from corrosion and the reason is the difficulty of using it as coating layer on metals and why it is difficult to stick to the surface of the metal, so in this study we focused the preparation of chemical and electrolytic coating of carbon nanotubes on metals copper and aluminum to protect metals from corrosion.

Effectiveness of carbon nanotube- metal coating is predestined by electrochemical procedure in artificial seawater (3.5% NaCl). The test serves to extract the corrosion inhibiting ability of carbon nanotube.

2. Materials & Methods

All chemicals and materials used in this work have been used as received.

2.1. Synthesis of carbon nanotube: Carbon nanotube was prepared from graphite, graphite (2.0 g) (Mesh100, Sigma-Aldrich, USA) , was added into (46 ml) concentrated sulphuric acid (BDH, 'AnalaR' sp. gr. 1.84) under stirring at laboratory temperature, then (1.0 g) sodium nitrate (BDH, 'AnalaR') was added while the reaction mixture was at 0 ° C. Under strong agitation, (6 g) potassium permanganate (BDH, GPR) was added slowly while the suspension temperature was maintained at approximately 2 ° C. The reaction mixture was heated to 35 ° C by a water bath with continuous stirring for 30 min. **Then, 100 ml deionized water DI (1µs.cm) was added, and the solution was stirred for 15 min at 90°C.**

Then add (332 ml) of water was added. After that, the mixture was then aged in the air for 10 days at room temperature. After the end of the aging period, it was noted that there was color change in solution from yellow to dark brown. This is an indication of the conversion of graphene oxide to carbon nanotubes and carbon nanofibers. Additional (10 ml) of Hydrogen peroxide (30%, Seema International) added to stop the oxidation. The mixture was filtered and rinsed with (170 ml) 4% of HCl aqueous solution followed by washing with (130 ml) of distilled water to remove the acid, then oxidation product washed until the pH reached 6, then filtered , dried. The above7mentioned method for preparing CNT is similar to the Hammers process [19] used for growing graphene oxide.

2.2. Deposition of carbon nanotube: The Copper and Aluminum were coated with carbon nanotubes using the electrophoresis deposition (EPD). Suspension of the produced carbon nanotubes, as described in step 1, was prepared by adding (1.0 g) graphene oxide powder to (200 ml) of distilled water, an ultrasonic probe homogenizer (MTI,USA,300W) was used to mix the solution for 10 min. The CNTO solution transferred into two electrodes glass cell body (25ml), a well cleaned and polished piece of of the metal under realization (Cu and Al) serves as cathode, while a piece of pure platinum act as anode , the two electrodes were connected to a D.C power supply. The deposition starts on applying several DC volts for several minutes. The deposited graphene layers were characterized using AFM and SEM.

2.3. Corrosion measurement: The corrosion tests were conducted with the use of advanced potentiostat, (Wenking MLab-200 ,Bank Elektronik–Intelligent controls GmbH ,Germany) with all accessories ,cell , electrode ,and working electrode holder. The corrosive media was artificial sea water made by dissolving 35 g of NaCl in 1 litter distilled water, the polarization curves were scanned between - 200 to + 200 mv from the open circuit potential and the corrosion currents i_{corr} , and corrosion potentials E_{cor} were evaluated by extrapolation of the anodic and cathodic Tafel lines from the polarization curves at four different temperatures; 20, 30, 40, and 50°C.

3. Results & Discussion

3.1. Carbon nanotube Characterization: The SEM images in Figure 1 show the morphology and topographic structure of the produced carbon nanotubes; they represent nanotubes and nanofibers. Through the SEM image shown, the graphene is transformed into carbon nanotube and carbon nanofiber. The same conclusions predicted from AFM images, they revealed continuous nanotube and nanofiber of carbon nanotube Figure 2.

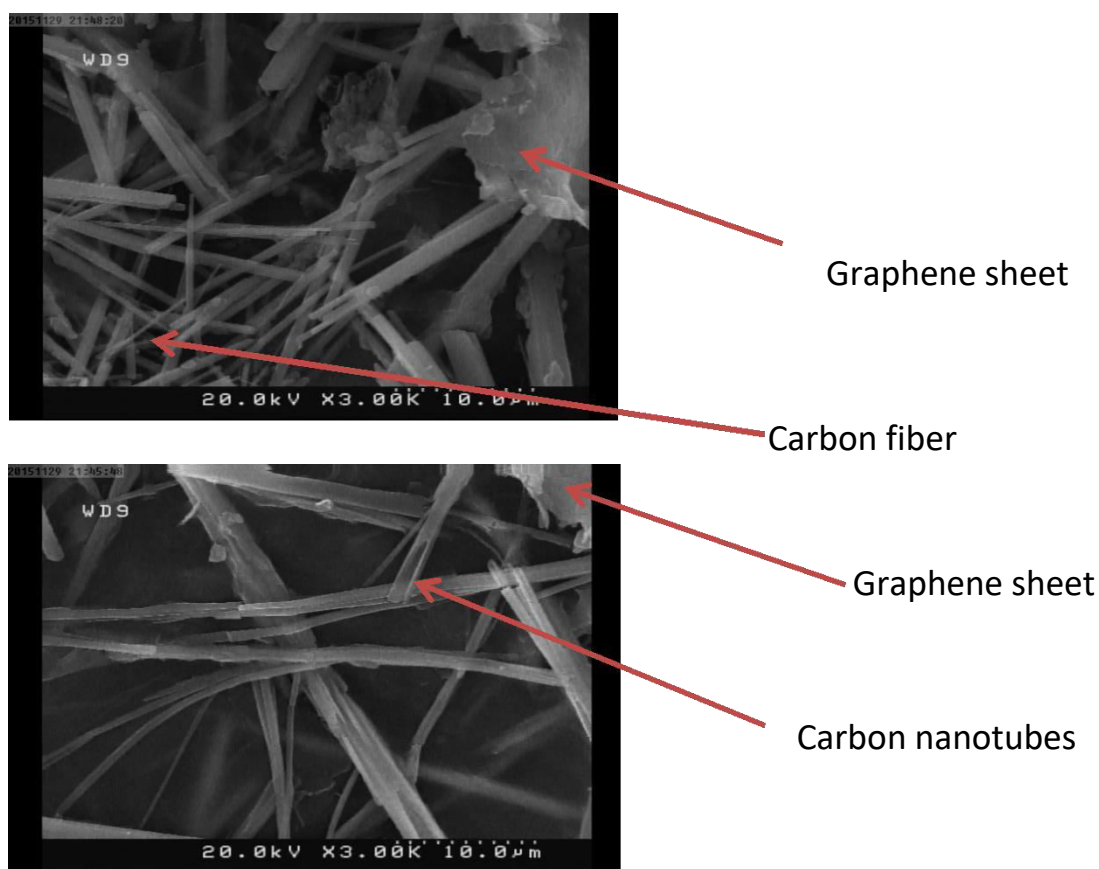


Fig. 1 SEM images of carbon nanotube and carbon nanofiber produced by new route from graphite flakes.

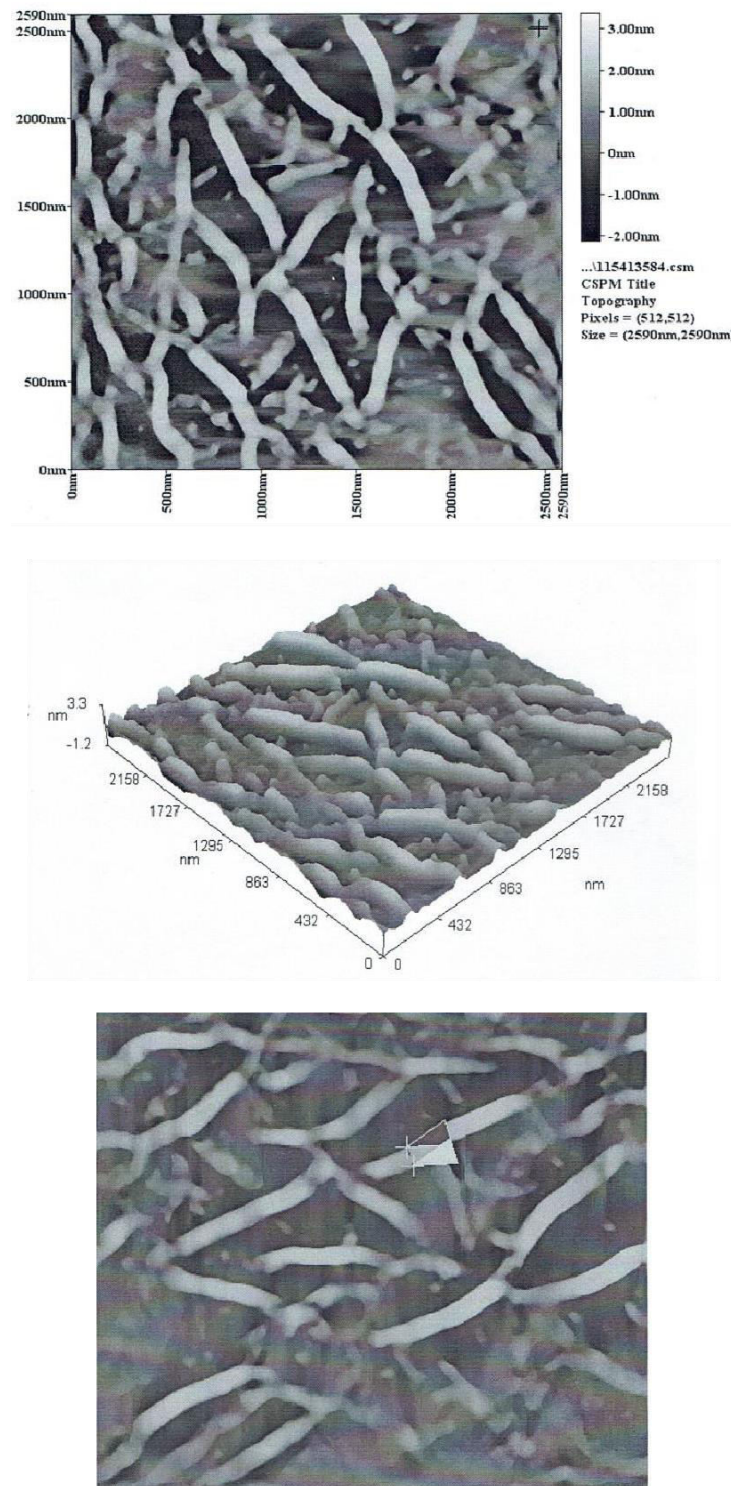


Fig. 2 AFM images show the 3D of carbon nanotube and carbon nanofiber produced by new route from graphite flakes.

3.2. Corrosion measurements:

Figures 3, 4, 5 and 6 show Tafel polarization curves of the uncoated and reduced of carbon nanotube coated copper and aluminum in artificial seawater (3.5% NaCl), respectively, in the temperature range (20–50 °C). The corrosion potentials (E_{corr}) in mV versus SCE, the corrosion current density (i_{corr}) in A cm^{-2} and α , and β in mV dec^{-1} were determined from Tafel plots (Figure 10), and weight loss (W_L) in g was calculated from Stern-Geary equation [20]:

$$R_p = babc / 2.303(ba + bc) i_{corr} \quad \text{--- (1)}$$

Surface coverage (θ) and protection efficiencies (% PE) were calculated from equations 2, 3, defined as [20]:

$$\%PE = \frac{(i_{corr})_{uncoated} - (i_{corr})_{coated}}{(i_{corr})_{uncoated}} * 100 \quad \text{--- (2)}$$

respectively, were

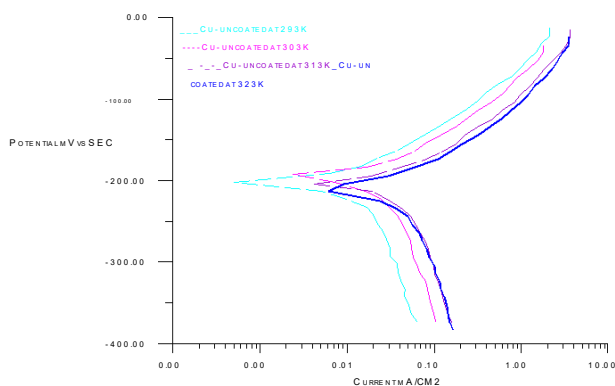


Fig. 3 Tafel plots of uncoated copper in 3.5% NaCl with different temperatures.

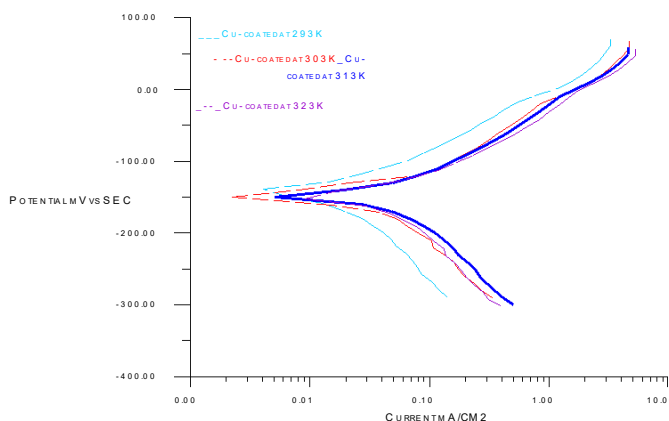
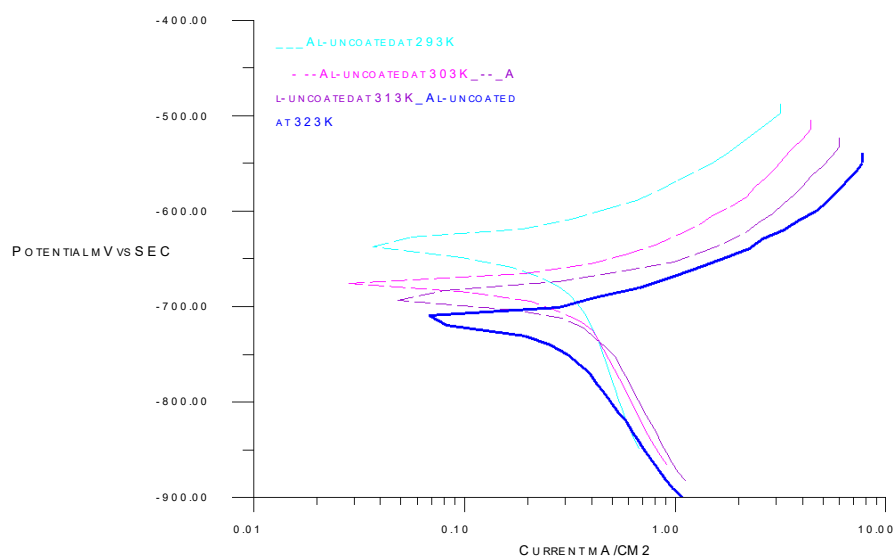
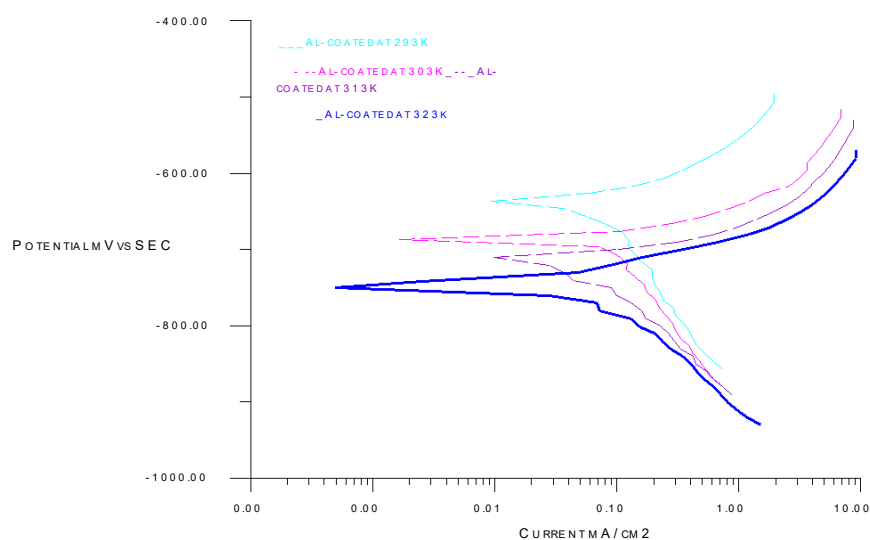


Fig. 4 Tafel plots of the coated copper in 3.5% NaCl with different temperatures.**Fig. 5** Tafel plots of the uncoated aluminium in 3.5% NaCl with different temperatures.**Fig. 6** Tafel plot of coated aluminium in 3.5% NaCl with different temperatures.

Surface coverage () and protection efficiencies (% PE) were calculated from equations 2, 3, respectively, were defined as [20]:

$$\%PE = \frac{(i_{corr})_{uncoated} - (i_{corr})_{coated}}{(i_{corr})_{uncoated}} * 100 \quad \text{--- (2)}$$

$$\theta = \frac{(i_{\text{corr}})_{\text{uncoated}} - (i_{\text{corr}})_{\text{coated}}}{(i_{\text{corr}})_{\text{uncoated}}} \quad \text{--- (3)}$$

where $(i_c)_{\text{uncoated}}$ and $(i_c)_{\text{coated}}$ are the uncoated and coated corrosion current density values, respectively, determined by extrapolation of Tafel lines. The thermodynamic parameters of the corrosion process, namely, the activation energy (E_a), the entropy (ΔS^*), the enthalpy (ΔH^*), and the Gibbs free energy (ΔG^*) of activation, were calculated using Arrhenius plot(4) and its derivative formulation(5) called transition state[21,22].

$$\log i_{\text{corr}} = -\frac{E_a}{2.303 \cdot R} \left[\frac{1}{T} \right] + \log A \quad \text{--- (4)}$$

$$\log \left(\frac{i_{\text{corr}}}{T} \right) = \log \left(\frac{R}{Nh} \right) + \frac{\Delta S^*}{2.303R} - \frac{\Delta H^*}{2.303R} \left(\frac{1}{T} \right) \quad \text{--- (5)}$$

the equation (3.7) of the activation free energy ΔG^* was calculated is;

$$\Delta G^* = \Delta H^* - T\Delta S^* \quad \text{--- (6)}$$

Where, (i_{corr}) is corrosion current density (h) is Planck's constant ($6.626 \cdot 10^{-34}$ J.s), (N) is Avogadro number ($6.022 \cdot 10^{23}$), R is the universal gas constant, ΔH^* is the enthalpy of activation, ΔS^* is the entropy of activation and ΔG^* is the Gibbs free energy. A plot of $\log(i_{\text{corr}} / T)$ Vs $1/T$ of Eq.(3.6) provide straight lines with a slope of $(-\Delta H^*/2.303RT)$ and an intercept of $(\log R/Nh) + \Delta S^*/2.303R$ as shown in fig. 7, 8, 9, 10.

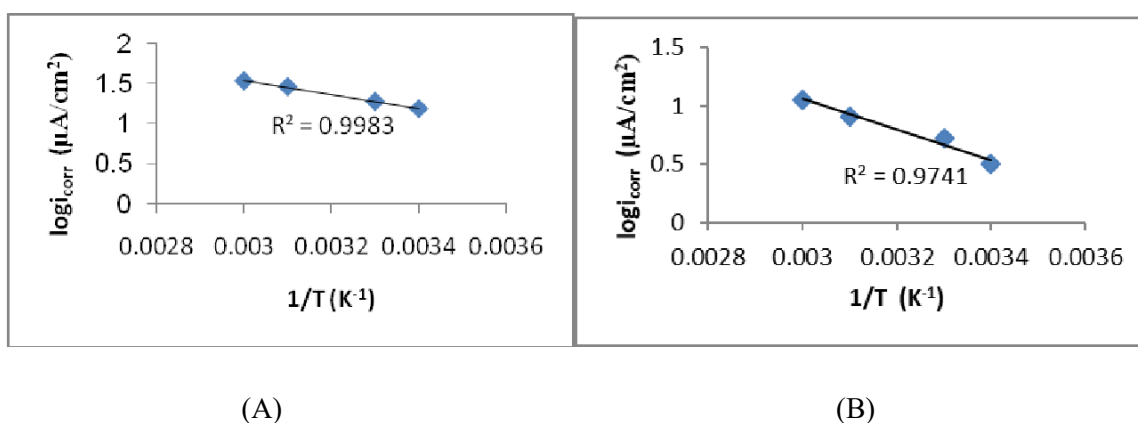


Fig. 7 $\log i_{\text{corr}}$ vs $1/T$ for (A) uncoated & (B) reduced carbon nanotube coated copper in seawater at different temperatures.

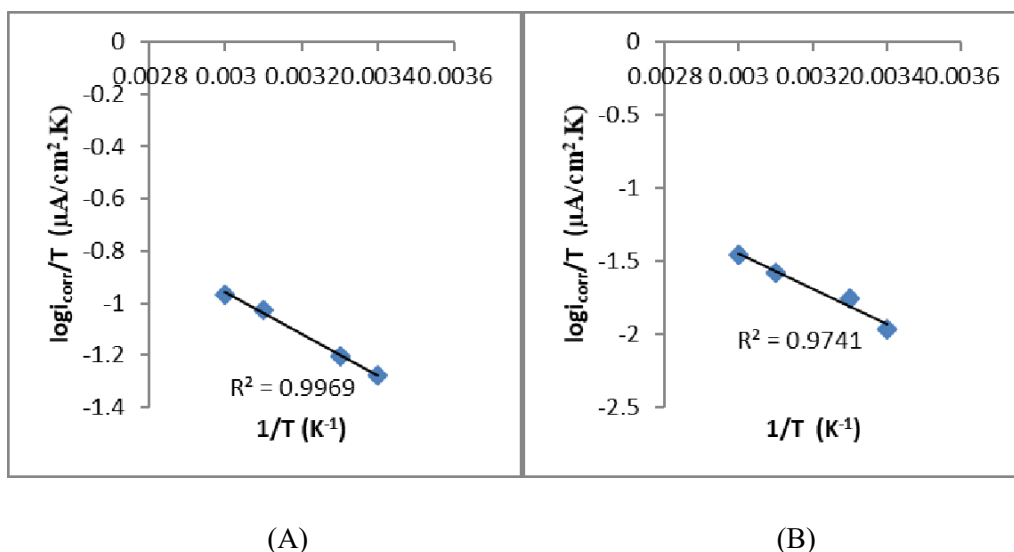


Fig. 8 Plot of $\log i_{\text{corr}}/T$ Vs $1/T$ for (A) uncoated & (B) reduced carbon nanotube coated copper in 3.5% NaCl to determined entropy (ΔS) and enthalpy (ΔH).

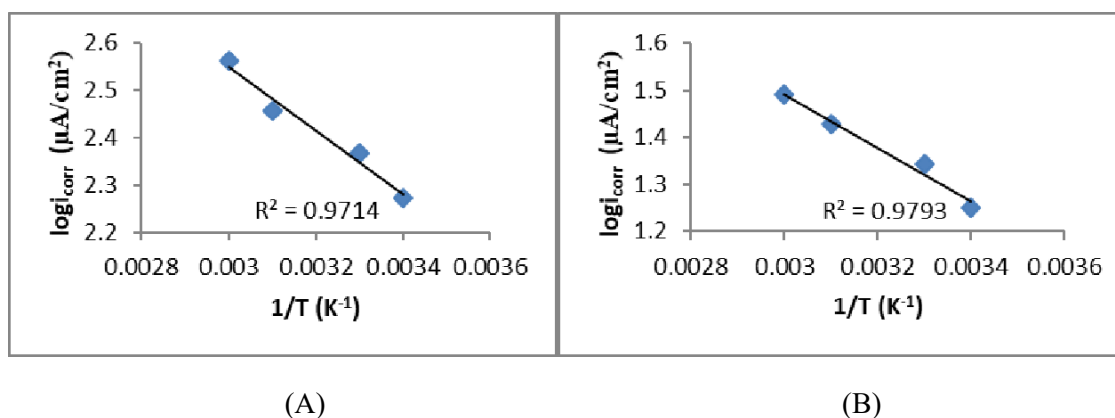


Fig. 9: $\log i_{\text{corr}}$ vs $1/T$ for (A) uncoated & (B) reduced carbon nanotube coated aluminum in seawater at different temperatures.

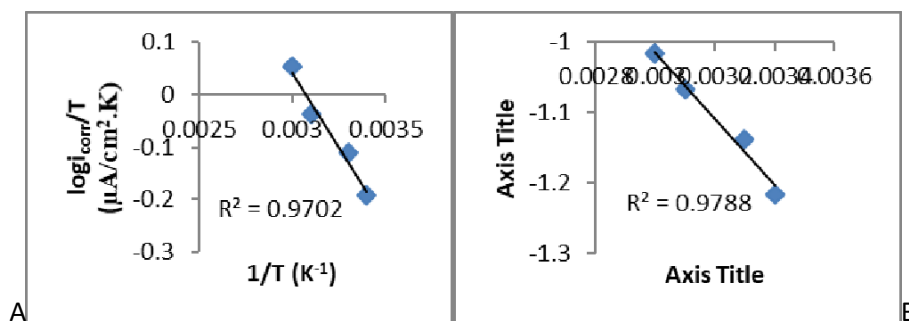


Fig.10: Plot of $\log i_{\text{corr}}/T$ Vs $1/T$ for (A) uncoated & (B) reduced carbon nanotube coated aluminium in 3.5% NaCl to determined entropy (ΔS) and enthalpy (ΔH).

System	T °C	E _{corr} mV	I _{corr} $\mu\text{A}/\text{cm}^2$	B _a (mV/Dec)	B _c (mV/Dec)	R _p $\Omega.\text{cm}^2$	WL g/(m ² d)	PL mm/y	PE%	E _a kJ/mol	ΔH_a kJ/mol	ΔS_a J/mol.K	ΔG_a kJ/mol	Θ In 20 °C
3. NaCl 5%	Uncoated Cu	20	-202.6	15.36	77	258.7	1677.5	4.37	0.179	17.27	15.4	-169.7	65.1	4.43
		30	-193.5	19	62	173	1043.1	5.41	0.221				66.8	
		40	-204.6	29.4	66.3	160	692.3	8.37	0.342				68.5	
		50	-213.8	34.9	73.7	186.5	657.2	9.93	0.406				70.2	

Coated _{Cu}	20	-139.7	3.2	64.3	134.2	5898.7	4.02	0.165	79.2	24.72	22.87	-156.6	68.8	
	30	-151.2	5.3	79.6	134.7	4099.1	9.83	0.402	72.1				70.3	
	40	-150.3	8.2	83.5	116.6	2633.7	11.4	0.468	72.1				71.9	
	50	-153	11.4	73.5	115.1	1708.5	9.69	0.397	67.3				73.5	

Table 1: Corrosion measurement parameters and the values of thermodynamic for uncoated and coated.

Table 2 corrosion measurements parameters and the values of thermodynamic for uncoated and coated aluminum with carbon nanotubes in one Media at different temperature range (20-50) 0C.

System		T °C	E _{corr} mV	I _{corr} μA/ cm ²	B _a (mV/ Dec)	B _c (mV/ Dec)	R _D Ω.cm ²	WL g/(m ² d)	PL mm/y	PE%	E _a kJ/mol	ΔH _a kJ/mol	ΔS _a J/mol.K	ΔG _a kJ/mol	Θ % In 20 °C
3.5% NaCl	Uncoated Al	20	-638.6	187.86	94.6	226.8	154.3	15.1	2.04	-	12.75	10.84	-164.14	58.93	11.9
		30	-677	233.68	80.7	230.8	111.11	18.8	2.54					60.57	
		40	-694.6	286.15	82	234	92.14	23	3.11					62.22	
		50	-710.7	365.2	65.4	237.9	60.99	17.3	2.34					63.89	
	Coated I	20	-637.2	17.8	64.4	254.6	1253.83	6.16	0.833	90.5 2	10.92	9.04	-189.76	64.64	
		30	-687.6	22	39.6	170	633.92	5.79	0.782	90.0 3				66.54	
		40	-711	26.8	30.9	156.2	417.96	4.73	0.639	90.6 3				68.43	
		50	-751.3	31.1	57.5	119	541.27	4.93	0.666	91.4 8				70.33	

These results reflects the following important facts; all samples coated with carbon nanotubes showed a good improvement to reduce the corrosion effect of the studied samples compared to the naked samples; corrosion protection values for aluminum are almost constant with increased temperature (around 90%), it is noted that there is no effect of increasing temperature on corrosion protection values as shown in the table 1, while copper shows the effect of increasing the temperature on the protection values as shown in the table 1 the range between(67-79%).

The thermodynamic and kinetics data show that the a values for the carbon nanotubes coated copper are higher than the uncoated ones which support the good protection efficiencies (67–79%) for copper, while the decrease in apparent activation energy for the carbon nanotubes coated aluminum leads to less protection efficiencies, around 65% in this case; the number of corrosion sites can be represented by preexponential factor in the Arrhenius equation:

$$R = A \exp(-E_a / RT) \text{ ----- (7)}$$

is a term which includes factors like the frequency of collisions and their orientation.

the corrosion potentials values of the reduced carbon nanotubes coated for copper went to less negative values as compared to the uncoated ones, while the aluminum shows the opposite.

The polarization resistances of the uncoated samples of copper and aluminum were always less than the coated samples, which can be attributed to the existence of some nonconductive carbon nanotubes oxide.

The range of E_a values (from 10KJ/mol to 24 KJ/mol) is lower than the threshold value of 80KJ/mol chemisorption. This means that adsorption is physical adsorption[23].

required for

Generally, the values of E_a lower than 40KJ/mol which attributed the physical adsorption and the value 100KJ/mol indicate chemical adsorption From Table 2; it was found that the negative sign and values (9).

04 KJ/mol 22.87 KJ/mol) of activation of enthalpy (ΔH) reflect the exothermic corrosion process[24].

The increase in E_a for the carbon nanotubes

controlled by E_a of coated copper The values of reveals that corrosion rate is mainly kinetic parameters of activation[25]. for the uncoated and carbon

nanotubes coated are negative. This implies that the activation complex is the rate determining step representing association rather than dissociation, indicating that a decrease in disorder takes place going from reactants to the activated complex [26].

4. CONCLUSIONS

This research presents a simple chemical method and cheap model for producing CNTs in liquid solution using method which is similar to the Hammers method used for growing graphene oxide. We expect that this new synthesizing method will produce cheap CNTs and as a result allow industrial applications based on CNTs to flourish. In addition, good adhered continuous layers of carbon nanotube were deposited electrochemically from solution of carbon nanotube oxide synthesized via a new method procedure, on surfaces of copper and aluminum; these layers exhibited good corrosion inhibition properties against artificial seawater in the temperature range of 20 to 50 C.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

Acknowledgments

The author thank Dr. Abdulkareem Mohammed Ali Al-Sammarraie, Head of Chemistry Department, College of science, University of Baghdad , for AFM analysis. I am also grateful for helpful discussion with him.

References

1. Iijima S. Nature (1991) ,354:56.
2. Lee BI, Rives JP. Colloids Surf (1991) ,56:25.
3. J.A.Isaacs et al; J Nanopart Res(2010) , 12:551-562.
4. T.Guo et al; Chem.Phys.Let. (1995) 243(1,2), 49-54.
5. C.Journet, W.K.Maser, P.Bernier and A.Loiseau et al; Nature (1997) 388, 756-758.
6. <http://students.chem.tue.nl/ifp03/synthesis.html>.
7. P.Nikolaev et al ; Chem.Phys.Let, (1999) 313(1,2), 91-97.
8. R.E.Smalley and B.Yakobson; Solid State Com (1998) 107(11), 597-606.
9. <http://www.ou.edu/engineering/nanotube/comocat.htm>.
10. P.L.Walker Jr et al; J. Phys. Chem. (1959)63 2 133.
11. M.José-Yacamán et al; Appl.Phys.Let. (1993) 62(6): 657.
12. http://en.wikipedia.org/wiki/Carbon_nanotube.
13. W.Z.Li et al; Science (1996) 274, 1701-1703.
14. H. Dai, Phys. World (2000).
15. J.Li et al; Appl.Phys.Let. (1999)75-3-19.07.
16. K.Hata et al; Science (2004)306 (5700): 1362-1365.
17. Cantoro et al; Nano Letters, (2006) 6 (6),1107-1112.

- 18.** Odom TW et al; J Phys Chem B (2000);104:2794.
- 19.** Hummers et al; (1958). Journal of the American Chemical Society 80 (6): 1339.
- 20.** F. Mansfeld, "Tafel slopes and corrosion rates from polarization resistance measurements," Corrosion, (1973) 29(10), 397–402.
- 21.** A. A. Khadom et al, "Electrochemical, activations and adsorption studies for the corrosion inhibition of low carbon steel in acidic media," Portugaliae ElectrochimicaActa (2009) 27(6), 699–712.
- 22.** E. A. Noor, "Temperature effects on the corrosion inhibition of mild steel in acidic solutions by aqueous extract of fenugreek leaves," International Journal of Electrochemical Science(2007) 2 (12), 996–1017.
- 23.** A. W. Adamson, Physical Chemistry of Surfaces, John Wiley & Sons, 6th edition(2006).
- 24.** L. Herrag et al., "Adsorption properties and inhibition of mild steel corrosion in hydrochloric solution by some newly synthesized diamine derivatives: experimental and theoretical investigations," Corrosion Science(2010) 52 (9), 3042–3051.
- 25.** A. M. Abdel-Gaber et al, "Inhibition of aluminium corrosion in alkaline solutions using natural compound," Materials Chemistry and Physics (2008) 109 (23), 297–305.
- 26.** S. A. M. Refaey et al, "Inhibition of stainless steel pitting corrosion in acidic medium by 2-mercaptobenzoxazole," Applied Surface Science(2004) 236 (1), 175–185.

Effect of educational exercises according to the technology of learning using glasses (3D) in learning some of the basic technical skills of female students

Abeer Ali Hussein¹, Riadjumaa Hassan^{1*}, Alikhoman Alwan¹

1. College of Physical Education and Sports Science.Karbala University

*Corresponding author: dr.riad2011@yahoo.com, College of Physical Education and Sports Science. University of Al-Qadisiya

Abstract

The importance of the research for recognizing the importance of educational exercises base on learning technology using 3D glasses in learning some basic skills in the technical gymnastics of female students. The subjects were divided into two groups: control or experimental of the skills in the research, 23 students as a control group and 23 students as experimental group, the control group took teaching courses in the subscribed teaching method while the experimental group took learning exercises according to the technology of learning using 3D glasses. The groups are equal to the number of learning units (skills in research in the technical gymnasium for female students). The study sample was divided by lottery method. The control group: practiced the learning exercises used by the curriculum of the third stage of the faculties of physical education and sports sciences of Karbala University and the extent of (12) educational unit. The experimental group: The students of the experimental group learned a new skill in jump on hands of the background on the ground movements and practice it and the development and use of an electronic device (laptop) using glasses using 3D glasses as well as DATA SHOW skill previously learned as skill on hands-on device casual The balance, where the curriculum took 24 educational units, each module takes 90 minutes by units in week to change learning environments, reduce learning time and stimulate students' enthusiasm. One of the most important conclusions (the use of learning technology according to 3D glasses has an effective effect in developing and improving the level of learning some of the skills on the students' gymnasiums).

Keywords: 3D glasses, gymnastics, DATA show, learning

How to cite this article: Huseein AA, Hassan R, Ahwan A (2019): Effect of educational exercises according to the technology of learning using glasses (3D) in learning some of the basic technical skills of female students, Ann Trop Med & Pub Health; 21: SP2031-19

Introduction

The unlimited scientific progress in the sciences of physical education and sports sciences, which harnessed to serve the field of teaching and learning in physical education because of its established fundamentals and based on it and derives the material that the teacher or teacher uses primarily in teaching or teaching ,where exercises emerged in ancient times with the first human race as it had to move in search of a livelihood in order to live where all the movements that was carried out is normal for the two men and arms and the body is the basic elements of movement in the exercise, as exercises

are purely organized exercises developed to be Special effects on the body and some of the foundations of modern dance training and motor training, as well as movements that make up the body and develop its motor ability in accordance with special rules that take into account the educational foundations and scientific principles to reach the greatest possible performance and work in different areas of life. The use of learning technology is necessary in our lives. We are witnessing a huge and rapid development in technology which encourages us to utilize it in the field of education in an innovative way, as it is no longer a field of knowledge and the use of technology in the process of learning an important role in it. As it is the nerve of the educational process, economic, social etc. Hence the learner can't ignore the effect of scientific and technical developments, especially those related to information technology in all aspects of life. Where it has been employed in the fields of work and all disciplines. This is why it is necessary to use it, and learn to use its various programs in all fields, including education and our educational system, an advanced system which keeps abreast of developments in the world in the use of technology and the use of 3D glasses. This system has been updated and reinforcement by learning technology and electronic simulation to be an essential element in this system to improve, develop, and guide it in line with new trends, therefore the researchers decided to prepare educational exercises according to learning technology with 3D glasses to explain the basic concepts of some of the technical skills of gymnastics and clarify, to improve the instruction process to the fullest.

1-2 - Research problem:

As a researcher in the college of Physical Education and Sport Sciences for artistic gymnastics for girls, noticed the difficulty in some basic skills in the technical gymnastics of the students (the back of the hands on the back of the ground movements, stand on the hands on the balance) because of the need for physical effort by the students as well as taking a long time from the educational process because they are skills that require muscle strength and flexibility of students. In spite of the process of teaching these skills, but the interface of some difficulty in teaching these skills to the difficulty of the performance of students to the skills to be applied to the application of the practical, the students found difficulty in performance, which led to access to this study.

1.3.1 Research Objectives

1 - Preparation of educational exercises according to the learning technology using glasses (3D) in learning some of the basic skills of the technical gymnastics of students.

2 - Recognize the importance of educational exercises according to the learning technology using the glasses (3D) in learning some of the basic skills in the technical gymnastics of students.

3 - Identify the superiority of the group (control or experimental) of the skills in search.

1-3-2- Research hypothesis:

1- There are significant differences between the tests of before and after and the control and experimental groups in the students' learning of the skills in question.

1.3.3. Research fields:

1 -Spatial field: Karbala University - College of Physical Education and Sports Sciences - Closed Hall.

2. Time field: from 2018 to 2019.

3 - Human field: students of the second stage - Karbala University - College of Physical Education.

3.1 – Community Research:

The researcher used the experimental method in the style of equal groups to suit the nature of the problem to be solved. The research society represents the students of the third stage (morning study) of the College of Physical Education and Sports Sciences – Karbala University for the academic year 2018/2019, the number of (56) students.

3.1.2 Research Sample:

After the exclusion of (10) students from the research community in order to achieve the requirement of homogeneity in the sample of learners became the number of the sample (46) students, or (21.7), which is appropriate for the representation of the community representation honestly, then the sample was divided into two groups are equal at random, (23 students) as experimental group, the control group takes teaching lessons in the teaching method followed while the experimental group takes learning exercises according to learning technology using 3D glasses. The groups are equal to the number of learning units (skills in research in the technical gymnasium for female students).

3.1.3 – homogeneity and Parity

Where the homogeneity of the sample and the parity of the two groups of research that affect the accuracy of the results of the search and to return the differences in the effect only to the independent variable, namely the use of 3D glasses, it first verify the homogeneity and equal sample of the research sample in variables similar to the skills in search as in Table (1).

Landmarks	Average	standard deviation	Standard error	Torsion coefficient
Statistical variables				
Rolling back on the floor mat	1.86	0.64	0.15	0.20
Stand on your hands on the floor	2.13	0.64	0.15	0.20

Table (1) shows the averages, standard deviations, standard errors and torsion coefficients for variables where the results showed that the values of the splicing coefficient of the above variables are less than (1) indicating the homogeneity of the research sample in these variables.

Table (2) shows the parity between the skills under consideration in the earlier tests of the control and experimental groups.

Table (2)

Statistical landmarks	The experimental group		Control group		Calculated t values	T value of t at significance level 0.05 and The degree of freedom (30)	Level of significance
Variables	س	±ع	س	±ع			
Leap back hands on the floor	2.81	0.83	2.44	0.73	0.47	2.04	Not significant
Stand on the casual balance	7.00	2.94	6.44	2.39	0.21		Not significant

3.3 The equipment and tools used in the research:

3-3-1 tools and equipment used

The researchers used the following equipment and tools:

1 – Laptop device. Number 20 Type Lenovo.

2 – the ground movements of the number 20

3. DATA SHOW .Number 2.

4 – wooden boxes. Number 20

5 – Glasses 3 D. Number 20. Type sony.

3.3.2. Means of data collection:

1. Information network

2 – Arab and foreign sources

3. Observation and experimentation

3.4. Field research procedures:

3.4.1 – Determination of my skill under consideration:

My skills in research were determined according to the vocabulary of artistic gymnastics curriculum of the third stage in colleges of physical education and sports sciences in accordance with the requirements of the research subject and the educational curriculum prepared for it (the skill of the jump of the hands on the back of the ground movements, Stand on the casual balance).

3-4-2 – Preparation of educational exercises:

3.4.3. Exploration Experience:

The Exploration experiment was conducted on 28/2/2018 on a sample of the research community of (8) students who did not participate in the main experiment, for several purposes including:

1- Organizing the work and timing of the procedures of the educational unit in all its departments.

2. Know the suitability of the tests for the research sample.

3 – Identify the obstacles that may appear when you perform the tests and educational curriculum and work to find solutions to them.

4 – Adjust the timing and repetitions necessary to implement the curriculum.

3.4.3.The Earlier Test:

The earlier test of the control and experimental groups were conducted on (9, 10/3/2018), after the completion of giving two educational units defined (**), where the first educational unit was given to the skill of the jump of the back hands on the ground floor, on 9/3/2018, which included explaining the skill with the use of exercise Prepared by the researcher and the use of some pictures and drawings of that skill and then displayed on the program of the laptop using 3D glasses where the research sample applied after the end of the educational unit was conducted tribal tests of the sample to evaluate the technical performance and accuracy of the same skill.

On the second day, on 10/3/2018, a second unit of handstand skill was given to the balance-balancing device. The unit included the same steps for the first skill of explanation, presentation and application.

(10) degrees have been given to each skill and this degree is given by a committee Consisted of threerectifiers. During extraction of the arithmetic mean for each rectifier. By extracting the mean for the best three grades, the final grade is calculated for each student.

In order to start the research procedures, the researcher organized the work of the control and experimental research groups as follow:

- The sample of the research (46) was divided into two experimental and experimental groups, each group (23) students.
- **The control group:** practiced the learning exercises used by the curriculum of the third phase of the colleges of physical education and sports sciences Karbala University and the extent of (12) educational unit.
- **The experimental group:** The students of the experimental group learned a new skill is the jump on the hands of the background on the ground movements and practice it and the development and use of an electronic device (laptop) using glasses using 3D glasses as well as the DATA SHOW skill previously learned is the skill of hands-on device casual The balance, where the curriculum took 24 educational units, each module takes 90 minutes per unit per week to change learning environments, minimize learning time and stimulate students' enthusiasm.

3.4.1-Subsequent Tests:

After the application and implementation of the educational units of the control and experimental groups, subsequent test was conducted to evaluate the technical performance of the skill of the jump of the hands on the back of the ground movements on Tuesday, 27/4/2018.

On Wednesday, 28/4/2018, subsequent test was conducted to evaluate the technical performance of the handstand skill on the balance-balance device. The researcher made sure that the conditions are similar to the tribal tests in terms of(place, time and rectifiers).

3-5 – Statistical Methods: The researcher used the statistical bag SPS.

Section Four

4. Results presentation and analysis and discussion:

In this section, will present the results of the earlier and subsequent test tests of the research sample through the presentation of the computational and standard deviations in explanatory tables after performing the necessary statistical operations for easy observation of the results, as well as comparing

the two groups by analyzing and interpreting the results of all the tests The differences and their statistical implications according to the exact scientific perspective.

4.1 Presentation of the results of the tests in the earlier and subsequent measurements of the two research groups and their discussion

Table (3)

Experimental		The average	the number	standard deviation	T	The degree of freedom	Significance
Leap back hands on the floor	Previous	2.5217	23	.84582	-	22.000	0.000
	dimension	7.8261	23	.88688	24.950		
Controls		The average	the number	standard deviation	T	The degree of freedom	Significance
Leap back hands on the floor	Previous	2.5217	23	.94722	-	22.000	0.000
	dimension	6.2609	23	86431	15.246		
Controls		The averages	the number	standard deviation	T	The degree of freedom	Significance
Leap back hands on the floor	Previous	2.8696	23	.81488	-	22.000	0.000
	dimension	7.5217	23	84582	22.719		
Controls		The average	the number	standard deviation	T	The degree of freedom	Significance
Leap back hands on the floor	Previous	2.4348	23	58977	-	22.000	0.000
	dimension	6.4783	23	1.08165	18.207		

4.2 Display and analyze the results of subsequent measurements between the research groups: Table (5)

Statistics show the variables in the subsequent measurement.

		Average	the number	standard deviation	T	The degree of freedom	Significance
Leap back hands on the floor	Experimental	7.8261	23	.88688	-7.391	44.000	0.000
	controls	6.2609	23	86431			
		Average	the number	standard deviation	T	The degree of freedom	Significance
Stand on the casual balance	Experimental	7.5217	23	.84582	-4.087	43.000	0.000
	controls	6.4783	23	1.08165			

4.2.1 Discussion of research results:

The researcher confirms through the statistical results that the educational method followed by the teacher of the material has an effect on students' learning of the basic skills on the ground movements and balancing device in the technical gymnastics, which depended on the teaching method adopted by the teacher of the article to the appropriate repetitions that accompanied the educational units, the performance of continuous exercises given their relevance to the capabilities and capabilities of students as well as the gradual level of difficulty of movements and skills, which ensures performance by everyone and this is in line with (Najah Mehdi Shalash and Akram Mohammed, 2000) pointed out " the practice and effort by continuous training and repetitions are necessary in the process of education and acquisition. Training is also a key factor in the interaction of the individual with skill and control of his movements. In order for the researcher to detect the differences between the earlier and subsequent measurements of the experimental group members sought to address the data statistically and extract the values of the arithmetic mean and the standard deviation and all the variables under study and research as shown in Table (3), and this confirms that there is a significant impact of learning technology in learning some of the basic skills on the ground movements and balance beam in the artistic gymnastics. And applied by the researcher because of the containment of educational units of exercises that activate the largest number of muscles as the educational units contained a variety of different movements of the trunk, arms, legs and head as well as various movements fast and slow according to the time of movement and frequency and distance in addition to the use of technology of glasses (3D) and a laptop computer. All of this helped to learn and gain a good level of scientific and legal knowledge of the technical gymnastic art of the students as well as the skills (Leap back hands on the floor , Stand on your hands on a casual balance machine).

The reason for this learning and acquisition of the skills sought is the response of students to all the requirements of learning through the educational units as the most effective means to highlight the capabilities and maintain the level and achieve the goals, as noted (Nizar student and Kamel Alwais, 2000) "The athlete who seeks towards a certain goal will have an incentive in his work, and working without a goal is a futile and neglected act, the sports educator should help the athlete in setting an appropriate goal for him to achieve so that the exercise is valuable and the athlete knows the extent of his progress.

As also pointed out (Ed. Lisa Yount) , "The use of learning technology in training because it reduces the risk of injury and also helps students who are hearing impaired and have little focus on learning basic skills, making the subjects more exciting and thus students are more likely to remember what they learn and from the side The VR is cheaper than other means "

The researcher believes that the use of learning technology creates an ideal environment for learning, especially in the gymnastic until the isolation of all external influences from the student and focus on the skill and also in line with the development of the process of learning and increase the suspense and excitement among students and break the usual routine when performing skills in the lecture.

The educational units contained a variety of different movements of the trunk, arms, legs and head as well as various movements fast and slow according to the time of movement and frequency and distance In addition to the use of virtual reality technology of the window on the virtual world through glasses D 3 glasses. All this helped to learn and acquire the skills.

This confirms that the exercises used in the educational units with the virtual world window have contributed to the learning and acquisition of basic skills (on the ground and parallel device) of the students and the researcher attributed the reason for this learning and acquisition of the skills sought to the responses of students to all the requirements of learning through the educational units as the most important means Effective to highlight the energies and maintain the level and achieve the goals, as the researcher sees through the presentation of skill on the screen is to keep pace with the scientific development taking place in all areas of science and learning through the correct model as this game lacks the Models in the many universities and leads.

In order to achieve the objective of the third study, which aims to identify the advantages of the differences between the use of electronic simulation environments and the curriculum adopted by the teacher in improving the performance of some skills on the gymnastic devices for female students, the researcher sought to extract the values of the average, the standard deviation and the standard error of the data of the two groups (experimental and control) the subsequent measurement (4) shows this.

Section Five

5. Conclusions and recommendations

5.1 Conclusions

- 1 - The use of learning technology according to 3D glasses effectively influence in the development and improvement of the level of learning some of the skills on the gymnastic devices for students.
- 2 - The results showed that there is a preference in the use of technology according to 3D glasses in the development and improvement of the level of learning some of the skills on the gymnastic devices for students.

5.2 Recommendations

The researcher made several recommendations as follows:

- 1 - Adopting the use of learning technology with 3D glasses in the improvement of some basic skills on the gymnastic devices for students.
- 2- Conduct studies that include the use of educational technology in learning the basic skills of sports in general.
- 3 - Emphasize the use of the educational curriculum which prepared by the researcher in the coming research on different age stages.

RE-WRITE THE REFERENCES PROPERLY

Arab and foreign sources and references and the Internet

.The Holy Quran.

.Ibtahal Hassan Abdul Amir Al-Tai: the effects of special exercises according to sensory preferences in learning some skills on the ground and platform jumping Gymnastic technical students, Master, college of Physical Education and Sports Sciences / University of Karbala, 2016.

.Ahmed Jasser: Gymnastics Instructor and Athletic Guide, 1, Syria, Aleppo, Shuaa Publishing and Science, 2000.

.Ahmed Mohamed Salem: Means and Technology Education, 2, Riyadh, Al-Rashed Library, 2006.

.Ahmed Mohamed Al - Zoghbi: Psychology of Individual Differences and Educational Applications, 1, Damascus, Dar Al - Fikr, 2007.

.Haidar Mahmoud Abdullah: The influence of the style of guided discovery and computer in the learning of some skills on the ground movements of the Men's Artistic Gymnastics, college of Physical Education and Sports Sciences, University of Babylon, 2010.

.Nizar Al-Talib and Kamel Lewis: Mathematical Psychology, University of Mosul, Dar Al Kut Books and Publishing, 2000.

.Heidi Mohamed: Design and Development of Virtual Reality (Analysis of the Challenges Faced by Educators), Master Thesis, college of Education, Department of Education Technology, Alexandria University, 2009.

.Siclovian, Conculul National De Edicatia Fizica Si Sport, (Bucuresti - Romania, Editera, 2001).

.The Virtual Classroom: Virtual Reality In Training And Education.

<http://vr-tec.blogspot.com>

The relation between Chemotherapy, Oxidative Stress and Apoptosis in Females with Breast Cancer

Nazar Ahmed Naji Abed¹, Jamal A Al-Jabbar Attawi^{2*}

1. Department of Chemistry, College of Science, Tikrit University, Baghdad, Iraq.

2. Medical Technology Institute in AL-Mansour, Middle Technical University, Baghdad, Iraq.

*Corresponding author Email: rusul.aljabbar@yahoo.com

ABSTRACT

The most common cancer among women is breast carcinoma. This study aimed to determine the effect of oxidative stress on breast cancer, heart markers, and apoptosis by caspase3. Moreover, the role of chemotherapy in the treatment of breast cancer and its mechanism through changing the measured markers would be studied. The present study was focused on using some biochemical markers in blood serum including creatine kinase (CK), Glutathione (GSH), Lactate dehydrogenase (LDH), Caspase-3 and Malondialdehyde (MDA). They were determined in 54 patients with breast cancer, 54 pathological control, and 54 normal healthy control, other fifteen patients selected from 54 breast cancer women after the end of the six cycles of chemotherapy. Result indicated that CK and GSH levels from breast cancer women were significantly lower when compared to normal healthy control, with ($P < 0.0001$, $P < 0.0001$ respectively). LDH, Caspase-3 and MDA levels of breast cancer patients were significantly higher compared to healthy control, with ($P < 0.001$, $P < 0.0001$, $P < 0.0001$ respectively). Therefore, their detection were important in oxidative stress and the accumulation of reactive oxygen species (ROS), during chemotherapy which offers an interesting therapeutic window. Chemotherapy increases the oxidative stress already present in patients with breast cancer, this inhibits caspase activity and apoptosis induced by the drug and thus interferes with the ability to kill tumor cells of antineoplastic agents.

Key words: Creatine kinase (CK), Caspase3 (CASP3), Glutathione (GSH), Lactate dehydrogenase (LDH), Malondialdehyde (MDA), Reactive oxygen species (ROS).

How to cite this article: Abed NAN, Attawi JAA-J (2019): The relation between Chemotherapy, Oxidative Stress and Apoptosis in Females with Breast Cancer, Ann of Trop Med & Pub Health; 21: SP2039-19

Introduction

In nearly all cancers, reactive oxygen species (ROS) are involved in breast cancer etiology. Increase of ROS has been detected, where they promote many aspects of tumor development and progression [Shiota Y, 2016]. Chemotherapeutic approach leads to increased ROS generation and increased oxidative stress in patients who

have breast cancer. Oxidative stress also causes free radicals to accumulate; resulting in more oxidative stress [Pragati et al., 2016]. Chemotherapy may adversely affect cardiovascular physiology, leading to heart failure. Specifically, anthracyclines, which are known to cause excessive free radical species build-up that, can lead to oxidative stress-induced cardiovascular apoptosis [Quryshi et al., 2018]. The potential therapeutic role of caspase 3 is therefore suggested. It has also been shown that tumors with low caspase levels have a better response to chemotherapy [Flanagan L., et al, 2016]. A family of enzymes called caspases elicit the initiating event, the apoptotic process [Thornberry N and Lazebnik Y., 1998]. Breast cancer therapy is associated with chemotherapeutic-induced cardiotoxicity diseases and increased oxidative stress [Fabricio B. S., et al.2017]. Excessive oxidative stress inhibits drug-induced caspase and apoptosis, interfering with antineoplastics ' ability to kill tumor cells [Lee, Shacter, 1999]. The electrophilic aldehydes bind covalently to the cysteine residue sulfhydryl group at the active caspases site and inhibit their activity [Wilson KP et al., 1994]. Aldehydes can also inhibit drug-induced apoptosis through inactivation of death receptors and inhibiting caspase activity [Conklin. 2004]. Cardiotoxicity was induced by doxorubicin (DOX) as a therapeutic agent as evidenced by serum parameters (e.g. levels of LDH and CK activity) [Amin et al., 2012]. (DOX) was accompanied by higher lipid peroxidation and lower GSH [Kim et al., 2017]. Elevated the level of LDH in serum indicates an increased leakage of this enzyme from mitochondria due to toxicity induced by doxorubicin treatment. Free radical generation causes membrane peroxidation and cardiac myocyte disruption, leading to increased CK release into the serum [Koti et al., 2009]. The levels of ROS that are frustrated in cancer cells by elevated antioxidant defense mechanisms, are higher than those found in normal cells, may be cancer cells more sensitive to ROS accumulation than cells which normal , providing an interesting therapeutic window [Diehn et al., 2009]. Increasing ROS to achieve a threshold that dissonates with cell viability and targets enhanced antioxidant mechanisms can kill cancer cells selectively and not affecting normal cells [Gorrini et al., 2013].

Materials & Methods:-

Fifty four women with newly diagnosed breast cancer were divided into 34 (stage 1) and 20 (stage II) patients with age range (25 -59) years, other 54 women with benign breast mass (pathological control) with age range (19 -60) years. These patients attended for treatment in Al - Amal National Cancer Hospital in Baghdad / Iraq from January, 2018 till September, 2018. Other 54 women were considered as a normal healthy control. The final diagnosis was checked by cytology, histopathological study (biopsy) and mammograph, under the Union for International Cancer Control Classification (UICC 1997). Cases have been classified according to Tumor Node Metastasis (TNM) classification and tumors have been diagnosed as invasive lobular and invasive ductal as shown in table (1) according to [American Cancer Society.2017]. Drugs used in chemotherapy were Doxorubicin, Paclitaxel and cyclophosphamide. Blood samples were withdrawn from all patients and coagulated at laboratory temperature and then centrifuged for 10 minutes at 3000 rpm. Separation of the resulting sera and assay has been manually done, for (CK), (LDH), (GSH), Caspase-3 and (MDA), The colorimetric method was used to measure the activity of CK and LDH using the kit from (Randox Company England) [Tietz. 1995]. Serum GSH was determined by modified procedure utilizing ,the reagent of Ellman (5,5-dithiobis(2-Nitrobenzoic acid DTNB) was added to the pH 8.0 phosphate buffer and the absorbance was measured at 412 nm [Sedlak, Lindsay. 1968]. Serum malondialdehyde (MDA) was measured according to Satoh

[Sato K.1978],an estimate of the reactive thiobarbituric acid product measuring the absorption at 534 nm.Serumcaspase-3 was determinat,by ELISA kit was pre-coated on well plates based on anti-casp3 antibodies and the biotin-conjugated anti-casp3 antibody was used to detect [Kumkum Jha et al., 2017].

Table 1: General features of patients with breast cancer

Features	patients. No	Features	patients. No
Total No. of subjects	54	T1N1M0 , T2N0M0 , T2N1M0 ,T3NOMO	stage II, (20)
Left breast	38	No malignant disease in family	22
Right breast	16	Malignant disease in family	32
Ductal cancer	36	Address Urban	35
Papillary cancer	18	Rural	19
T1N0M0	Stage 1,(34)	Marital status Married	48
		Single	6

The results were statistically analyzed using the ANOVA program. The mean (± SD) values were used. The test-t test was used to compare groups to analyze and compare the outcomes of patients and healthy patients. At a potential level ($P < 0.01$, $P < 0.001$, $P < 0.0001$ and $P < 0.05$), using Excel (2010) and (2013).

Ethical Aspects

According to Iraq's ethical guidelines, the informed consent was obtained from all patients included with the study protocols.

Results& Discussion:-

Table(2) showed that CK level was significantly decreased in serum from breast cancer women in comparison with normal healthy control ($P < 0.0001$), whereas there was no significant decrease comparison pathological control with normal healthy control ($P \geq 0.084$).Results indicated a negative correlation between the levels of serum CK and the stage of breast cancer. It also showed that there was decrease CK level, which was incomitant with that reported by Aminet al., 2012.CK level was increased in serum from breast cancer women compared to normal healthy control.

Table 2: Biostatistical calculation and student t-test for CK in sera of normal healthy control, pathological control and breast cancer women.

Serum (CK) (U/L)	Newly Breast cancer	Pathological control	Normal healthycontrol
Sample size	54	54	54
Mean±SD	31.51±21.7	45.08±12.6	48.90±10.6
P-value	0.0001 *	0.084	-

* Significant at 0.05 level of significance between two independent means.

This was consistent with the study by Vivier et al .The immune system protects the host by innate and adaptive responses from infection or disease, while decrease serum CK levels can be attributed to the host immune response in cases of breast cancer. In both caspase-dependent and independent apoptosis pathways, and provide potential cellular mechanism for the effects of certain genes on tissue damage and creatine kinase circulating [Sangkylu et al.,2016]. Table 3 showed that the LDH level from breast cancer women was significantly higher than in normal healthy control with ($P<0.001$), whereas there was no significant increase in comparison normal control with pathological control ($P\geq 0.586$).

Table3: Biostatistical calculation and student t-test for LDH in sera of normal healthy control, pathological control and breast cancer women.

Serum (LDH) (U/L)	Newly Breast cancer	Pathological control	Normal healthy control
Sample size	54	54	54
Mean±SD	71.71±26.2	59.08±17.3	57.49±12.6
P-value	0.001 *	0.586	-

* Significant at 0.05 level of significance between two independent means.

LDH was a vital metabolism enzyme associated with cancer development, invasion and metastasis [Yang et al., 2014]. LDH play a key role in the progression and maintenance of tumors. Consequently, LDH inhibition can inhibit tumor progression [Le A., et al.2010].LDH correlated with clinical characteristics and survival outcome to multiple cancers. LDH is another important enzyme for cancer cell metabolism. It is important marker for several types of cancer. These results were consistent with the results by [Girgis, H. et al.2014].It was concluded that LDH inhibition has an anti-proliferative effect on breast tumors as well as a therapeutic target for breast cancer. LDH inhibition was considered the therapeutic target of cancer energy metabolism. Table (4) showed in post-therapeutic study, a significant increase level in the serum CK and LDH after treatment compared with its level before treatment in patients with breast cancer ($P<0.0003$, $P<0.05$ respectively).

Table 4: Biostatistical calculations and student t-test for CK and LDH in sera of breast cancer before and after treatment.

Serum (CK) (U/L)	Before treatment	After treatment	Serum(LDH) (U/L)	Before treatment	After treatment
Sample size	15	15	Sample size	15	15
Mean±SD	30.17±10.05	46.79±12.02	Mean±SD	70.21±15.2	82.91±13.1
P-value	0.0003 *		0.05		

* Significant at 0.05 level of significance between two independent means.

It has been observed that the decrease in LDH inhibits glycolysis has an anti-tumor effect in cancer cells. The inhibition of LDH also confirmed a pro-apoptotic effect, which was likely due to increased activated caspase-3, which in turn inhibits cell growth, suppresses tumor invasion and leads to apoptosis in cancer cells. [Xiaojun Liu X, et al. 2015]

Doxorubicin's chemotherapy protocol to induce generation of free radicals that triggers the membrane peroxidation and cardiac myocyte derangement that can lead to increase the release of CK in serum [Koti et al., 2009]. Results showed that cardiac enzymes, LDH and CK were increased after chemotherapy. The main side effect of chemotherapy is cardiotoxicity, especially when using doxorubicin's chemotherapy [Kaithwas et al., 2011]. High serum LDH levels suggest that increased leakage of the enzyme from mitochondria due to induced toxicity through doxorubicin treatment [Koti et al., 2009]. It has been concluded that LDH inhibition inhibits tumor progression by increasing activated caspase-3, inhibits cell growth, and may be a potential therapeutic target. Table (5) showed that GSH level from breast cancer women significantly decreased in comparison to normal healthy control ($P < 0.0001$), while there was no significant decrease when compared normal control to **pathological control ($P \geq 0.062$)**.

Table 5: Biostatistical calculation and student t-test for GSH in sera of normal healthy control, pathological control and breast cancer women.

Serum (GSH) (mg/dl)	Newly Breast cancer	Pathological control	Normal healthycontrol
Sample size	54	54	54
Mean $\pm$ SD	25.64 $\pm$ 4.1	34.7 $\pm$ 5.8	36.4 $\pm$ 3.2
P-value	0.0001*	0.062	-

* Significant at 0.05 level of significance between two independent means.

The results indicated that increased oxidative stress can be attributed to increased levels of ROS in breast cancer patients, decreased glutathione content activity during these periods. These results were in agreement with that reported by Pragati et al., 2016. More oxidative stress can be generated in patients with breast cancer by the accumulation of free radicals during chemotherapy. Results indicated that the generation of ROS can cause GSH depletion. The depletion of glutathione stimulates the initiator of caspases and activates caspase-3 [Armstrong JS, and Jones DP 2002]. Results in this study showed agents that limited of glutathione precursors enhance both glutathione depletion and cell killing by inhibit glutathione biosynthesis, these findings support the potential of targeting the glutathione biosynthetic pathway as a therapeutic strategy. These results were in agreement with that reported by Alexander B, et al. 2018. In Triple-negative breast cancer (TNBC), agents that limit the availability of glutathione precursors increase both glutathione depletion and in vitro TNBC cell killing by glutathione biosynthesis inhibitors. Table (6) showed that the MDA level from breast cancer women significantly increased compared to normal control ($P < 0.0001$), while there was no significant increase in comparison normal control to **pathological control ($P \geq 0.0532$)**.

Table 6: Biostatistical calculation and student t-test for MDA in sera of normal healthy control, pathological control and breast cancer women.

Serum (MDA) (nmol/ml)	Newly Breast cancer	Pathological control	Normal healthy control
Sample size	54	54	54
Mean±SD	12.21±0.38	9.3±0.63	9.1±0.41
P-value	0.0001 *	0.0532	-

* Significant at 0.05 level of significance between two independent means.

Lipid peroxidation was the metabolic process that causes oxidative of lipids by reactive oxygen species. MDA represent the end product of lipid oxidation. These studies were consistent with our study. Lipid peroxidation forms a number of oxidation products, including lipid hydroperoxides and aldehydes like MDA. This process can degrade the lipids within the cell membrane resulting in damage to the cells and eventually cell death [Stoakes, 2018]. MDA level is the marker of oxidative damage for lipids in patients with breast cancer [Khoshbin et al., 2015]. Results in table (7) showed a significant decrease in GSH level in serum after treatment ($P < 0.0001$). Also there was significant increase in MDA level after treatment with ($P < 0.0001$), when compared to its level before treatment in patients with breast cancer.

Table 7: Biostatistical calculations and student t-test for GSH and MDA in sera of breast cancer before and after treatment

Serum (GSH) (mg/dl)	Before treatment	After treatment	Serum (MDA) (nmol/ml)	Before treatment	After treatment
Sample size	15	15	Sample size	15	15
Mean±SD	26.5±4.2	16.3±0.66	Mean±SD	12.6±0.33	14.3±0.82
P-value	0.0001 *		0.0001 *		

* Significant at 0.05 level of significance between two independent means.

Chemotherapy is most commonly used for the treatment of malignant cancer. The production is excessive of reactive oxygen species (ROS) and subsequent oxidative stress build-up is one of the major reasons of adverse reactions, particularly for drugs which target DNA [Singh et al., 2018]. Results indicated that MDA in breast cancer patients after chemotherapeutic was an important indicator of damage caused by oxidation. The aldehydes inhibits caspase-3 activity, this adverse reactions reduces antineoplastic agents effectiveness.

These studies were consistent with our study. The electrophilic aldehydes bind covalently to the cysteine residue sulfhydryl group at the active caspases site and inhibit their activity [Wilson KP et al., 1994]. Antioxidants can enhance the cancer chemotherapy activity by reducing the production of aldehyde [Conklin, 2004]. Table(8) showed that significant increased in CASP3 level before and after treatment ($P < 0.0001$) also the CASP3 level from breast cancer women significantly increased in comparison to normal healthy control ($P < 0.0001$). Anti-cancer can prevent the growth of breast cancer cells by stimulating apoptosis through caspase-dependent pathways. Caspase-3 was found to play a role in chemotherapeutic induced apoptosis in breast cancer.

Table 8: Biostatistical calculations and student t-test for CASP3 in sera of breast cancer before and after treatment, normal healthy control, and breast cancer women.

Serum (CASP3) (ng/ml)	Before treatment	After treatment	Newly Breast cancer	Normal healthy control
Sample size	15	15	54	54
Mean±SD	2.44±0.041	3.62±0.025	2.42±0.067	2.12 ±0.054
P-value	0.0001 *		0.0001 *	

* Significant at 0.05 level of significance between two independent means.

These studies are consistent with our study. Anticancer drugs eventually lead to caspases activation, a family of enzymes called caspases perform the initiating event, the apoptotic process [Thornberry N and Lazebnik Y., 1998]. Antiapoptotic mechanisms regulating caspase activation were also assumed to be involved in drug resistance of tumor cells, and activation of caspases has to be tightly controlled [Los M., et al. 1997]

.Chemotherapy also leads to increased oxidative stress. ROS levels are still higher in cancer cells than those observed in normal cells. Rising ROS to achieve a threshold that contradict with cell viability and target enhanced antioxidant mechanisms can kill cancer cells selectively and not affecting normal cells, providing an interesting therapeutic breast cancer window. Our results are consistent with that study. [Diehn et al., 2009; Gorrini et al., 2013]. From this study, it has been concluded that ROS, which is responsible for the oxidative stress caused by chemotherapy, needs to be tightly controlled as the uncontrolled increase leads to increased lipid peroxidation forming a number of oxidation products, including malondialdehyde (MDA), which reduces the effectiveness of antineoplastic agents. Identifying defects in the activation of caspase may provide molecular targets to overcome drug resistance in tumor cells. This suggests that caspase-3 has a potential therapeutic role.

CONCLUSION

From the results from this study, we can conclude that breast cancer therapy is associated with cardiotoxicity induced by the chemotherapeutic drugs and the increase in oxidative stress. Elevating of ROS to achieve a threshold that dissonates with cell viability can kill cancer cells selectively and not affecting normal cells. Oxidative stress inhibits the activity of caspase and apoptosis induced by the drug and therefore interferes with the ability of antineoplastic agents.

References

- 1-Shiota M, Yokomizo A. (2016) Prostate cancer and oxidative stress. *Nihon Rinsho*. 74. 71–4.
- 2-Pragati S, Brij Raj S, and Archana S. (2016) Nonenzymatic Evaluation of Antioxidant and Lipid Peroxidation Level in Breast Cancer Patients Receiving Juice of Wheat Grass during Treatment. *Recent Advances in Biology and Medicine*, 2, 1-4.
- 3-Quryshi N, Norwood Toro LE, Ait-Aissa K, et al. (2018) Chemotherapeutic-Induced Cardiovascular Dysfunction: Physiological Effects, Early Detection-The Role of Telomerase to Counteract Mitochondrial Defects and Oxidative Stress. *Int J Mol Sci*. 19(3). p: E797.
- 4-Flanagan L, Meyer M, Fay J et al. (2016) " Low levels of Caspase-3 predict favourable response to 5FU-based chemotherapy in advanced colorectal cancer: Caspase-3 inhibition as a therapeutic approach". *Cell Death Dis* .7: e2087.
- 5-Thornberry N and Lazebnik Y. (1998) " Caspases: Enemies within. *Science*".281:1312–1316.

- 6-Fabricio B. S, Walckiria G.R, Ana Ligia, et al (2017). " Effects of treatment with chemotherapy and/or tamoxifen on the biomarkers of cardiac injury and oxidative stress in women with breast cancer". *Medicine (Baltimore)*. 96(47): e8723.
- 7- Lee Y-J, Shacter E. (1999) Oxidative stress inhibits apoptosis in human lymphoma cells. *J Biol Chem*. 274. 19792-19798.
- 8-Wilson KP, Black J-AF, Thomson JA, et al.(1994) Structure and mechanism of interleukin-1 β converting enzyme. *Nature*.370:270-275.
- 9-Conklin KA (2004) Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. *Integrative Cancer Therapies*. 3(4). 294-300.
- 10-Amin KA, Mohamed BM, El-Wakil MA, Ibrahim SO. (2012) Impact of breast cancer and combination chemotherapy on oxidative stress, hepatic and cardiac markers. *J Breast Cancer*. 15(3). 306-312.
- 11-Kim SH, Kim KJ, Kim JH, et al. (2017) Comparison of doxorubicin-induced cardiotoxicity in the ICR mice of different sources. *Lab Anim Res*. 33(2): 165–170.
- 12-Koti BC, Vishwanathswamy AH, Wagawade J, Thippeswamy AH. (2009) Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats. *Indian J Exp Biol*. 47. 41–46.
- 13-Diehn M, Cho RW, Lobo NA, et al. (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 458(7239). 780–783.
- 14-Gorriani C., Harris I.S., Mak T.W. (2013) Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery*. 12(12). 931–947.
- 15- The American Cancer Society medical and editorial content team.Last Revised: December 20, 2017 .
- 16-Tietz N.W. (1995) Clinical guide to laboratory tests. 3rd ed., W.B. Saunders company, Philadelphia, PA 19106.
- 17-Sedlak J., Lindsay R.H. (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 25(1). 192-205. 192.
- 18- Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method.*Clin Chim Acta* 1978;90:37-43.
- 19-Kumkum Jha, Mridula Shukla, Mohan Kumar, et al. (2017) Expression of Caspase 3 and Inhibition of Apoptosis Lowers Survival in Breast Cancer". *World J Surg Med Radiat Oncol* .(2017);6:7-16.
- 20-Vivier E, Raulet DH, Moretta A, et al. (2011) Innate or adaptive immunity? The example of natural killer cells. *Science*. 331. 44–49.
- 21 -Sangkyu Kim, Eric Simon, Leann Myers, et al. Programmed cell death genes are linked to elevated creatine kinase levels in unhealthy male nonagenarians". *Gerontology*. (2016); 62(5): 519–529.
- 22-Yang Yang, Dan Su, Lin Zhao, et al. (2014) Different effects of LDH-A inhibition by oxamate in non-small cell lung cancer cells. *Oncotarget*. 5. 11886–11896.
- 23- Le A, Cooper CR, Gouw AM, et al." Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression" *Proc Natl Acad Sci USA*. (2010);107:2037–2042.
- 24-Girgis, H. et al.(2014). "Lactate dehydrogenase A is a potential prognostic marker in clear cell renal cell carcinoma". *Mole cancer* . **13**, 101.
- 25- Xiaojun Liu X, Yang Z C,Rui C D, et al." Effects of the suppression of lactate dehydrogenase A on the growth and invasion of human gastric cancer cells".*Oncol Rep*. (2015);33(1):157-62.
- 26-Kaithwas G, Dubey K, Pillai KK. (2011) Effect of aloe vera (*Aloe barbadensis* Miller) gel on doxorubicin-induced myocardial oxidative stress and calcium overload in albino rats". *Indian J Exp Biol*. 49. 260–268.
- 27-Armstrong JS, and Jones DP. (2002) Glutathione depletion enforces the mitochondrial permeability transition and causes cell death in Bcl-2 overexpressing HL60 cells. *Faseb J*. 16:1263–1265.
- 28-Alexander B, Lauren S. F, Tanu S, et al.(2018)." Metabolite Profiling Reveals the Glutathione Biosynthetic Pathway as a Therapeutic Target in Triple-Negative Breast Cancer". *Mol Cancer Ther* .**17**(1):264-275.

- 29-**Stoakes S.F. (2018) Lipid Peroxidation. [https://www.news-medical.net/life-sciences/Lipid-Peroxidation-\(French\).aspx](https://www.news-medical.net/life-sciences/Lipid-Peroxidation-(French).aspx)
- 30-**Khoshbin A.R., Mohamadabadi F., Vafaeian F., et al. (2015) The effect of radiotherapy and chemotherapy on osmotic fragility of red blood cells and plasma levels of malondialdehyde in patients with breast cancer. Reports of Practical Oncology & Radiotherapy. 20(4). 305-308.
- 31-**Singh K., Bhori M., Kasu Y.A., et al. (2018) Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity – Exploring the armoury of obscurity. Saudi Pharm. Journal, 26(2). 177-190.
- 32-**Los M, Herr I, Friesen C. et al. Cross-resistance of CD95- and drug-induced apoptosis as a consequence of deficient activation of caspases (ICE/Ced-3 proteases). Blood. (1997);90:3118–3129.

Aqueous Extract of Date Palm Fruit (*Phoenixdactylifera*) protect liver Against Cyproterone Acetate Toxicity in male mice

Nuha Yaarub Al-Harbi*¹, Eman Fadhel Abbas Awadh¹, Wasan Najim Abdul-Sada²

1. University of Babylon, College of Science, Dept. of Biology, Iraq.
2. Basic Science Department, College of Dentistry, Babylon University, Hillah, Iraq

*Corresponding author

Abstract:

Cyproterone acetate (CPA) is an antiandrogen drug that is used in the treatment of prostate cancer, which is related to drug-induced liver injury (DILI). The aim of this study is to evaluate the effect of the water extract of one of the local dates on liver tissue in white mice. Forty albino mice male were divided into 8 equal groups to receive some of the extract orally in one ml as follows. Group 1: distilled water passive control, group 2: corn oil positive control, group 3: received with 5 mg/kg body weight CPA, group 4: received, with 20 mg/kg CPA, group 5: received with 5 mg/kg CPA & 60 mg/kg date palm extract, group 6: received, with 5 mg/kg CPA & 120 mg/kg date palm extract, group 7: received, with 20 mg/kg CPA & 60 mg/kg date palm extract, group 8: received with 20 mg/kg CPA & 120 mg/kg date palm extract lasted for 21 days. Showed a histological study of the liver remarkable degeneration of hepatocytes associated with interstitial necrosis and blood vessel congestion. The current study proved that the water extract of dates has a weak effect in the repair of damage in the liver tissue to treatment for the low dose of cyproterone acetate only. In conclusion, the present study has shown that CPA has a toxic effect and some histopathological changes have been detected, so care should be taken when CPA is prescribed as antiandrogenic treatment.

Keywords: Cyproterone acetate, Date palm fruit, hepatotoxicity, histopathology.

How to cite this article: Al-Harbi NY, Abbas Awadh EF, Abdul-Sada WN (2019): Aqueous Extract of Date Palm Fruit (*Phoenixdactylifera*) protect liver Against Cyproterone Acetate Toxicity in male mice, Ann Trop Med & Pub Health; 21: 2040-19

Introduction

CPA is synthetic progesterone and antiandrogenic component administered. It is used to treat many physical conditions in prostate cancer and also in breast cancer, serious acne, womanly hirsutism, precocious puberty, hypersexuality (1). Drugs can have direct toxic effects (dose-dependent) or elicit hypersensitivity or metabolic distinctive reactions (dose-independent) that can take place at any time during the course of therapy (2). Hepatotoxicity signs resulting from the use of both steroidal androgens and nonsteroidal antiandrogens causes, many cases immunoallergic cytotoxic reactions, cholestasis, autoimmune hepatitis (3 & 4), acute hepatitis, fulminant hepatic fail (5) cirrhosis (6) and ultimately, CPA has imputed a hepatocellular mutagenic capacity leading to hepato-carcinogenesis (7). Many treatments with natural products can use because they are cheap and easy to obtain. The Prophet Mohammed (Peace Be upon Him) recommended the use of natural products as medicine for certain diseases (8). In traditional medicine, herbal medicines are widely used around the world, where palm pollen grains are widely used as anti-hepatotoxicity (9 & 10). *Phoenix dactylifera* L. plant is one of the cultivated plants in the Middle East and North Africa. The fruits of dried dates contain 8 phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid (11 & 12). In addition, date fruits contain Ascorbic acid, β -carotene, nicotinic acid, riboflavin and thiamine (13) and contains twenty-one free amino acids (leucine, α -alanine, and proline are predominant), and the amides asparagine and glutamine are abundantly found in *P. dactylifera* (14). Another study indicated that date palm fruit contains cholesterol, campesterol, stigmasterol, β -sitosterol, and fucosterol (15). Every date types are a user provenance of natural antioxidants. It can be considered effective food (16) since date fruit extract had a powerful antioxidant and antimutagenic specialty (17). Various studies have that the date fruit extract has been

shown to ameliorate liver damage in rat, inhibit swelling and tumors, suppress the growth of *Streptococcus pyogenes* and improve sperm parameters (18 & 19). The aim of this study is to evaluate the effect of aqueous extract of date palm fruit against CPA toxicity in mice.

Materials and Methods

Preparation of extract

Fresh date palm fruits (*Phoenix dactylifera* L., *Palmae*) were provided from a local market in Hilla (Babil, Iraq), dried at room temperature, and was manually isolated from the pits. The flesh of the dried *P. dactylifera* fruits was ground. About 650 g of the powder was soaked in 2 L of cold distilled water. After 24 h, the solution was filtered and evaporated under vacuum and dried to a constant weight using a freeze-drier. The dry extract of the fruit was dissolved in distilled water instantaneously before treated the mice (20).

Preparation of cyproterone acetate

Obtained cyproterone acetate anti-androgen from local pharmacies and called Androcur as a trading name and equipped from a subsidiary company of Filiale de Schering AG Germany, 20mg concentration for each disc. Dissolved the drug used in this study in absolute ethyl alcohol and left exposed to the air until drought then added to the powder pure corn oil. solute each disc in 12.5 ml from corn oil to obtained 20mg/ml this study in absolute ethyl alcohol and left exposed to the air until drought then added corn oil and calculated required concentrations to conduct experiments depending on the dose given to human(21).

Animals of the experiment

In the current study, 40 male white mice have used range ages from used 2-3 months. The mice were provided with eating and water *adlibitum*.

Experimental protocols

Mice were randomly divided into eight groups each consisting of 5 animals that were treated daily with one milliliter orally as follows:

1-First control group: treated with distilled water for 21 days, as a negative control.

2-Second control group: treated with corn oil for 21 days, as a positive control.

3-Third group: treated with cyproterone acetate 5 mg/kg/BW for 21 days.

4-Fourth group: treated with cyproterone acetate 20mg/kg/BW for 21 days.

5-Fifth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 60mg/kg/BW for 21 days.

6-Sixth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

7-Seventh group: treated with cyproterone acetate 20mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

8-Eighth group: treated with cyproterone acetate 20 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

Animals were sacrificed 24 hours after of the last dose, use diethyl ether to drugged mice, open the abdominal cavity and remove the liver, then fixed the fresh small pieces of each mouse liver in the formalin solution 10 % until the histological preparation.

Histological study

Ordinary histological processing is prepared for the liver in order to study the histopathological changes that may be found in the experimental groups as compared with negative and positive control groups. Based on recommended histological techniques (22), the processing steps and staining technique was as follow: small pieces of livers were

dehydrated using a graded ethanol series, subsequently embedded in paraffin, wax blocks were cut by the microtome to prepare 5µm thick sections and stained with hematoxylin after deparaffinization of sections in xylene and hydrated in progressive descending ethanol series and stained with eosin after the washing and differentiate, then wash again, dehydrate, cleared in xylene and mount with Canada balsam on glass slide for light microscopic examination.

Results and Discussion

Several studies have indicated liver enzyme aberration in experimental animals treated with cyproterone acetate (23). Our study showed treated mice of 5 mg/kg body weight of CPA (group 3, fig. 1) revealed degeneration and necrotic changes in hepatocytes, sections of liver of CPA 20 mg/kg/BW treated mice (group 4, fig. 2) showed congested blood vessels, necrosis in hepatocytes and moderate inflammatory cell infiltration in the portal triad as compared to other treated groups. The hepatotoxicity depend on both the dose and the duration of xenobiotics exposure will impact the type and grade of toxicity, there is often susceptibility to the toxicity based on the intralobular site of hepatocytes for xenobiotics that immediately affect the liver and hepatocellular necrosis, evidence of necrosis is generally apparent within forty-eight hour or previously(24).

One study noted an increase in serum aspartate aminotransferase (AST) and alanine aminotransferase. (ALT) activities in all patients with in progress prostate cancer who were treated with CPA-induced liver damage, in 91% of those cases, the type of hepatic injury was hepatocellular damage. This damage is frequently involved hepatocytes damage that is associated with an elevated ALT level (25). It is generally synched progress that hepatocellular hypertrophy may be a serious qualitative metric, but classify the severity of hypertrophy is less accurate than relying on liver weight or quantitative measurement of enzyme induction. There is a substantial relationship between hepatotoxicity and enzyme induction with clinical pathology measurements are described (26).Changes may occur in liver histology without enzyme reduction but include fluid aggregation, fatty change in hepatocytes, inflammatory cell infiltration, fibrosis, and probably granuloma formation (27).

Examination of stained liver sections of animals treated with 5 mg/kg/BW of CPA and crude aqueous palm date fruit extract 60 mg/kg/BW (group 5, fig.3) showed a noticeable degeneration in hepatocytes, sections of the liver of CPA 5 mg/kg/BW and crude palm date fruit extract 120 mg/kg/BW treated mice (group 6, fig.4) revealed congested blood vessels and necrosis of hepatocyte as compared with CPA treated mice with reducing severity in two groups. "The mechanism by which the aqueous date palm fruit extract induces its hepatoprotective activity versus oxidative damage caused by any drug is not clear. However, it is potential that polyphenolic compounds (flavonoids, anthocyanins, and phenolic acids), and trace elements (selenium, copper, zinc, and manganese), an extension to vitamin C present in the date palm fruit are the responsible compounds for this protection(28, 29, 30).

One of the studies conducted on the aqueous extract of date fruits demonstrated that has antioxidant and antimutagenic activities. This extract also has an inhibition effect on lipid peroxidation and protein oxidation and also by the aptitude to scavenge superoxide and hydroxyl radicals (31).In addition to that, there are many studies which indicates that the hepatoprotective activity of any drug is its ability to block the aromatase activity of cytochrome P-450. On that basis, it is proposed that flavonoids in *Phoenix dactylifera* could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase (32).

Tissue sections of the liver of CPA 20 mg/kg/BW and palm date fruit extract 60 mg/kg/BW treated mice (group7, fig. 5) showed congested blood vessels and severe degeneration in hepatocyte, in the liver of animals treated with CPA 20 mg/kg/BW and palm date fruit extract 120 mg/kg/BW (group 8, fig. 6) . Although comparative studies indicated that dried date palm fruit with phenolic content was higher than fresh date palm fruits (33). However, the water extract of dried palm fruit used in this study did not have the ability to preserve the liver from the toxic effect of the drug CPA especially at a high dose. The direct effect of cyproterone is attributed to increase of hepatocytes of placental glutathione S-transferase, which are believed preneoplastic elements (34-35). Where it works on Growth Factor-beta 1 (TGF) expression on the induction of apoptosis might account for both the liver damage and the expansion of liver tumors observed after giving a drug of CPA (36).

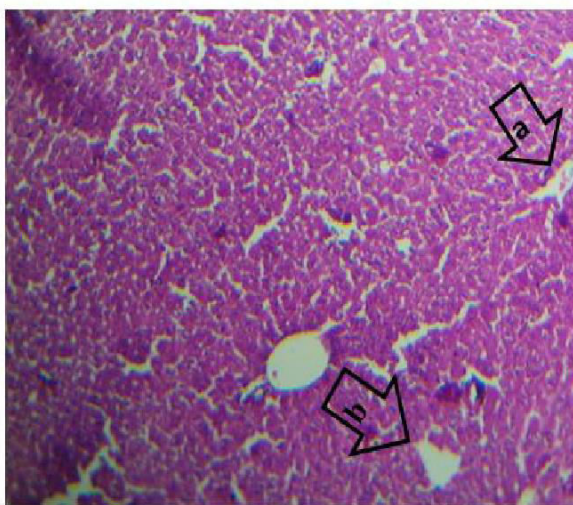


Figure-1: Histological liver section of (CPA) 5 mg/kg, group showed (a) Marked activation kuppfer cell . (b) degeneration of the hepatocyte necrosis of hepatocyte.



Figure-2: Histological liver section of (CPA) 20 mg/kg group showed (a) sever degeneration of hepatocyte (b) hypertrophied hepatocytes with deeply stained shrunken nuclei .(c) congested blood vessels.

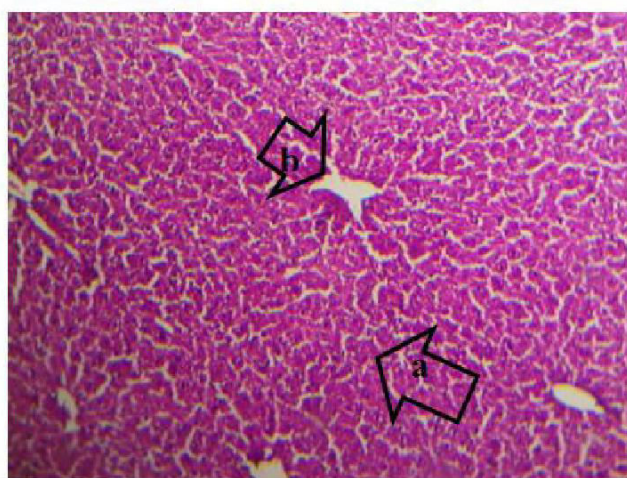


Figure-3: Histological liver section of (CPA) 5 mg/kg + 60 mg/kg date palm extract, group showed (a) Some hepatocytes were free from nuclei and others contained pyknotic nuclei.

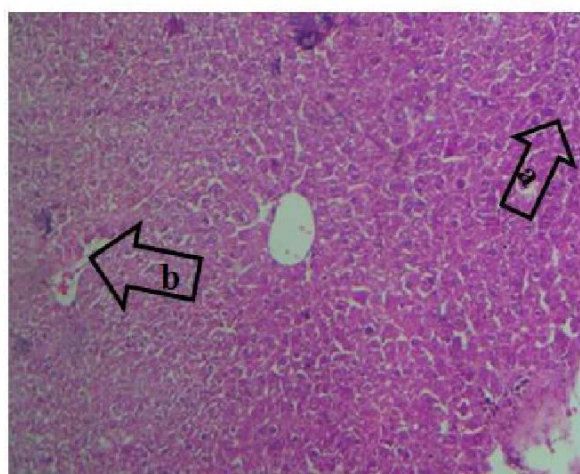


Figure-4: Histological liver section of (CPA) 5 mg/kg + 120 mg/kg date palm extract, group showed (a) moderate hypertrophy of cells and (b) moderate hemorrhagic area .



Figure-5: Histological liver section of (CPA) 20 mg/kg + 60 mg/kg date palm extract, group showed sever degeneration of hepatocytes (b) and multihemorrhagic Areas.

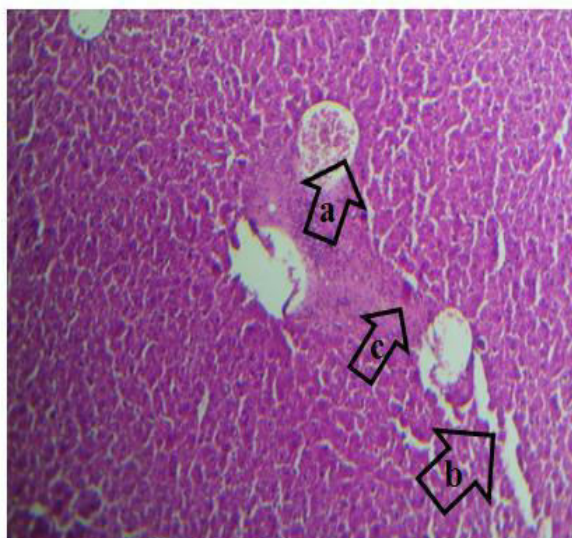


Figure-6: Histological liver section of (CPA) 20 mg/kg+120 mg/kg date palm extract, group showed (a) congested blood vessel (b) necrosis of hepatocyte (c) moderate inflammatory cells infiltration in the portal triad

References:

1. Levesque, H.; Manchon, N. D.; Moore, N.; Courtois, H.; Trivalle, C.; Vinel, J. P. & Bourreille, J. (1989). Fulminant hepatitis due to cyproterone acetate. *The Lancet*, 333(8631), 215-216.
2. Kaplowitz, N. (2004). Drug-induced liver injury. *Clinical Infectious Diseases*, 38(Supplement_2), S44-S48.
3. Murphy, B. J., & Collins, B. J. (1996). Severe hepatitis and liver failure induced by cyproterone acetate. *Australian and New Zealand journal of medicine*, 26(5), 724-724.
4. Kacar, S., Akdogan, M., Kosar, Y., Parlak, E., Sasmaz, N., Oguz, P., & Aydog, G. (2002). Estrogen and cyproterone acetate combination-induced autoimmune hepatitis. *Journal of clinical gastroenterology*, 35(1), 98-100.
5. Drakos, P. E., Gez, E., & Catane, R. (1992). Hepatitis due to cyproterone acetate. *European journal of cancer (Oxford, England: 1990)*, 28(11), 1931-1932.
6. Garty, B. Z., Dinari, G., Gellvan, A., & Kauli, R. (1999). Cirrhosis in a child with the hypothalamic syndrome and central precocious puberty treated with cyproterone acetate. *European journal of Pediatrics*, 158(5), 367-370.
7. Rüdiger, T., Beckmann, J., & Queiber, W. (1995). Hepatocellular carcinoma after treatment with cyproterone acetate combined with ethinyloestradiol. *The Lancet*, 345(8947), 452-453.
8. Marwat SK, Khan MA, Rehman F, Bhatti IU (2009). Aromatic plant species mentioned in the Holy Qur'an and Ahadith and their ethnomedicinal importance *Pak J Nut* 8: 1472-1479
9. Abdelouahhab, Z., & Arias-Jimenez, E. J. (1999). Date palm cultivation. *FAO Plant Production and Protection Paper*, (156).
10. Khare, C. P. (2007). *Indian Medicinal Plants: An Illustrated Dictionary* Springer-Verlag. Berlin pg, 699-700.

11. Al-Farsi*, M. A., & Lee, C. Y. (2008). Nutritional and functional properties of dates: a review. Critical reviews in food science and nutrition, 48(10), 877-887
12. Regnault-Roger, C., Hadidane, R., Biard, J. F., & Boukef, K. (1987). High-performance liquid and thin-layer chromatographic determination of phenolic acids in palm (Phoenix dactylifera) products. Food Chemistry, 25(1), 61-71.
13. Mahran, G. H., Abdel-Wahab, S. M., & Attia, A. M. (1976). A phytochemical study of date palm pollen. Planta Medica, 29(02), 171-175
14. Al-Shahib, W., & Marshall, R. J. (2003). The fruit of the date palm: its possible use as the best food for the future?. International journal of food sciences and nutrition, 54(4), 247-259.
15. Garcia, B., Marco, J. A., Seoane, E., & Tortajada, A. (1981). Triterpenes, waxes, and tricin in Phoenix canariensis. Journal of Natural Products, 44(1), 111-113
16. Saafi, E. B., Louedi, M., Elfeki, A., Zakhama, A., Najjar, M. F., Hammami, M., & Achour, L. (2011). Protective effect of date palm fruit extract (Phoenix dactylifera L.) on dimethoate induced-oxidative stress in rat liver. Experimental and Toxicologic Pathology, 63(5), 433-441
17. Hasan, M. Z. (2010). MZ Hasan and CL Kane, Rev. Mod. Phys. 82, 3045).
18. Al-Qarawi, A. A., Mousa, H. M., Ali, B. H., Abdel-Rahman, H., & El-Mougy, S. A. (2004). Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J Appl Res Vet Med, 2(3), 176-180.
19. Bahmanpour, S., Talaei, T., Vojdani, Z., Panjehshahin, M. R., Poostpasand, A., Zareei, S., & Ghaemini, M. (2015). Effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. Iranian Journal of Medical Sciences, 31(4)
20. Jahromi, A. R.; Rasooli, R.; Kamali, Y.; Ahmadi, N., & Sattari, E. (2017). Short-term effects of date palm extract (Phoenix dactylifera) on ischemia/reperfusion injury induced by testicular torsion/detorsion in rats. Pharmacognosy research, 9(1), 69.
21. Abd-Al-Ameer, E. H. (2008). The Effects of Cyproterone Acetate on the Sexual Performance and Fertility of the White Rats *Rattus rattus*. Ph. D. thesis. College of sciences. The University of Babylon.
22. Bancroft, J. D., & Gamble, M. (Eds.). (2008). Theory and practice of histological techniques. Elsevier health sciences.
23. Blake, J. C., Sawyerr, A. M., Dooley, J. S., Scheuer, P. J., & McIntyre, N. (1990). Severe hepatitis caused by cyproterone acetate. Gut, 31(5), 556-557
24. Evans, J. (1987). Principles of toxicological pathology: Edited by John R. Glaister. Taylor & Francis, London, 1986. pp. vi+223. £ 25.00. ISBN 0-85066-316-4. Food and Chemical Toxicology, 25(4), 337-338
27. Lake, B. G., & Evans, J. G. (2003). The Digestive System II: The Hepatobiliary.
28. Hong, Y. J., Tomas-Barberan, F. A., Kader, A. A., & Mitchell, S. (2006). The flavonoid glycosides and procyanidin composition of Deglet Noor dates (Phoenix dactylifera). Journal of agricultural and food chemistry, 54(6), 2405-2411.
29. Allaith, A. A. A. (2008). Antioxidant activity of Bahraini date palm (Phoenix dactylifera L.) fruit of various cultivars. International Journal of Food Science & Technology, 43(6), 1033-1040
30. Saafi, E. B., El Arem, A., Issaoui, M., Hammami, M., & Achour, L. (2009). Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. International journal of food science & technology, 44(11), 2314-2319
31. Vayalil, P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Arecaceae). Journal of Agricultural and Food Chemistry, 50(3), 610-617.
32. Kowalska, M. T., Brandt, M. E., & Puett, D. (1990). Inhibition of cytochrome P-450 aromatase activity by plant extracts. Planta Medica, 56(06), 675-675.

- 33. Al-Farsi, M.; Morris, A. and Baron, M.(2007).** Functional Properties of Omani dates (*Phoenix dactylifera* L.). *Acta Hort.*, 736, 479-487.
- 34. Fasciati, R., & Maier, P. (1997).** EGF-and CPA-induced mitogenic stimuli are differentially down-regulated by TGF-beta1 in .cultured rat hepatocytes. *Carcinogenesis*, 18(5), 911-917
- 35. Lw-Baselli, A., Hufnagl, K., Parzefall, W., Schulte-Hermann, R., & Grasl-Kraupp, B. (2000).** Initiated rat hepatocytes in primary culture: a novel tool to study alterations in growth control during the first stage of carcinogenesis. *Carcinogenesis*, 21(1), 79-86.
- 36. Oberhammer, F. A., & Qin, H. M. (1995).** Effect ofthree tumourr promoters on the stability of hepatocyte cultures and apoptosis after transforming growth factor- β 1. *Carcinogenesis*, 16(6), 1363-1371.

.

Colorimetric determination of iron and zinc in people with gallstones in Diyala Governorate

Nabaa H. AL-Obiady¹, Firas T. Maher¹, Abbas Shebeeb Al-kadumi²

1. Tikrit University, College of science.
2. Al - Nahrain University, College of pharmacy.

Abstract

In this study, iron and zinc were determined in 100 samples of gallstones, where 100 samples (17 males and 87 females), the ranged age between (17-78) were collected from Baquba Educational Hospital from December 2017 to March 2018. Iron level was determined in two ways, the first method reduction Fe(III) to Fe(II) by Hydroxyl ammine hydrochloride and using reagents 1,10-phenanthroline, and the second method was determined both iron and zinc respectively by using 2,2'-Bi pyridyl , Dithizone respectively. The results have been given Linearity range (1-260),(2-300)(2-200) $\mu\text{g.ml}^{-1}$ for each of the Total Iron ,Fe(II) and Zn(II) respectively , Detection limits(0.112),(0.303) and (0.396) for each of the Total Iron ,Fe(II) and Zn(II). The results showed the highest concentration of total iron on the white stone but the lowest concentration on the white stone, While the highest concentration of Fe^{+2} on the yellow stone but the lowest concentration on the white stone and the highest concentration of Zn^{+2} on the white stone but the lowest on the yellow stone .As for the sex, the highest concentration of total iron , Fe^{+2} and Zn^{+2} was recorded in the female. As for the color has been recorded highest concentration of total iron on the green stone , While the highest concentration of Fe^{+2} on the yellow stone and the highest concentration of Zn^{+2} on the white stone .

Keywords: Gallstone, Iron, Zinc, gallbladder, Cholecystitis, Colorimetric, Determination

How to cite this paper: Al-Obiady NH, Maher FT, Al-Kadumi AS (2019): Colorimetric determination of iron and zinc in people with gallstones in Diyala Governorate, Ann Trop Med & Pub Health; 21: SP2041-19

Introduction

Gallstone disease is one of the most popular pathological entities in the digestive system and stone is a solid crystalline deposits created in the gallbladder⁽¹⁾. Bile stones are one of the most important surgical problems in many hospital admittance and surgical interferences⁽²⁾. Quantitative chemical analysis of total cholesterol, total bilirubin, fatty acids, triglycerides, phosphatic acids, bile acids, soluble proteins, calcium, sodium potassium, magnesium, copper, oxalates and chlorides are also secondary components set in all kinds of gallstone⁽³⁾. Gallstones are classified in cholesterol, pigment and mixed stones. Other materials found contain calcium salts of phosphates, myosin, glycoprotein, phosphates and some minerals⁽⁴⁾. Fourteen elements were reported: sulfur- chloride- potassium- calcium- titanium- vanadium- chromium- manganese- iron- nickel- copper- zinc- bromide and lead are found in the bile stone and played an important part in the formation of gallstones⁽⁵⁾. Analysis of

the chemical structure of gallstones has made an important reference to the treatment of recurrence prohibition⁽⁶⁾.

In the state of fasting, the correct diagnosis achieved in more, than 90% of the cases; however, the bile duct, stones may be, lost in up to 50% of the, status. The exact number and, size of the stones were not determined by ultrasound⁽⁷⁾.

This similarity and susceptibility in cholangial-pancreatic, (MRCP, noninvasive) and endoscopic ultrasound (EUS) or ERCP⁽⁷⁾ Studies on the structure of metastases in different parts of the world mark a close relate to dietary and ethnic habits⁽⁸⁾. Other studies have shown that dietary intake of total calories in the form of carbohydrates and fat was connected with high levels of triglycerides in Gallstone patients⁽⁹⁾. The formation of stone is increased dependably with the consumption of dietary fats rich in saturated fatty acids⁽¹⁰⁾.

This study describes an extensive quantitative analysis of gallstones, including Total Iron, Zinc and Fe⁺². Here, the objective, of this study was to define the chemical, structure of gallstones among gallstone patients.

Iron (Fe) is the most abundant element, in the earth and, the fourth most, abundant element in the crust, nearly 5 percent of the mass. The total iron content in the body is 3.5 g. 70% of this iron is present in hemoglobin. Biologically important compounds of iron are hemoglobin, myoglobin, cytochromates, catalases, and peroxidase. In all these compounds, iron is set, in the form of hemi or porphyrin. In addition to the presence, of these iron, in the form of non-hemi called non-hemi iron. There is a non-ferment iron in the form, of ferritin (in the form of red iron) and transported (iron transfer form). Iron works such as hemoglobin, in the transport, of oxygen. In cellular respiration, it acts, as an essential component, of enzymes involved in biological oxidation like cytpchromesc c2a

(11).

tissue	blood	brain	liver	muscle	bone	dd-intake*
Fe contet	450 mg/dm ³	20-2000 ppm	250- 1400 ppm	180 ppm	3-380 ppm	6-40 mg

Zinc (Zn) is a trace element, essential for all life forms. Many proteins contain structures called "zinc fingers" that assistance regulate genes. Zinc insufficiency is known to lead to dwarfism in developing countries. Zinc is an paramount component of the pancreas. Zinc is desired for activity of more than 300 enzymes, covering all six layers of enzymes. Zinc binding sites in proteins are typically tetrahedral, or triglyceride-engineered, consisting of cysteine sulfate, histidine nitrogen, aspartate, glutamate, or combination. Zinc functions are a component of certain enzymes like carbon anhydrase, carboxylic peptidease, alkali phosphatase, dehydrograz lactate, alcohol dehydrogase, super-dimautase, retinase⁽¹²⁾.

Materials and methods

The study included 100 patient with age ranged (17-78) years undergoing cholecystectomy at the department of general surgery in Baquba Teaching Hospital December 2017 during March 2018.

Gallstones from 100 patients of cholelithiasis were collected after cholecystectomy. 100 gallstones were collected from 13 males and 87 females. The stones were divided into 5 groups depending upon their color: yellow stone, white stones, black, brown and green stone. Other relevant information was obtained about patients such as age, gender, family history of the patient and the presence of any other disease from hospital records.

The current work describes spectrophotometric method for the determination of Fe, Fe⁺² and Zn (II) in the Gallstone. The method is based on formation of colored complexes of Fe and Zn (II) with 2,2'-Bi-pyridyl and Dithizone respectively. Fe forms deep red colored chromogen with 2, 2'-Bi-pyridyl having maximum absorbance at 520 nm and Zn (II) complexes with dithizone to develop cherry red color having maximum absorbance at 525 nm.

While (Fe⁺²) is based work with the 1,10-phenanthroline (C₁₂H₈N₂, Ortho-phenanthroline or O-phen) to form strongly colored complexes at 508 nm.

Reagent:-

- 1-Primary standard solid Ferrous ammonium sulfate hexahydrate (NH₄)₂Fe(SO₄)₂·6H₂O, 1 gm resolved in 100 ml of Distill water. The stock solution was further diluted to get range of 2-500 ppm.
- 2-Primary standard solid of Zinc sulphate ZnSO₄, 1 gm resolved in 100 ml of distill water. The stock solution was further, diluted to get range 2-200 ppm.
- 3-Preparation of 2,2'-Bi-pyridyl reagent (1% w/v): 1 gm of 2,2'-Bi-pyridyl was resolved in 100 ml of methanol.
- 4-Preparation of Dithizone (0.1% w/v): 10 mg of dithizone was resolved in 100 ml of chloroform.
- 5-Preparation of Buffer solution (Alkaline ammonium citrate): 5 gm of dibasic ammonium citrate was resolved in 100 ml of 5% Ammonium Solution.
- 6- 1, 10-Phenanthroline solution: 0.1 gm is dissolved in the 100 ml of distill water with the simple heating to complete the melting process.
- 7- Hydroxyl amine hydrochloride: 10 gm is dissolved in 100 ml of distill water.
- 8- Sodium acetate buffer 1.2 M: 9.84 gm is dissolved in 100 ml of distill water.

Procedure:-

For determination of Fe(II) and Zn (II) following step was followed:

- 1-1 ml of gallstone solution that resolved in the special solvent.
- 2-2 ml of 2,2'-Bi-pyridyl reagent was added, followed by 1 ml of buffer solution and 1 ml of Dithizone reagent.
- 3-The solution was shaken and separation of two layers.
- 4-The aqueous layer was diluted with methanol up to mark in 10 ml volumetric flask.
- 5-The organic layer was diluted with chloroform up to mark in 10 ml volumetric flask.
- 6-Dithizone reagent for chloroform layer and bi-pyridyl reagent for aqueous layer.

7-Fe(II) is found in the aqueous layer that read the absorbance in 520 nm.

8-Zn(II) is found in the organic layer that read the absorbance in 525 nm⁽¹³⁾.

While the procedure of determine the Fe⁺².by 1,10-phenanthroline is :

1-Add 1 ml of sample on the conical flask . To each flask add:

A-1 ml of the hydroxylamine hydro chloride solution

B-5 ml of the 1, 10 –Phenanthroline solution

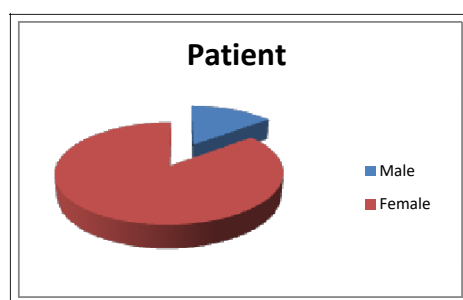
C-8 ml of the sodium acetate solution

2-Allow the solution to stand for 10 minutes to fully develop the color.

3-Read the Absorbance in the Wave length 508 nm⁽¹⁴⁾ .

Results and discussion

This study carried on 100 cholecystectomy patients from December 2017 and March 2018 in which cholecystectomy was carried on 100 patients: 87(87%) were women, 13(13%) men. Similar finding have been observed in prior studies Figure(1).



It was reported that, the concentration of bile salts in bile is reduced by estrogen, so making it a lithogenesis⁽¹⁵⁾. It is presumed that under the effect of the female sex hormone, muscles may relax, the amplification of bile matrices and the contents of the twelve pancreatic excretion deteriorate into the gallbladder and elevate conditions that support the formation of gallstones⁽¹⁶⁾. Females were more probable to develop gallstone disease, particularly if oral contraceptives were used and / or had four or more children⁽¹⁷⁾.

The gallstone collected from 100 gallstone patients were divided into 5 groups based on their color: Pale yellow, whitish stones, black, blackish brown and brownies yellow or greenish. The most common type of gallstone was cholesterol stone 46 (46%), followed by greenish stone 23 (23%), whitish stone 15(15%).black stone 10(10%) and blackish brown stone 6(6%). Out of the total number of stones collected, the incidence of gallstone was higher in age group 31-50 years (Table 1). These results are in a various way related with the results of study carried in Babylon by Al-Khafeji⁽¹⁸⁾,

who showed that the most common type of gallstone was of cholesterol type (40%) followed by pigment stone (35%), and mixed stone(25%). However, the results differs with studies performed in other countries like southern India by Jayanthi⁽¹⁹⁾ who showed that the most common type of gallstone was of pigment stone(63.8%) followed by mixed type(34.8%)and then by cholesterol stone(1.9%).

The comparison of our data with those in similar studies in different countries may not be valid because of geographic, dietary and ethnic differences Bedirli⁽²⁰⁾.

(18)(20) Age period 31-50 showed high percentage of calculi in two other studies that is in agreement with ours (Al-khajeji,2006 ; Jayanthi *ET.AL.*,1998).

Figure 6: The standard curve of Fe⁺²

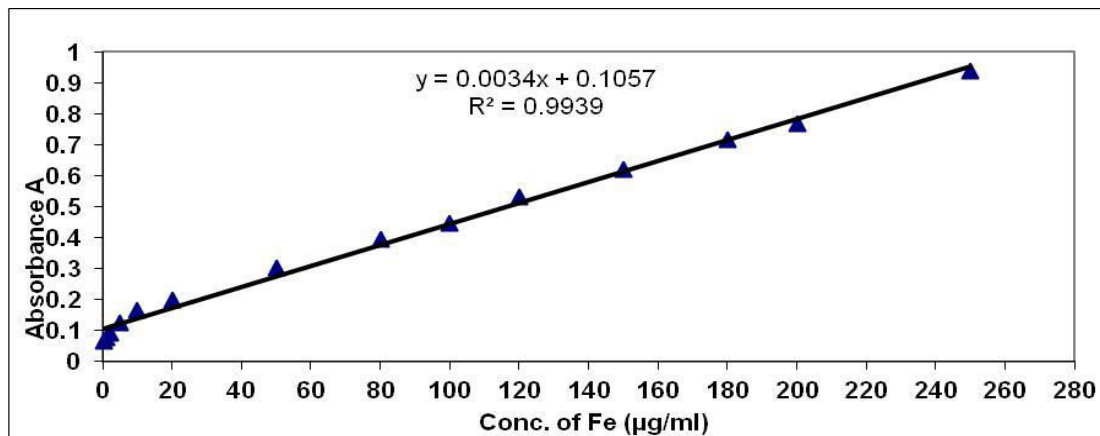
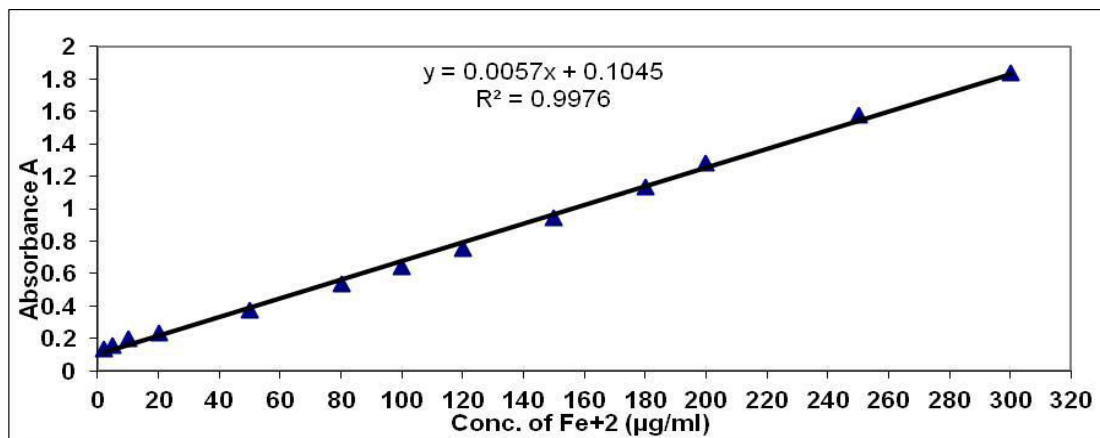


Figure 5: The standard curve of Total Iron



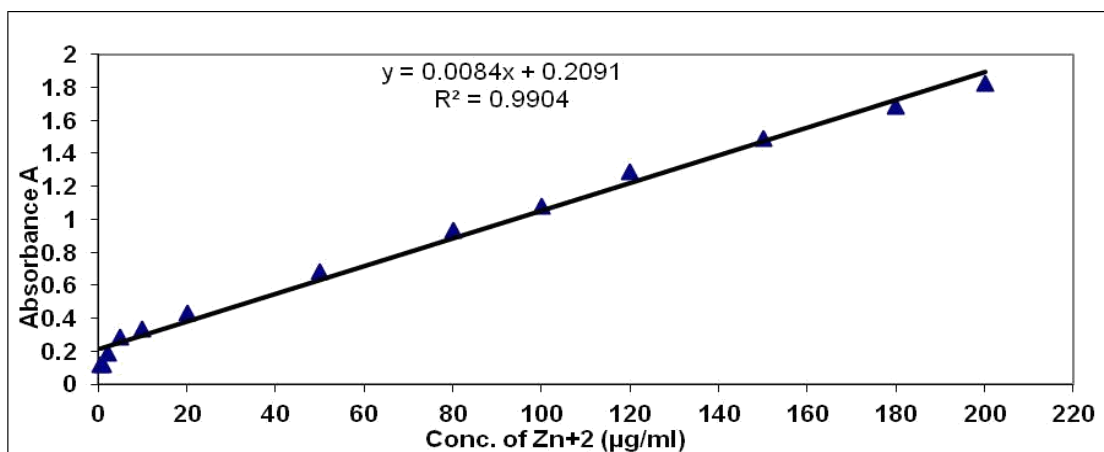


Figure 7: The standard curve of Zn^{+2}

Table 1: incidence of different types of gallstone in relation to age and sex

Age group (years)		Type of gallstone					Total gallstone
		Yellow stone	Green stone	White stone	Black stone	Brown stone	
11-20		3		1	1		5
21-30		9	6	3	2		20
31-40		13	4	2	4	1	24
41-50		10	6	6	1	1	24
51-60		11	5	1	2	1	20
61-70			2	2		2	6
71-80						1	1
Total	Men	6	4	1	2		13
	Women	40	19	14	8	6	87
		46	23	15	10	6	100

Table 2: Analytical data for determination of Fe, Zn in Gallstone

Statistical analytical factors	Values		
	Iron	Iron II	Zinc
Linearity rang ($\mu g.ml^{-1}$)	1 – 260	2 – 300	2-200
Linear equation	$A=0.0034[C]+0.1057$	$A=0.0057[C]+0.1045$	$A=0.0084[C]+0.2091$
Slope ($\Delta A/\Delta C$)(m)	0.0034	0.0057	0.0084
Intercept standard error	0.0090	0.0012	0.0014
Correlation coefficient (R^2)	0.9939	0.9976	0.9904

Intrcept standard deviation			0.028			0.037			0.0044		
Percentage linarity (R^2 %)			99.390			99.760			99.040		
Correlation coefficient	Zn	Fe	Fe ⁺²	Zn	Fe	Fe ⁺²	Zn	Fe	Fe ⁺²	No.	
		yellow			yellow			Black			
(LOQ)	56.535	130.088	99.21	38.321	159.075	127.807	177.369	94.205	12.543	1	
(LOD)	96.892	95.97	88.684	43.44	113.911	100.964	177.607	116.264	7.105	2	
	108.797	83.911	66.578	162.607	46.558	37.807	94.273	96.264	16.228	3	
Relativ	91.773	198.323	83.421	38.559	87.147	56.578	175.107	116.558	17.105	4	
Calculated value	69.154	93.029	85.701	173.678	70.676	43.245	88.797	121.558	22.543	5	
			109.73								
	85.702	127.147	6	162.964	85.382	67.631	121.178	113.029	17.631	6	

153.321	260.088	13.245	110.94	125.088	115	166.178	95.382	11.491	7
162.964	142.441	32.192	130.226	94.5	79.912	178.559	174.5	66.929	8
45.464	124.794	99.035	44.154	153.323	99.21	118.202	175.088	50.438	9
151.416	88.911	80.087	43.083	216.558	129.385	137.369	156.264	71.491	10
107.25	138.911	122.192	44.154	99.794	13.245		Green		
96.892	138.029	120.263	45.345	14.794	7.807	39.273	95.576	92.719	11
	brown		91.892	142.441	19.912	103.916	105.088	100.807	12
100.464	182.441	115.526	8.678	107.147	60.263	116.416	186.558	128.684	13
107.369	129.205	90.614	150.226	121.264	86.929	157.964	251.264	115	14
118.321	151.558	95.175	90.464	112.441	107.631	49.75	124.794	88.684	15
143.559	120.97	110.263	72.488	96.558	79.035	146.059	135.088	124.473	16
175.464	113.029	6.052	45.107	25.676	23.596	38.083	158.911	137.456	17
171.059	180.676	115.877	102.726	58.323	35.35	63.202	215.97	127.105	18
	White		77.488	93.029	57.982	63.678	166.558	103.421	19
17.845	75.97	65.175	121.654	118.911	96.754	92.964	145.97	109.035	20
65.821	50.088	39.736	42.25	90.088	64.298	101.178	142.735	125.175	21
90.107	83.911	50.087	98.083	49.794	37.631	54.75	133.911	110.789	22
84.511	129.205	99.736	127.488	104.205	63.245	175.464	146.264	125.701	23
94.273	118.911	106.228	81.178	145.97	8.798	39.154	107.735	104.473	24
110.94	45.676	45.35	68.797	87.735	60.614	44.273	177.735	109.561	25
89.392	148.911	108.333	156.416	121.852	86.929	173.797	105.382	95.701	26
93.083	33.911	29.385	157.369	103.323	94.298	48.559	154.205	83.421	27
94.511	112.735	48.859	77.488	162.441	98.333	122.726	142.147	89.035	28
182.369	92.147	80.614	176.535	93.617	84.824	84.511	135.97	120.614	29
112.369	53.617	2.017	162.964	100.97	93.245	116.535	114.794	80.263	30
121.654	133.029	126.929	45.345	143.323	120.263	105.94	174.205	102.368	31
91.773	25.676	151.14	121.654	132.441	116.754	156.178	113.029	107.982	32
44.273	80.676	65.877	133.559	120.676	98.333	144.154	140.088	27.28	33
34.75	57.735	36.052							34

References

- 1- Doherty GM, Way LW,(Current Surgical Diagnosis & Treatment), TheMcGraw-Hill Companies,12th ed, 2006
- 2- Jaraari AM, Peela J, Trushakant NP, et al .(Quantitative analysis of gallstones in Libyan patients). Libyan J Med. 2010; 5: 9672-9633
- 3- Chandran P, Kuchhal NK, Garg P, et al. (An extended chemical analysis of gallstone). *INDIAN J CLIN BIOCHEM*,2007;22(2):145-50.
- 4- Pundir CS, Chaudhary R, Rani K, Chandran P, Kumari M, Carg P.(Chemical analysis of biliary calculi in Haryana), *Ind. J. Surg.*2001, 63(5), 370-373
- 5- Rautary TR, Tapash R, Venkatathri. (Analysis of Indian cholesterol Gallstones by Particle induced x-ray emission and thermogravimetry derivative) *Eur J Gastroenterol Hepatol*. 2006;18(9):999-1003.
- 6- Wang Q, Zhang H, Ouyang J. (Progress in the study of application of modern instruments to investigation of gallstone).*Guang Pu Xue Yu Guang Pu Fen Xi*. 2007;27(1):202-6
- 7- Leopold, GR.; Amberg, J.; Gosink, BB. et al (Gray scale ultrasonic cholecystography: a comparison with conventional radiographic techniques), *Radiology* 1976,121(2):(445–8).

- 8- Verma GR, Pandey AK, Bose SM, Prasad R. (Study of serum calcium and trace elements in chronic cholelithiasis). ANZ J Surg. 2002;vol 72:issu8 , 596-999.
- 9- Lee Hooper, Asmaa Abdelhamid, Helen J Moore, Wayne Douthwaite, C Murray Skeaff, Carolyn D Summerbell. (Effect of reducing total fat intake on body weight: systematic review and meta-analysis of randomised controlled trials and cohort studies). BMJ 2012;345:7666.
- 10- P.Chandran, N. K. Kuchhal, P. Garg and C.S. Pundir, (An ExtendedChemical Analysisof Gallstone) ,Indian Journal of Clinical Biochemistry, 2007 / 22 (2) ,pp145-150.
- 11- Sigismund Lasocki , Thomas Gaillard and Emmanuel Rineau, (Iron is essential for living), Critical Care 2014, 18:678, p1-2.
- 12- Kuljeet Kaur, Rajiv Gupta, Shubhini A. Saraf, and Shailendra K. Saraf, (Zinc: The Metal of Life), Comprehensive Reviews in Food Science and Food Safety.2014 ,Vol13, pp 358-376.
- 13- Pratik S. Mehta and Vandana B. Patel, (Spectrophotometric method for determination of Fe(II) and Zn (II) in Multi vitamin soft gel capsule),International journal of pharmaceutical research and analysis ,2012 , 2,(2),87-91.
- 14- Harris, 7th ed., Chap. 18. Skoog, West, Holler and Crouch ,(Spectrophotometric determination of Fe in Drinking Water) ,7th ed., Chap 23,pp. 594-601.
- 15- Rains AJH.(1964). Gallstones. Heinemann Monography. 1st ed
Roitt,IBrostoff ,J and Male, D. (2001). Immunology 6th ed.W.B Saunders
- 16- Horn G. (Observations on the aetiology of cholelithiasis).Bri.1965,29(2),732-737
- 17- Moore EW. (The role of calcium in the pathogenesis ofgallstones. Hepatology),1984,4(2):228-243.
- 18- Al-khajeji F. A .(Bacteriological and Clinical study of gallbladder stones and biles after cholecystectomy) .Msc. Thesis. Babylon university. Collage of medicine,(2006).
- 19- Jayanthi V ., Palenivela U. C., Prasanthi R., Mathew S.,and Srinivason V. (Composition of gallstones in Coimbatore District of Tamil Nadu Sate). Ind.J. Gastroenterol.1998,17(4):134-135.
- 20- Bedirli A, Sakrak O, Sözüer EM, et al. (Factors effecting the complicationsin the natural historyofacutecholecystitis)Hepatogastroenterology,2001, 48(41):1275-1278.

The Impact of Basketball Education Program according to the patterns of FARC on some of the kinetic characteristics of slow learners (6 - 8) years

Alikohman Alwan¹, Riadjumaa Hassan¹, Abdulhussein Majed Mohammed¹

1. College of Physical Education and Sports Sciences, University of Al-Qadisiya,

Iraq *Corresponding author Email: handball.2014@yahoo.com

Abstract

A slow learner child learn in the same way as normal children (they learn by using their previous experiences), but they need more time and effort from school, home and the teacher has to choose the best method for teaching material. FARC Patterns of Modern Learning research aims to apply this experimental research sample and to identify the educational effect of basketball on some of the kinetic characteristics of the students (6-8 years). The sample of the research was determined by the slow learners in Al Rajaa institute of mental disable in Qadisiyah governorate with 35 students. It was concluded that the practice of sports activity according to the FARC sensory - visual style in the institute instills the spirit of teamwork and the participation of others and thus develops kinetic qualities. The researchers recommended to adopt the modern and constant patterns derived from the slow learnerschild environment which is commensurate with his abilities and abilities.

Key words: FARC, slow learners, basketball, educational effect

How to cite this paper: Alwan A, Hassan R, Mohammed AM (2019): The Impact of Basketball Education Program according to the patterns of FARC on some of the kinetic characteristics of slow learners (6 - 8) years, Ann Trop Med & Pub Health; 21: SP2042-19

Definition of research:

1.1: Introduction and Importance of Research:

Caring for children with special needs is a social responsibility whose functions should be entrusted to public institution, private sector, as well as civil society institutions,

which stems from the legitimacy of their right to integrate into society and to have equal opportunities with others in all life fields and contribute to the provision of conditions appropriate to launch their capabilities, in particular people with special needs generally have available capabilities and latent potential that could be made available through training, rehabilitation, practice and patience that must begin from the family through educational institutions rehabilitation, training and operating to reach various aspects of society.

It has become clear to everyone that the process of development of motor qualities in humans has become a matter of modern science concerned with close relationship between them and the development of man. Therefore, this attention requires full preparation from the young. Kinetics are an important topic which is related to the performance of the human motor to do research and studies to reveal the best ways to reach the human to adjust its kinetic potential to the fullest.

By considering childhood as an important stage which is passed by children with disabilities a stage that is susceptible to various factors which surround him and thus leave an impact either to be positive or negative on his future life, and because of the importance of the early years in the formation of this character which requires educational institutions to take an active role in care and embrace and attract the largest number of children

especially slow learning in private institutes as one of the first educational institutions

And the basic structure that contributes to the building of society, where they contribute effectively to child-rearing according to the principles and methods of psychological, educational, health and FARC style one of the modern methods of learning after the classification of students according to the nature of reception and preference sensory.

Hence, the importance of research in identifying the impact of basketball education program according to FARC patterns on some of the kinetic characteristics of slow learning (6-8) years.

1-2: Research problem

The motion activity is an integral part of a slow-learning life, and the development of basic movements and growth have a basis in developing their future lives.

The interest in slow learning methods, especially mobility, leads to the completion of the link between the most sensitive stages in human life, which is the beginning of its inception and the later stages of life and vice versa.

The success of these curricula and activities depends on the type of activity practiced - the specialized staff - the place and the equipment. All of these can be referred to as the job competencies of the institutes of special needs and their programs. This study is concerned with the extent of these activities and activities in the development of some kinetic characteristics in children who entered the Institute of Raja, so the researcher formulated the problem of his research with the following question:

Is that approach basketball according to the sensory preference and the activities in which it is practiced have a tangible effect some of the kinetic qualities in children, thus finding answers in logical terms and scientific give clear indication and adequate answer to such a question.

1-3: The Research Objective:

Learning about the effect of an educational program on basketball according to FARC patterns on some of the kinetic characteristics of slow learning (6 - 8) years.

1-4 The Search hypothesis

There are statistically significant differences between the pre-test and post-test samples in the program implemented by the researcher.

1-5: The Research Areas:

1-5-1 Human Field: First and second graders at the Raja Institute for Mental Disability in Al-Qadisiyah Governorate for the academic year (2017-2018).

1.5.2. The Time domain: The period from 6/3/2017 to 10/5/2017

1-5-3 The spatial field: the squares and the sports hall at the Institute of mental retardation Al-Qadisiyah province.

3. The Research methodology and field procedures

Research Methodology:

The researchers use the experimental method in the form of two parallel samples with tribal and remotetests.

3.2 The research community and its sample :

The research community are students of the Institute of Mental Retardation for the academic year 2017-2018 and those between the ages of 6-8 years.

The size of the research community (35) students and excluded a group of students from the research community of (15) students for the pilot experiment as well as students who are not committed, and therefore the number of the study sample (20) students and divided by lot on two experimental groups after all children chose this pattern through their desire and the

questionnaire distributed to them by the FARC patterns and a group of female officers who practiced in the method of the methodological institute.

3.2.1 Homogeneity and equivalence the members of the research community

The researchers conducted a homogeneity and equivalence of members of the research community as shown in Table (1)

Table (1)
Shows the homogeneity and equivalence of the control and experimental groups

No.	Variables	First experimental group			Second experimental group			T value	Significance
		middle	deviation	Sprain	middle	deviation	Sprain		
1	Age	7.63	0.52	-0.64	7.38	0.92	-1.00	0.67	Moral
2	Weight	47.25	0.89	-0.62	47.38	0.92	-1.00	0.28	Moral
3	Length	145.38	2.92	-0.86	145.88	2.59	-0.94	0.36	Moral
4	Speed Test(Running 25 m)	8.5	0.41	-0.82	8.63	0.50	-0.64	1.34	Moral
5	Broad jumping of stability	.78	0.21	0.07	.79	0.22	0.07	0.00	Moral
6	Hockey Throwing Test	9,9	0.43	-0.58	9,8	.42	0.07	1.01	Moral
7	The test of sitting from lying down (30) seconds	9.9	1.3	-0.90	10	1.4	-0.26	0.47	Moral

&(T) tabular value = 1.734 at an indication level (0.05)

3.3 Means of gathering information

- Arab and foreign references. -the exams.
- Assist team to carry out the tests. *
- Test vocabulary and registration form.
- Measurement of length and weight (measuring tape - medical balance).
- Stopwatch number (2) - tennis ball.

3.3.1 Tests used (1)

The researcher used the Hibleneck and Bormes test to measure motor performance, a Belgian test.

Test 1: Speed Test (25m)

Start from the high stand position.

Time is measured up to the fraction of a second.

Give one try.

The second test: the board jumping of stability

The pupil jumps together with the feet together behind the starting line.

Allows the arms to swing backwards.

The distance between the start line and the end of the heels is measured on the landing.

The distance is measured to the nearest centimeter.

Two attempts are given and the best attempt is calculated.

Third Test: Hockey Throwing Test

No running or approaching space is allowed.

The distance from the starting line is measured to where the ball first falls to the nearest centimeter.

Two attempts are given and the best attempt is calculated.

The fourth test: the test of sitting from the bowel (30) seconds

The test results from the position of the backbone with the back of the neck. The knees are bent so that the soles of the foot are on the ground with the joint of the foot joint with the help of a colleague. The correct number of times is calculated in only 30 seconds.

The knee should be touched at the elbow each time.

• Supporting team:

1. Ali Salam Al-Oussi Master student –college of Physical Education and Sports Sciences - Al-Qadisiyah University.

2. Lecturer. Majed Abdel Hamid PhD student - college of Physical Education and Sports Sciences - Al-Qadisiyah University.

3. Lecturer Ahmed Ghalab Master –college of Physical Education and Sports Sciences - Al-Muthana University.

(1) Amin Anwar Al-Khouli, Osama Kamel: Motion Education, Cairo, Dar Al-Fikr Al-Arabi, 1983 p.414

It is not allowed to leave the soles of the foot of the ground.

3 - 5 scientific tests for the test:

The researcher sought to adopt the scientific bases in the process of standardization of the tests, although they are standardized tests for the purpose of determining the extent of the science of these tests selected, A good test must have scientific foundations

Which is the stability, honesty and objectivity, and the results were as follows:

3.5.1 Honesty:

The researcher relied on the validity of the test used the content (s) are required to display the test on a number of specialists in sports science and special needs, they referred to the validity of the test with the approval of all experts

3.5.2 Stability :

The researchers used the test and retest method to find the stability coefficient, the test method was used to find the measurement by applying the kinetic tests on 4/2/2017 at 9:00 am on the playground of the Al-Rajaa Institute of Mental Retardation in Qadisiyah governorate on a sample of (5) students from the same sample. The tests were re-applied on the same sample and under (12/2/2017) at 9 am, ie, seven days after the first test, and created the simple correlation coefficient (Pearson correlation) for the test scores as shown in Table (3).

3.5.3 the Objectivity Tests :

The tests were carried out under the supervision of two neutral arbitrators, with the same conditions and the method of testing. The results were collected and then treated statistically. The correlation coefficient was calculated and the correlation coefficient values were high, This means that all tests are highly objective and as shown in Table (2).

- Prof. Dr. Jameel Kadhim Jawad
- Asst. Prof. Dr. Mushtaq Abdul Radha

Table (2)
The value of the coefficient of stability and objectivity of the tests

Tests	Variables	Stability	Subjectivity
Motion capacities	Speed Test (25m)	0.82	0.88
	Board jumping of stability	0.82	0.81
	Throwing hockey	0.82	0.89
	The test of sitting from lying down (30) seconds	0.92	0.89

3.6 Exploration Experience:

In order to identify the problems and difficulties facing the research during the application of the tests, and to determine the time period required by the test, a pilot experiment was carried out on 6/3/2017 at the Raja Institute for mental retardation and classification of the sample according to the FARC questionnaire form (2) Per student in the sample (which was filled by the Institute's supervisors).

After collecting the results of the questionnaire, most of the sample preferred the visual sensory style and the second sensory method. The sample was divided into two experimental groups and according to the above two types. Therefore, the emphasis in his exercises and his methodology was considered to increase the visual and auditory model for each experimental group.

3.7 Field research procedures:

3.7.1 The Pre-Test:

The pre-test was conducted on the sample of the research and the number of (20) students on 8/3/2017 variables (under research) for the purposes of homogeneity between the same group and equivalence.

3-7-2 Educational program :

Objective of the program:

- Development of the kinetic characteristics of the research sample.

Through the extensive readings of the scientific references that dealt with the nature and characteristics of people with special needs class (slow learning) and their needs and abilities to practice motor activity and the use of studies that have been implemented programs in this category and through the consultation of experts in this field and the exploratory experiment and its results that include modules curriculum:

The implementation of the educational curriculum (8) weeks with (16) educational units and the existence of (2) units of education per week and time (45) minutes per unit of education divided as follows:

Preliminary part (5 s):

It aims to prepare and prepare the students psychologically and activate the circulatory system, which is a collection of simple movements represented by Swedish warmth.

Main part (35 Q): It has two parts

A - Explain one of the basic skills selected basketball and practical application with the help of children as a model and display skill Fdoa and pictures:

B - the performance of students to skill with continuous guidance and participation of the researcher and the team to perform the full skill and continuously:

The closing part (5 s):

Aiming to prepare the sample members to return to normalcy and return them to a state of relaxation and return to their ranks.

3.5.3 - Post-tests

The researcher conducted the post-tests after the completion of the implementation of the educational curriculum and training for one day in the same sequence on Sunday and Monday, 10/5/2017

The researcher was concerned that the conditions in which the tests were carried out are similar to the conditions in which the tribal tests were conducted, as mentioned above. They are similar in terms of place, time and method of testing.

2-8 Statistical means:

The researcher used statistical bag spss to address data statistically "

4- Present and discuss the results

4.1 Presentation and analysis of results

Table (3)

The differences between the averages of the pre and post measurements show the first experimental group in the variables under consideration

No.	Statistical treatments Variables	Pre measurement		Post measurement		T value Secluded	Significance
		S	+_A	S	+_A		
1	Speed Test (25m)	8.5	0.41	7.5	0,46	2.4	Moral
2	Board jumping of stability	0.78	0.21	1.5	1	2.59	Moral
3	Throwing hockey	5	0.43	11	1	2.56	Moral
4	The test of sitting from lying down (30) seconds	9.9	1.3	13.7	1.5	2.4	Moral

* Tabular value (t) at a significance level of 0.05 and a degree of freedom (9) = 2.26

On the variable kinetic tests it has shown the result of statistics , there is a difference between post-test and pre testing in computational circles for the four kinetic tests, the researcher attributes this to the thrill repetition and facilitation of skill, as well as the development of the sample to educational units that were rich in visual learning tools And the model and encouragement where the enthusiasm of the sample was increased

of their learning effectiveness and race among the sample members , the award was awarded to the winner of the impact of the great and in this confirms (Nader Fahmy)

child promotion is an important point which indicates a positive trainer when implementing any successful program. (1)

Alaa Abdul Baqi also mentioned " . The child with a mental disability needs to constantly repeat performance so that the child can absorb the multiple skills. "(2)

Table (4)

The significance of the differences between the averages of the tribal and remote measurements shows the second experimental group in the variables under consideration

No.	Statistical treatments Variables	Pre measurement		Post measurement		T value Secluded	Significance
		S	A	S	A		
1	Speed Test (25m)	8.5	0.41	8.2	0.9	2.43	Moral
2	Board jumping of stability	0.78	0.21	0.88	0,94	3.4	Moral
3	Throwing hockey	5	0.43	7	0,69	2.65	Moral
4	The test of sitting from lying down (30) seconds	9.9	1.3	10.1	0,95	3.84	Moral

* Tabular value (t) at a significance level of 0.05 and a degree of freedom (9) = 2.26

Table (4) shows that there are significant differences in all search variables and that the differences for the benefit of telemetry on the variable of kinetic tests showed that the results of the statistics there is a difference between the tribal test and the remote in the arithmetic and kinetic tests and the researcher attributed this to the program of researcher and method in Exercise, repetition and exercise during the exercise of the FARC sound pattern in the repair and pumping of the sample by speech and return.

-
- (1) Nader Fahmi Zayyud: **Education of Mentally Retarded Children** ", 3prining , Dar Al-Fikr Al Arabi, Publishing and Distribution, Jordan, 1995, p. 104.
 - (2) Alaa Abdul Baki Ibrahim: **A training program for children with mental disabilities**, college of Education, Ain Shams University, I 1,1993, p.11

Table (5)

The significance of the differences between the averages of the dimension measurements shows the first experimental and experimental groups in the variables under study

No.	Statistical treatments Variables	Pre measurement		Post measurement		T value Secluded	Significance
		S	A	S	A		
1	Speed Test (25m)	7.5	0,46	8.2	0.9	2.35	Moral
2	Board jumping of stability	1.5	1	0.88	0,94	2.76	Moral
3	Throwing hockey	11	1	7	0,69	2.2	Moral
4	The test of sitting from lying down (30) seconds	13.7	1.5	10.1	0,95	2.87	Moral

* Tabular value (t) at a significance level of 0.05 and a freedom degree of 18 = 2.10

Table (5) shows the computational and standard deviations of the two tests for the kinetic tests of the four test skills of the experimental groups. The value of t is calculated to be greater than the scale of 2.10 at the level of 0.05 and the freedom level of 18) For all kinetic skills, the significance of differences is significant for the first experimental group

- The researcher believes that the reason for this is due to the nature of visual motor activity practiced in the approach followed by the researcher compared to the other sample that the researcher used the pattern of hearing to convey information, as the activity of visual movement is the best representative of motor motives that lead to the development of some of the motor characteristics and It is one of the factors that are affected by the child, where he returns to running, playing and the continuous movement because the child's contact with the environment in which he lives on a regular basis makes him plenty of time to practice various activities so that his movement is abundant and therefore has a great motivational motivation cannot achieve Its playing it alone.

- In addition, he believes that through the exercise of continuous visual activity by children, the movement of the arms with the movement of the two legs is consistent and in a larger area where jogging takes its normal position , it is automatic and hence the velocity potential develops clearly and this development remains low when the setting is ineffective. (1)

- The researcher believes that the reason for the progress of the sample of the sample is the pattern of sight in the tests of jumping from the stability of the consistency between the arms movements and the two men obtained by this sample, which resulted from the exercise of many movements which is characterized by complex performance through their many observations and imitation of the model and this is what pointed out .

Some references , where Dr. Farida Ibrahim Othman said that "the activities practiced by the child increase the confidence and proficiency of some skills(1)

- In addition, the great correlation between speed and jump from the stability that the source of these two qualities are the muscles of the legs is one of the reasons for the progress of the sample of experimentation in these tests which is confirmed by (Mainl) that the development is fast in the jump when the child gets the appropriate learning because children are doing jumping big desire (2).

5. Conclusions and recommendations

5.1. Conclusions:

In light of the results of the tests, analysis and discussion, the researcher reached the following conclusions:

1. There are statistically significant differences between the first and second groups in the tests of motor characteristics and for the benefit of the experimental group.
2. The sports curriculum and activities have a positive impact on the development of motor skills in children
4. The practice of sports activity according to FARC visual sensory style inside the institute instills the spirit of teamwork, participation of others, and thus develops and develops kinetic qualities.

5.2 Recommendations:

2. Adopting modern and constant patterns derived from the child's slow learning environment and which are commensurate with his abilities and abilities, especially visual.
- 3 - To prepare all the supplies (hardware - tools - halls) for the activity within the Institute in addition to increasing the use of drawings and images to develop the abilities of the child kinetic.
4. Extensive studies (in different learning styles) for students of special needs institutes. 5 -
To find standard scores for tests of motor characteristics and age (6-8) years in Iraq
6. Paying attention to cadres supervising institutes of special needs, taking into account the strict specialization in recruitment.

(1) Hassan Sayed Moawadh - Methods of Teaching Physical Education - Cairo - 1982 (p. 47)

(2) Wajih Mahjoub - Movement science - motor development from birth to old age - I 2 - Baghdad - 1987 (p. 101)

(3) Amin Al-Khuli -Ibid – p136

(4) Farida Ibrahim Othman - Education of the kinetic stage of kindergartens and primary stage - Kuwait - Dar Al-Qalam - 1989 (p. 47)

(5) Kurt Maynell - Ibid - p. 195

References

1. Ahmed Abdul Rahman Al-Sarhid - Farida Ibrahim Othman - The scientific foundations of motor education and its applications for kindergartens and primary stage - Kuwait - Dar Al-Qalam - -1printg - 1993.
2. Assama Kamel Rateb - The Growth of Movement in Childhood and Adolescence - Cairo - Dar Al-Fikr Al-Arabi - printing 2 - 1994.
3. Amin Al-Khouli - Osama Kamel Rateb - Kinetic Education - Cairo - Arab Thought House - 1983.
4. Hamed Abdel Salam Zahran - Growth Psychology (childhood and adolescence) - Cairo - World Books - 1995.
5. Hassan Sayed Moawad - Methods of Teaching Physical Education - Cairo - 1982.
6. Toukan Obeidat - Abdul Rahman Adas - Scientific Research - Jordan - Dar Al Fikr Publishing and Distribution - 1988.
7. Samira Talib Sultan - The Effect of Proposed Curriculum for Motor Education in Some Basic Movements of Riyadh Children (4-5 years) in Basrah Governorate - Unpublished Master Thesis - Basrah University –college of Physical Education - 2005.
8. Shabel Badran - Recent trends in the education of pre-school children - Lebanese Knowledge House-2000.
9. Adnan Khalaf al-Jubouri et al. - Basic Principles in Teaching Physical Education - Dar Al-Kutub - 1986.
10. Afaf Abdel-Karim - Programs of mobility and teaching for young people - Knowledge facility in Alexandria - 1995.
11. Omar Al-Toumi - The development of theories and educational ideas - Beirut - House of Culture - 1981.

12. Freida Ibrahim Osman - motor education for kindergarten and primary stage - Kuwait - Dar Al-Qalam - 1989.
13. Qasem Hassan Al-Mandalawi - Ahmed Saeed - Mathematical training between theory and practice - Baghdad - 1976.
14. Qasem Hassan Hussein et al. - Theories of Physical Education - Baghdad - University Press - 1979.
15. Kort Manil - Motor Learning - Translation (Abdel Ali Nasif) –printing 1 - University of Mosul - 1980.
16. Najemuddin Badran - Evaluation of the curricula of kindergartens from the point of view of teachers - Research Journal - Issue (6) - in 1989.
17. Mahjoob - Movement science: motor development from birth to old age - I 2 - Baghdad - 1987.
18. Yassin Yassin - Hassan Mohamed Ali Al-Obeidi - Statistical Applications and Computer Applications in Physical Education Research - Dar Al Kutab for Printing and Publishing - Mosul - 1999.

Annex (1)

Registration Form

Student Name:

Al-Rajaa Institute :

Height Weight

No.	Test	Achievements	Units
1	Speed Test (25m)	-----	Stability
2	Board jumping of stability	-----	To nearest cm.
3	Throwing hockey	-----	To nearest cm.
4	The test of sitting from lying down (30) seconds	-----	No. of times in (30) seconds

The tested name :

Date :

Signature :

FARC Questionnaire Annex (2)

- Choose the answer that explains your best choice. You can choose more than one answer if the one answer does not match your perception. Leave the question that does not apply to you unanswered.

- You have to choose food in a restaurant or café. You are:
- Choose from the description in the meal list.
- You choose something you ate before.
- Listen to the waiter or ask your friends to make choices.
- Look at what others eat or look at the food.
- Preferred teacher or provider who uses:
- Questions, newly asked questions, a group discussion, or hosting a speaker.
- Worksheets, books, or readings.
- Graphs, charts, or graphic maps and images.
- Offers, models, or application sessions.
- You are going to attend a special feast for your family. You can:
- Cook something you know without the need for instructions.
- Ask friends to give you suggestions.
- Consider the cookbook to take ideas from the pictures.
- Use a cookbook where you learn that there are good recipes.

Detection of *tox*A gene in clinical isolates of *Pseudomonas aeruginosa* using real time PCR

Sana MH AL-Shimmary^{*}, Neam Hadier Jawad^{*}, Taibat Adnan Wahhab^{*}

^{*}College of Science for Women, University of Baghdad

^{*}Corresponding author Email: Sana.m.alshimmary@gmail.com

Abstract

P. aeruginosa is an opportunistic pathogen that infects human, animals, plants. It causes many infections in patients with immunocompromised patients by surgery, burn, wounds, urinary tract infections, Keratitis and otitis external. It has many virulence factors which aid it to attack the host. The prevalence of these virulence factors are various among different infections. In this study, one hundred clinical isolates of *P.aeruginosa* were collected from burns, wounds and urine infections in Baghdad hospitals from August 2018 to March 2019 from patients, their ages ranged between 20 to 45 years. By using real time PCR technique, rapid detection of *tox*A gene was performed. The results showed the prevalence of *tox*A gene of *P.aeruginosa* from wounds and burns was higher than from urine which may belonged to the mechanism of its action.

The aim of the study: to estimate the presence of *tox*A gene of *P.aeruginosa* isolates from different sources

Keywords: *Pseudomonas aeruginosa*, *tox*A gene, real time PCR

How to cite this paper: Al-Shimmary S, Jawad NH, Wahhab TA (2019): Detection of *tox*A gene in clinical isolates of *Pseudomonas aeruginosa* using real time PCR, Ann of Trop Med & Pub Health; 21: 2043-19

Introduction

One of the gram negative bacterium which represents a ubiquitous microorganism and could be alive in different environments like hospitals is *Pseudomonas aeruginosa* [1]. There are various origins for this bacterium like toilets, sinks, equipment of respiratory therapy and even detergent solutions. *P.aeruginosa* are present in skin and intestine of some carriers [2]. It causes many infections like Folliculitis, keratitis and pneumonia in healthy and immune-deficient individuals [3]. This bacterium has diverse virulence factors that play role in bacterial toxicity and invasion [4]. One of these virulence factors is exotoxin A which is encoded by *tox*A gene. Exotoxin A represents a main member of type II secretion system (T2SS) for this bacterium [5]. This toxin has enzymatic activity and belongs to the family of mono-ADP ribosyl transferase so it is definite as NAD⁺-diphthamide-ADP-ribosyl transferase [6]. That inhibits synthesis of protein by ADP-robosylation of eukaryotic elongation factor 2 [5] and enables this bacterium destroy tissue for prevalence, nutrition supply and to rise its survival rate[6].

The expression of *P. aeruginosa* exotoxin A (PE) is complicated; many studies settled a relation between iron metabolism and PE expression. *tox*A gene. At the transcriptional level, is regulated by the products of *regAB* operon, so they are mediated by Fur-regulated alternative sigma factor, which had initially been identified as a positive activator for the

production of the siderophore pyoverdine (PvdS). After induction of the Ptac-pyds gene, the production of regAB and *tox*A was regulated in mature cell in the medium with high-iron, *P. aeruginosa* produces pyoverdine that chelate ions of iron in high affinity, so in the abundance of iron ions, pyoverdine was activates signaling pathwayfor regulation of PE expression, so the important factor that allows the colonization of *P.aeruginosa* in the host is the efficient uptake of iron. [6, 7]. Presence of exotoxinA may vary among various clinical isolates as referred by many studies like Khattab *et al.* (2015) and Nikbin *et al.* (2012)[8,9].

Materials and Methods:

Bacterial isolation

One hundred *P.aeruginosa* was collected from wounds (40 isolates), burns (40 isolates) and urine (20 isolates) from August 2018 to March 2019 from patients their age ranged between 20 to 45 years in Baghdad's hospitals. All isolates were tested by the conventional laboratory testsincluding cultural and biochemical tests then the identification was ensured by vitek2 tests.

DNA extraction

Genomic DNA of all isolates was extracted using Wizargenomic DNA purification kit (Promega, Madison, WI, USA). Depending on manufacturer's protocols, DNA extraction was performed.

PCR method

By using specific primers for *tox*A gene of *P .aeruginosa* (table 1), real time PCR was performed by using Smart cycler Real-Time PCR from Cephied (USA).

Table 1: PCR Oligonucleotide Primers

tox A gene	Sequence 5'→3'	Product	Reference
Forward primer	CTGCGCGGGTCTATGTGCC	270bp	[10]
Reverse primer	GATGCTGGACGGGTCGAG		

Real time PCR was performed to detect this gene in *P.aeruginosa* in total volume 25 µl consisting of 12.5 µl of PCR premix,2 µl of each primers, 2 µl of the extracted DNA,1 µl of SYBR green dye and 5.5 µl of deionized water, the PCR mixture was transported to smart cycler to start the reaction according to the program designed to amplify the *tox*Agene (shows in table 2)

Table (2) Amplification Program of *tox*A gene

Stage	Temperature(time)	cycles
Stage 1	95°C (900 sec.)	1 cycle
Stage 2	95°C (15 sec.)	40 cycles
	62°C (30 sec.)	

Results and Discussion

All collected isolates which gave positive results with Vitek 2 tests, were assessed by DNA extraction then the products checked by gel electrophoresis (figure 1).

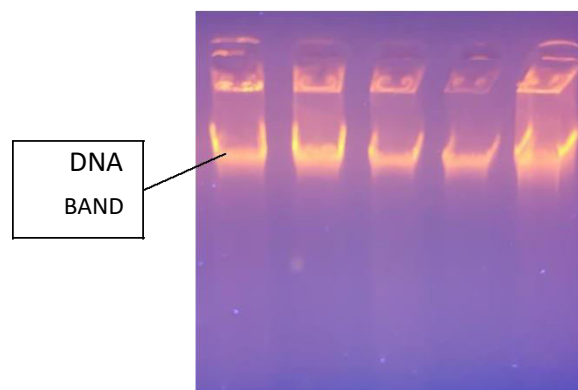


Figure 1: agarose gel electrophoresis of genomic DNA using 0.8% agarose, TBE (1x) and visualized by ethidium bromide stain

In this study, all isolates were checked for the prevalence of *tox* A gene in *P.aeruginosa*, the results showed that only 37 isolates and 35 isolates from burns and wounds gave positive respectively while only 8 isolates from urine gave positive results (figure 2).

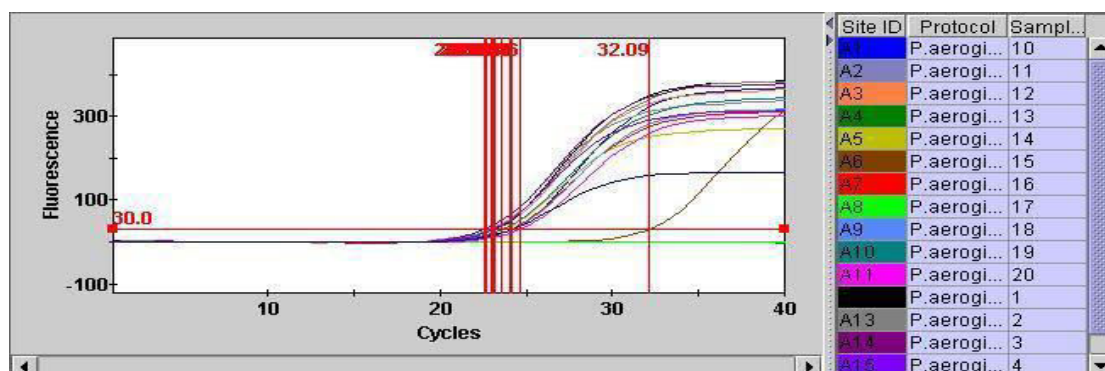


Figure2: Shows the results of some isolates in real time PCR technique depending on *tox* A gene using SYBR green dye in the detection

Pseudomonas aeruginosa has different virulence factors which help it to colonize in the host and cause community acquired and nosocomial infections worldwide [11]. These virulence factors include exotoxine A, exoenzyme S, alginate, phospholipase and elastase which are controlled by particular signaling systems[12].

In epidemiologic investigation, the main problem of health system in the world is the spread of highly virulent of *P.aeruginosa* [13]. Clinical results of *P. aeruginosa* infections rely on suitable response of the host and the virulence factors of bacteria that operate to weaken the host response[14]

In this study, the aim is to determine the prevalence of *tox*A gene in all isolates and with the other studies results. Presence of exotoxinA may vary among various clinical isolates as referred by many studies like Khattab *et al.* (2015) and Nikbin *et al.* (2012). Khattab *et al.*(2015)found that present of tox A gene in their collected samples was various, they showed that isolates from blood was significantly lower than that from burn and pulmonary tract[8]. Nikbin *et al.*(2012) found that the presence of *tox*Agene in isolates from pulmonary tract was significantly lower than from burn [9]. Yousefi *et al.*(2015)recorded that the frequency of *tox*A gene from wound isolates and other samples was significantly higher than from urine isolates [5]. These studies results were rather like our study results which show the prevalence of *tox*A gene in *P. aeruginosa* from urine is lower than from wounds and burns. Exotoxin A efficiently harm tissues and reducing the phagocytic activity ofimmunological cells (white blood cells)[15].

References

1. K.D. Mena, C.P. Gerba, Risk assessment of *Pseudomonas aeruginosa* in water. (2009). Rev. Environ. Contam. Toxicol. 201: 71-115.
- 2.E.A. Figueiredo, H. Ramos, M.A. Maciel, C. Vilar Mdo, N.G. Loureiro,R.G. Pereira, *Pseudomonas aeruginosa*: frequency of resistance to multiplerugs and cross-resistance between antimicrobials in Recife/PE.(2007). Rev. Bras. Ter.Intensiva. 19: 421-427.
3. S.D. Aaron, D. Kottachchi, W.J. Ferris, K.L. Vandemheen, M.L. St Denis,A. Plouffe, S.P. Doucette, R. Saginur, F.T. Chan, K. Ramotar, Sputum versus bronchoscopy for diagnosis of *Pseudomonas aeruginosa* biofilms in cystic fibrosis.(2004). Eur. Respir. J. 24:631-637.
4. I. Bucior, C. Tran, J. Engel, Assessing *Pseudomonas* virulence using host cells, Methods.(2014). Mol. Biol. 1149:741-755.
- 5.Yousefi-Avarvand, A., Khashei, R., Ebrahim-Saraie, H.S., Emami, A., Zomorodian, K. and Motamedifar, M. The Frequency of Exotoxin A and Exoenzymes S and U Genes Among Clinical Isolates of *Pseudomonas aeruginosa* in Shiraz, Iran. (2015). International journal of molecular and cellular medicine,4(3):167.
- 6.Michalska, M. and Wolf, P.*Pseudomonas* Exotoxin A: optimized by evolution for effective killing. (2015). Frontiers in microbiology, 6.
7. Ochsner, U.A., Johnson, Z., Lamont, I.L., -Cunliffe, H.E. and Vasil, M.L. Exotoxin A production in *Pseudomonas aeruginosa* requires the iron regulated *pvdS* gene encoding an alternative sigma factor.(1996).Molecular microbiology, 21(5):1019-1028.

8. Khattab, M.A., Nour, M.S. and ElSheshtawy, N.M. Genetic identification of *Pseudomonas aeruginosa* virulence genes among different isolates. (2015). J Microb Biochem Technol. 7:274-277.
9. Nikbin, V.S., Aslani, M.M., Sharafi, Z., Hashemipour, M., Shahcheraghi, F. and Ebrahimipour, G.H. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. (2012). Iranian Journal of Microbiology, 4(3):118.
10. K. Wolska, P. Szweda, Genetic features of clinical *Pseudomonas aeruginosa* strains. (2009). Pol. J. Microbiol. 58:255-260.
11. S. de Bentzmann, P. Plesiat, The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. (2011). Environ. Microbiol. 13: 1655-1665.
12. M. Alhede, T. Bjarnsholt, M. Givskov, *Pseudomonas aeruginosa* biofilms: mechanisms of immune evasion. (2014). Adv. Appl. Microbiol. 86: 1-40.
13. L.M. Deshpande, T.R. Fritsche, R.N. Jones, Molecular epidemiology of selected multidrug-resistant bacteria: a global report from the SENTRY Antimicrobial Surveillance Program. (2004). Diagn Microbiol. Infect. Dis. 49: 231-236.
14. A. Ben Haj Khalifa, D. Moissenet, H. Vu Thien, M. Khedher, Virulence factors in *Pseudomonas aeruginosa*: mechanisms and modes of regulation. (2011). Ann. Biol. Clin. Paris 69 (4):393-403.
15. Z. Hosseini Doust, T.G. van de Ven, N. Tufenkji, Evolution of *Pseudomonas aeruginosa* virulence as a result of phage predation. (2013). Appl. Environ. Microbiol. 79: 6110-6116.

Molecular Study of Resistant Microorganisms in Post-Operative Patients on Meropenem Treatment

Alli Ahmmmed Mahde Alanbage¹, Burooj M. Razooqi Al-aajem^{1*}

1. Department of Microbiology, College of Medicine, Diyala University, Diyala, Iraq.

*Corresponding author: E-mail: m.rburooj@yahoo.com

ABSTRACT

Introduction: Surgical site infection is defined as an infection that occurs within 30 days of the operation if no implant is left in place (or within 1 year if an implant is left in place after the procedure). It affects either incision or deep tissues at the operation site. These infections may be superficial or deep incisional infection or infections involving organ or body space.

Aim of the study: -to evaluate the efficacy of meropenem in treatment of wound infection and relation to microorganism's resistance. To detect resistance gene of microorganisms to this drug, reduce the incidence of wound infections after surgery,

Materials and Methods: The populations of this study included (80) patients with different age groups who attended Baquba Teaching Hospital in Diyala Province. The patients were suffering from post-operative wound infections. A questionnaire with a form was prepared for this purpose. This form includes information regarding age, sex, date, surgery type, site of surgery and duration, meropenem treatment duration. Swab samples were collected from patients during the period between November 2017 to June 2018.

Results: - The results of the present study showed that the total number of classes two days treatment 13(16.2%), were 8(16.7%) males within gender and 5(15.6%) females within gender. The result revealed that the number of sensitive and resistant bacterial isolate in patients have five days treatment was 6(75.0%) sensitive and 2(25.0%) resistant within classes for meropenem treatment duration. While the result revealed that the number of sensitive and resistant bacterial isolate in patients who had six days treatment was 4(80.0%) sensitive within classes for meropenem treatment duration. (10) resistant samples were amplified for the gene blaNDM, blaIMP, blaVIM, blaKPC and blaOXA-48. However, the amplification of these isolates showed that (5) contained blaNDM gene resistant.

Conclusion: -*Acinetobacter baumannii*, *Staphylococcus aureus* and *E. coli* were the most resistant microorganism to meropenem drug in Baquba Teaching Hospital. The gene resistant of *Acinetobacter baumannii* to meropenem treatment was (blaNDM).

Keywords: Meropenem, Post-Operative Patients, *ACINETOBACTER BAUMANNII*, blaVIM.

Introduction

Surgical site infection is defined as an infection that occurs within 30 days of the operation if no implant is left in place (or within 1 year if an implant is left in place after the procedure). It affects either incision or deep tissues at the operation site¹. These infections may be superficial or deep incisional infection or infections involving organ or body space². Postoperative SSI is the most common nosocomial infections in surgical patients. They lead to increased morbidity and mortality, prolonged hospital, and on average double the cost of medical care³. Studies on the magnitude of SSI are complicated by the heterogeneous nature of these infections. The prevalence and incidence rates of post-operative wound infections vary widely between procedures, hospitals, surgeons, patients and

geographical locations^{4, 6}. In Iraq. Studies have shown that the introduction of minimal invasive surgery like laparoscopic surgery has resulted in a decrease in incidence of SSI^{5,7}. Recently published papers reported the incidence of SSIs as 2.8% in laparoscopic and 4.6% in open appendectomies in Korea, and 7.2%, 6.2%, and 5.9% in Brazil, China, and Sweden, respectively⁸. A post-operative surgical site infection (SSI) is an infection of a wound from a surgery. Many micro-organisms live in and on our bodies and also in our environment. From endogenous flora of the patient's skin, mucous membranes, the air, soil or water⁹. The most commonly isolated bacterial pathogens are *S. aureus*, *Enterobacteriaceae*, Coagulase Negative Staphylococci (CoNS), *Enterococci* and *Pseudomonas aeruginosa*¹⁰. There is an increase the incidence of SSIs attributed to antimicrobial resistant pathogenic bacteria like methicillin resistant Staphylococcus aureus (*MRSA*)¹¹. A study at a University hospital in Iran, reported *S. aureus* to be the commonest bacteria pathogen (43%), followed by *Escherichia coli* (21%), *Klebsiella spp* (13%), *Pseudomonas* (10%) and CoNS (5%) among a surgical patients. In that study MRSA accounted for a high rate of 78.9% of all *S. aureus* isolates¹². Meropenem is a broad-spectrum carbapenem with high levels of activity against Gram-positive and Gram-negative pathogens. It is one of the most prescribed antibiotics for the empirical treatment of severe infections¹³. **Meropenem, like other β -lactam antibiotics**, binds to penicillin-binding proteins, disrupts bacterial cell wall synthesis, and causes death of susceptible microorganisms. The activity of Meropenem is excellent *in vitro* for a wide variety of aerobic and anaerobic microorganisms. *Streptococci* (including penicillin-resistant *S. pneumonia*), enterococci (excluding *E. faecium* and non- β -lactamase-producing penicillin-resistant strains), *Staphylococci* including penicillinase-producing strains¹⁴. The most common adverse effects are diarrhea (4 – 5 %), nausea and vomiting (1 – 4 %), injection-site inflammation (2 %), headache (2 %), rash (2 – 3 %), and thrombophlebitis (1%). With increasing resistance to many antibiotics worldwide, an understanding of the known mechanisms of resistance to meropenem is important to guide appropriate prescription in severe infections. Even though meropenem is reported to be poorly associated with the development of bacterial resistance *in vitro*¹⁵. **Like many other broad spectrum β -lactams**, resistance to meropenem, is becoming more common. Knowledge of resistance can assist the clinician to determine clinical scenarios when meropenem should not be commenced, or alternatively, should be ceased due to possible resistance development¹⁶.

Materials and Methods

The populations of this study included (80) patients with different age groups who attended Baquba Teaching Hospital in Diyala Province. The patients were suffering from post-operative wound infections. A questionnaire with a form was prepared for this purpose. This form includes information regarding age, sex, date, surgery type, site of surgery and duration, meropenem treatment duration, drug dosage and the presence of some risk factors that could be a possible predisposition for infection. Swab samples were collected from patients during the period between November 2017 to June 2018.

Age range from ≥ 1 year to ≤ 72 years, they were 48 males and 32 females.

Statistical analysis: -Data was analyzed statistically using SPSS 20.0. Chi-square and Fischer Exact tests were applied and **p-value ≤ 0.05 was considered to be statistically significant.**

The Scientific Ethical Committee in the College of Medicine, University of Diyala and Baquba Teaching Hospital was approved that the research proposal submitted by Assistant prof. Burooj Mohammed Razooqi Al-aajem, entitled (Molecular study of resistant microorganisms in post-operative patients on meropenem treatment). Under the scientific research with the necessary rules and regulations governing the ethics of scientific research, scientific ethical committee decided to approve the research project and give the ethical number (MD42 January 2016BMR).

Results

The study was conducted on 80 post-operative patients recumbent at Baquba Teaching Hospital from **November 2017 to June 2018. Their ages ranged between ≥ 1 years to ≤ 72 years, 48 were males and 32** were females. Most of them were subjected to wound infection, burn infection, diabetic foot infection, blast injury and abdominal surgery. In Table (1), the results illustrated the statistical variation to surgery duration according to gender. The results show that the classes of duration after surgery(1-5) days in males was 25 cases within gender (52.1%) and within classes of surgery duration(56.8%), while in females the duration after surgery was (19) cases within gender (59.4%) and within classes of surgery duration(43.2%). The present study showed that the classes of duration after surgery(6-10) days in males was 14 cases within gender was (29.2%) and within classes of surgery duration was (66.7%), while in females the duration after surgery was (7) cases within gender was (21.9%) and within classes of surgery duration was (33.3%). The results showed that the classes of duration after surgery(11-25) days in males was 9 cases within gender (18.8%) and within classes of surgery duration (60.0%), while in females the duration after surgery was (6) cases within gender (18.8%) and within classes of surgery **duration(40.0%)**. So, the result showed no significant differences at ($p \leq 0.05$).

Table (1): Statistical variations to surgery duration according to gender

		classes of surgery duration			Total	
		1 - 5 days	6 - 10 days	11 - 25 days		
Gender	Male	Count	25	14	9	48
		Expected Count	26.4	12.6	9.0	48.0
		% within Gender	52.1%	29.2%	18.8%	100.0%
		% within classes of surgery duration	56.8%	66.7%	60.0%	60.0%
	Female	Count	19	7	6	32
		Expected Count	17.6	8.4	6.0	32.0
		% within Gender	59.4%	21.9%	18.8%	100.0%
		% within classes of surgery duration	43.2%	33.3%	40.0%	40.0%
Total	Count	44	21	15	80	
	Expected Count	44.0	21.0	15.0	80.0	
	% within Gender	55.0%	26.2%	18.8%	100.0%	
	% within classes of surgery duration	100.0%	100.0%	100.0%	100.0%	

*P value = 0.750

The results of the present study showed that the total number of classes two days treatment 13(16.2%), they were 8(16.7%) males within gender and 5(15.6%) females within gender. Also the total number of classes three days treatment 23(28.8%), they were 17(35.4%) males within gender and 6(18.8%) females within gender. The results shows that the total number of classes four days treatment 14(17.5%), they were 9(18.8%) males within gender and 5(15.6%) females within gender. The results of the present study showed that the total number of classes five days treatment 10(12.5%), they were 3(6.2%) males within gender and 7(21.9%) females within gender. The results revealed that the total number of classes six days treatment 9(11.2%), they were 6(12.5%) males within gender and 3(9.4%) females within gender. Also the total number of classes seven days treatment 11(13.8%), they were 5(10.4%) males within gender and 6(18.8%) females within gender. So, the results **showed no significant difference at ($p \leq 0.05$)** is shown in Table (2).

Table (2): Statistical variations to meropenem treatment duration according to gender

*P value = 0.231

		classes for Meropenem treatment duration(day)						Total	
		Two days treatment	Three days treatment	Four days treatment	Five days treatment	Six days treatment	7		
Gender	Male	Count	8	17	9	3	6	5	48
		Expected Count	7.8	13.8	8.4	6.0	5.4	6.6	48.0
		% within Gender	16.7%	35.4%	18.8%	6.2%	12.5%	10.4%	100.0%
		% within classes for Meropenem treatment duration(day)	61.5%	73.9%	64.3%	30.0%	66.7%	45.5%	60.0%
	Female	Count	5	6	5	7	3	6	32
		Expected Count	5.2	9.2	5.6	4.0	3.6	4.4	32.0
		% within Gender	15.6%	18.8%	15.6%	21.9%	9.4%	18.8%	100.0%
		% within classes for Meropenem treatment duration(day)	38.5%	26.1%	35.7%	70.0%	33.3%	54.5%	40.0%
	Total	Count	13	23	14	10	9	11	80
		Expected Count	13.0	23.0	14.0	10.0	9.0	11.0	80.0
		% within Gender	16.2%	28.8%	17.5%	12.5%	11.2%	13.8%	100.0%
		% within classes for Meropenem treatment duration(day)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

In Table (3) the result was illustrated statistical variation to type of bacteria according to type of surgery. The results shows that the number of no growth cases was 7(43.8%) in wound infection, 6(40.0%) burn infection, 2(16.7%) diabetic foot, 4(57.1%) blast injury, 2(40.0%) abdominal surgery, 1(33.3%) testicular abscess and 10(45.5%) in others cases within type of surgery. The results revealed that the number of *Staphylococcus aureus* was 1(6.2%) in wound infection, 4(26.7%) burn infection, 6(50.0%) diabetic foot, 1(14.3%) blast injury, 2(40.0%) abdominal surgery and 2(9.1%) in others cases within type of surgery. The results of the present study showed that the number of *Acinetobacter. Spp* was 3(18.8%) in wound infection, 4(26.7%) burn infection, 2(16.7%) diabetic foot, 1(20.0%) abdominal surgery and 3(13.6%) in others cases within type of surgery. In the study the results showed that the number of *E. coli* was 2 (12.5%) in wound infection, 1(6.7%) burn infection, 1(8.3%) diabetic foot, 2(28.6%) blast injury, 1(33.3%) testicular abscess and

3(13.6%) in others cases within type of surgery. The results revealed that the number of other type of bacteria was 3(18.8%) in wound infection, 1(8.3%) diabetic foot, 1(33.3%) testicular abscess and 4(18.2%) in others cases within type of surgery. So, the results showed no significant difference at ($p \leq 0.05$).

Table (3): Statistical variations to type of bacteria according to type of surgery

			Type of bacteria					Total
			No growth	Staphylococcus aureus	E. coli	Acinetobacter . Spp	others	
Type of surgery	Wound infection	Count	7	1	2	3	3	16
		Expected Count	6.4	3.2	2.0	2.6	1.8	16.0
		% within Type of surgery	43.8%	6.2%	12.5%	18.8%	18.8%	100.0%
		% within Type of bacteria	21.9%	6.2%	20.0%	23.1%	33.3%	20.0%
	Burn infection	Count	6	4	1	4	0	15
		Expected Count	6.0	3.0	1.9	2.4	1.7	15.0
		% within Type of surgery	40.0%	26.7%	6.7%	26.7%	0.0%	100.0%
		% within Type of bacteria	18.8%	25.0%	10.0%	30.8%	0.0%	18.8%
	Diabetic foot	Count	2	6	1	2	1	12
		Expected Count	4.8	2.4	1.5	2.0	1.4	12.0
		% within Type of surgery	16.7%	50.0%	8.3%	16.7%	8.3%	100.0%
		% within Type of bacteria	6.2%	37.5%	10.0%	15.4%	11.1%	15.0%
	Blast injury	Count	4	1	2	0	0	7
		Expected Count	2.8	1.4	.9	1.1	.8	7.0
		% within Type of surgery	57.1%	14.3%	28.6%	0.0%	0.0%	100.0%
		% within Type of bacteria	12.5%	6.2%	20.0%	0.0%	0.0%	8.8%
	Abdominal surgery	Count	2	2	0	1	0	5
		Expected Count	2.0	1.0	.6	.8	.6	5.0
		% within Type of surgery	40.0%	40.0%	0.0%	20.0%	0.0%	100.0%
		% within Type of bacteria	6.2%	12.5%	0.0%	7.7%	0.0%	6.2%
	Testicular abscess	Count	1	0	1	0	1	3
		Expected Count	1.2	.6	.4	.5	.3	3.0
		% within Type of surgery	33.3%	0.0%	33.3%	0.0%	33.3%	100.0%
		% within Type of bacteria	3.1%	0.0%	10.0%	0.0%	11.1%	3.8%
	others	Count	10	2	3	3	4	22
		Expected Count	8.8	4.4	2.8	3.6	2.5	22.0
		% within Type of surgery	45.5%	9.1%	13.6%	13.6%	18.2%	100.0%
		% within Type of bacteria	31.2%	12.5%	30.0%	23.1%	44.4%	27.5%
Total		Count	32	16	10	13	9	80
		Expected Count	32.0	16.0	10.0	13.0	9.0	80.0
		% within Type of surgery	40.0%	20.0%	12.5%	16.2%	11.2%	100.0%
		% within Type of bacteria	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

*P value = 0.386

The results as in Table (4) revealed that the frequency of sensitive bacterial isolate was 3(33.3%) in wound infection, 7(77.8%) burn infection, 5(50.0%) diabetic foot, 2(66.7%) blast injury, 2(66.7%) abdominal surgery, 2(100.0%) testicular abscess and 7(58.3%) other cases within type of surgery. Also, the rate of resistant bacterial isolate was 5(55.6%) in wound infection, 2(22.2%) burn infection, 5(50.0%) diabetic foot,

1(33.3%) blast injury, 1(33.3%) abdominal surgery and 4(33.3%) other cases within type of surgery. While the frequency of one type resistant and other sensitive was 1(11.1%) in wound infection and 1(8.3%) in other cases within type of surgery.

Table (4): Rate of sensitive and resistant bacterial isolate according to type of surgery

			Sensitive and Resistance			Total
			Sensitive	Resistant	one type resistant and other sensitive	
Type of surgery	Wound infection	Count	3	5	1	9
		Expected Count	5.3	3.4	.4	9.0
		% within Type of surgery	33.3%	55.6%	11.1%	100.0%
		% within Sensitive and Resistance	10.7%	27.8%	50.0%	18.8%
	Burn infection	Count	7	2	0	9
		Expected Count	5.3	3.4	.4	9.0
		% within Type of surgery	77.8%	22.2%	0.0%	100.0%
		% within Sensitive and Resistance	25.0%	11.1%	0.0%	18.8%
	Diabetic foot	Count	5	5	0	10
		Expected Count	5.8	3.8	.4	10.0
		% within Type of surgery	50.0%	50.0%	0.0%	100.0%
		% within Sensitive and Resistance	17.9%	27.8%	0.0%	20.8%
	Blast injury	Count	2	1	0	3
		Expected Count	1.8	1.1	.1	3.0
		% within Type of surgery	66.7%	33.3%	0.0%	100.0%
		% within Sensitive and Resistance	7.1%	5.6%	0.0%	6.2%
	Abdominal surgery	Count	2	1	0	3
		Expected Count	1.8	1.1	.1	3.0
		% within Type of surgery	66.7%	33.3%	0.0%	100.0%
		% within Sensitive and Resistance	7.1%	5.6%	0.0%	6.2%
	Testicular abscess	Count	2	0	0	2
		Expected Count	1.2	.8	.1	2.0
		% within Type of surgery	100.0%	0.0%	0.0%	100.0%
		% within Sensitive and Resistance	7.1%	0.0%	0.0%	4.2%
	Others	Count	7	4	1	12
		Expected Count	7.0	4.5	.5	12.0
		% within Type of surgery	58.3%	33.3%	8.3%	100.0%
		% within Sensitive and Resistance	25.0%	22.2%	50.0%	25.0%
Total		Count	28	18	2	48
		Expected Count	28.0	18.0	2.0	48.0
		% within Type of surgery	58.3%	37.5%	4.2%	100.0%
		% within Sensitive and Resistance	100.0%	100.0%	100.0%	100.0%

*P value = 0.816

Table (5) result showed that the number of sensitive bacterial isolate was 12(42.9%) *Staphylococcus aureus*, 5(17.9%) *Acinetobacter Spp*, 6(21.4%) *E. coli* and 5(17.9%) others within sensitive and

resistant. While the result showed that the number of resistant bacteria isolate was 4(22.2%) *Staphylococcus aureus*, 8(44.4%) *Acinetobacter .Spp*, 4(22.2%) *E .coli* and 2(11.1%)others within sensitive and resistant. Also the result showed that the number of one type resistant and other sensitive was 2(100.0%) others bacterial isolate within sensitive and resistant. So, the results showed a high significant difference at ($p \leq 0.05$).

Table (5): Statistical variations to type of bacteria according to sensitive and resistant

			Type of bacteria				Total
			Staphylococcus aureus	E.coli	Acinetobacter . Spp	others	
Sensitive and Resistance	Sensitive	Count	12	6	5	5	28
		Expected Count	9.3	5.8	7.6	5.3	28.0
		% within Sensitive and Resistance	42.9%	21.4%	17.9%	17.9%	100.0%
		% within Type of bacteria	75.0%	60.0%	38.5%	55.6%	58.3%
	Resistant	Count	4	4	8	2	18
		Expected Count	6.0	3.8	4.9	3.4	18.0
		% within Sensitive and Resistance	22.2%	22.2%	44.4%	11.1%	100.0%
		% within Type of bacteria	25.0%	40.0%	61.5%	22.2%	37.5%
	one type resistant and other sensitive	Count	0	0	0	2	2
		Expected Count	.7	.4	.5	.4	2.0
		% within Sensitive and Resistance	0.0%	0.0%	0.0%	100.0%	100.0%
		% within Type of bacteria	0.0%	0.0%	0.0%	22.2%	4.2%
Total	Total	Count	16	10	13	9	48
		Expected Count	16.0	10.0	13.0	9.0	48.0
		% within Sensitive and Resistance	33.3%	20.8%	27.1%	18.8%	100.0%
		% within Type of bacteria	100.0%	100.0%	100.0%	100.0%	100.0%

*P value = 0.035



Figure (4.1): Bacteria resistant to meropenem antibiotic disk on Muller Hinton agar

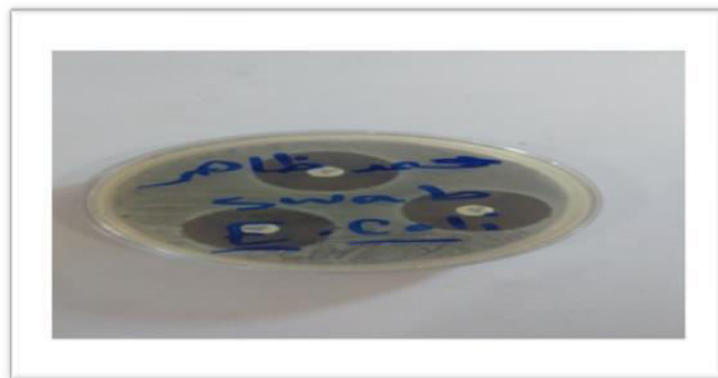


Figure (4.2): Bacteria sensitive to meropenem antibiotic disk on Muller Hinton agar.

The result regarding to meropenem treatment duration showed the frequency of sensitive and resistant bacterial isolate in patients have two days treatment was 3(50.0%) sensitive and 2(33.3%) resistant and 1(16.7%) one type resistant and other sensitive within classes for meropenem treatment duration. While the frequency of sensitive and resistant bacterial isolate in patients have three days treatment was 6(46.2%) sensitive and 7(53.8%) resistant within classes for meropenem treatment duration. Also the result showed the frequency of sensitive and resistant bacterial isolate in patients have four days treatment was 4(44.4%) sensitive, 4(44.4%) resistant and 1(11.1%) one type resistant and other sensitive within classes for meropenem treatment duration. The result revealed that the number of sensitive and resistant bacterial isolate in patients have five days treatment was 6(75.0%) sensitive and 2(25.0%) resistant within classes for meropenem treatment duration. While the result revealed that the number of sensitive and resistant bacterial isolate in patients have six days treatment was 4(80.0%) sensitive and 1(20.0%) resistant within classes for meropenem treatment duration. Also the result showed the frequency of sensitive and resistant bacterial isolate in patients have seven days treatment was 5(71.4%) sensitive and 2(28.6%) resistant within classes for

meropenem treatment duration. **So, the results showed no significant difference at ($p \leq 0.05$) is shown in Table (6).**

Table (6): Rate of sensitive and resistant bacterial isolate according to meropenem treatment duration

			Sensitive and Resistance			Total
			Sensitive	Resistant	one type resistant and other sensitive	
classes for Meropenem treatment duration(day)	Two days treatment	Count	3	2	1	6
		Expected Count	3.5	2.3	.3	6.0
		% within classes for Meropenem treatment duration(day)	50.0%	33.3%	16.7%	100.0 %
		% within Sensitive and Resistance	10.7%	11.1%	50.0%	12.5%
	Three days treatment	Count	6	7	0	13
		Expected Count	7.6	4.9	.5	13.0
		% within classes for Meropenem treatment duration(day)	46.2%	53.8%	0.0%	100.0 %
		% within Sensitive and Resistance	21.4%	38.9%	0.0%	27.1%
	Four days treatment	Count	4	4	1	9
		Expected Count	5.3	3.4	.4	9.0
		% within classes for Meropenem treatment duration(day)	44.4%	44.4%	11.1%	100.0 %
		% within Sensitive and Resistance	14.3%	22.2%	50.0%	18.8%
	Five days treatment	Count	6	2	0	8
		Expected Count	4.7	3.0	.3	8.0
		% within classes for Meropenem treatment duration(day)	75.0%	25.0%	0.0%	100.0 %
		% within Sensitive and Resistance	21.4%	11.1%	0.0%	16.7%
	Six days treatment	Count	4	1	0	5
		Expected Count	2.9	1.9	.2	5.0
		% within classes for Meropenem treatment duration(day)	80.0%	20.0%	0.0%	100.0 %
		% within Sensitive and Resistance	14.3%	5.6%	0.0%	10.4%
	7	Count	5	2	0	7
		Expected Count	4.1	2.6	.3	7.0
		% within classes for Meropenem treatment duration(day)	71.4%	28.6%	0.0%	100.0 %
		% within Sensitive and Resistance	17.9%	11.1%	0.0%	14.6%
Total		Count	28	18	2	48
		Expected Count	28.0	18.0	2.0	48.0
		% within classes for Meropenem treatment duration(day)	58.3%	37.5%	4.2%	100.0 %
		% within Sensitive and Resistance	100.0%	100.0%	100.0%	100.0 %

*P value = 0.598

(10) resistant samples were amplified for the gene blaNDM, blaIMP, blaVIM, blaKPC and blaOXA-48. However, the amplification of these isolates showed that (5) contained blaNDM gene resistant.

While blaIMP, blaVIM, blaKPC and blaOXA-48 that showed none result. The successful and non-successful amplification of these genes was shown in figures (1, 2).

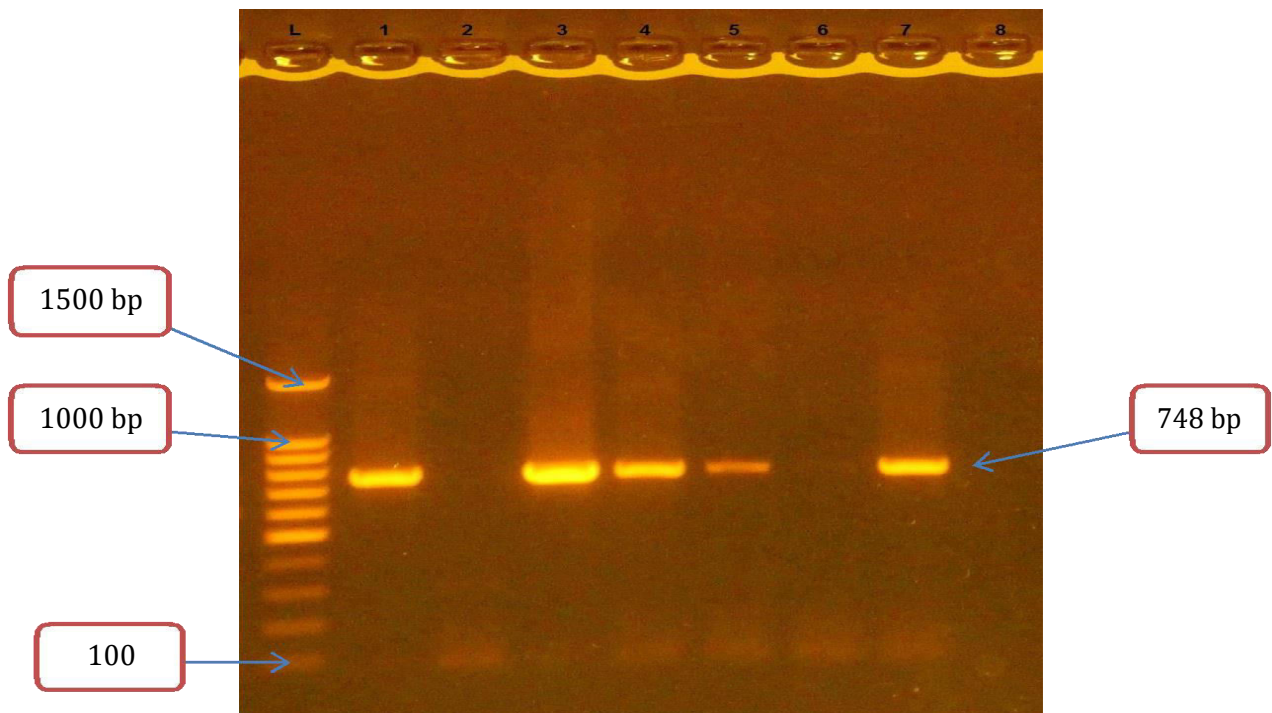


Figure (1): blaNDM resistant gene in *Acinetobacterbaumannii* for meropenem, 1gm (1%) agarose gel stained with 1µl of Ethidium Bromide (10mg/ml). Lane's L: 100-DNA ladder; lanes 1, 3, 4, 5, 7: PCR product of blaNDM resistant gene (band 748bp) from examined samples.

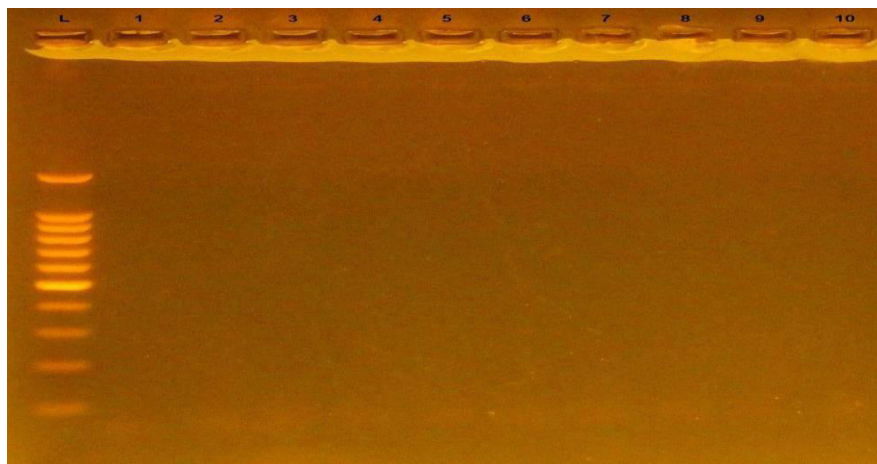


Figure (2): blaIMP, blaVIM, blaKPC and blaOXA-48 resistant genes in *Acinetobacterbaumannii* for meropenem show negative result.

Discussion

Surgical site infection are the second most common type of adverse events occurring in hospitalized patients following surgery and are one of the most common surgical complications¹⁷. SSI surveillance is integral to hospital infection control and quality improvement programs, with feedback of SSI rates

being an important component of SSI reduction strategies. The incidence of SSI differs widely from hospital to hospital and from one geographic location to another¹⁸. The obtained results from this study revealed that the number of male cases was higher than of female numbers in 1-5classes surgery of duration. Also the number of male cases was higher than of female numbers in 6-10classes surgery of duration and the number of male cases was higher than of female numbers in 11-25classes surgery of duration. This was related to type of surgery studied samples. The results showed that the highest hospital stay is 1-5 days and the number of cases is 44(55.0%). In the current study the results was showed that the meropenem treatment duration divided into seven day according to gender and the result showed that the number of male cases treatment with two, three, four and six days was higher than of females number treatment with same days. While the results were showed that the meropenem treatment duration with five and seven days the number of females was higher than male number treatment with same days. The results showed that the highest meropenem treatment duration is three days and the number of cases is 23(28.8%). In the current study, 48 bacterial isolates were investigated to determine their types and antimicrobial susceptibility pattern. Our finding demonstrates the predominance of gram negative bacterial isolates in SSIs, *S. aureus* being the commonest isolated organism followed by *E. coli*, *Streptococcus. Spp*, *Klebsiella. Spp*, *Proteus. Spp*, *Pseudomonas. Spp*, *Pantoea. Spp*. This pattern of organisms causing SSIs in the current study agrees with a previous study^{10,17}. Regarding the frequency of the isolation of organisms in different types of infection in the present study, *Acinetobacter. Spp* was the most common isolate from wound infection types, while *S. aureus* and *Acinetobacter Spp* were among the most common isolates from burn infection types. The results obtained from this study showed that the *S. aureus* was the most common isolate from diabetic foot infection. The results also showed that the *E. coli* and *Acinetobacter Spp* were among the most common isolates from other cases infection type. These findings suggest that the etiologic agents of SSIs depend on where the procedures are performed and whether skin was incised or gastrointestinal tract was opened. When gastrointestinal tract is opened, organisms usually include aerobic Gram negative rods. In the present study, anaerobic organisms were not isolated from culture despite the measures taken to recover such organisms from surgical wounds. The probable reasons could be delayed in inoculation of the swabs into the fresh blood agar media; some patients received prophylactic metronidazole which kills anaerobes and the use of dry cotton wool swabs for specimen collection. A previous study documented that the use of dry swabs for collection of specimens could hinder the isolation of the anaerobes¹⁹. This results may be due to spread of a single *A. baumannii* isolate as a source of a nosocomial outbreak is often linked to contamination of respiratory equipment and transmission via the hands of hospital staff²⁰. Numerous studies showed that the hospital environment is a preferential setting in which *A. baumannii* isolates can persist and develop. *A. baumannii* seems to have unique characteristics among nosocomial Gram-negative bacteria that enhance its environmental persistence. The possibility that the community represents a reservoir for *A. baumannii* was evaluated by analyzing and comparing isolates recovered from patients in two hospitals in New York, USA, with isolates recovered from the hands of individuals in the community²¹. This result may be due to different geographical study or the timing of study. The results obtained from this study showed that the higher number of resistant bacterial isolate from patients have three and four day's meropenem treatment duration. The result also showed that the higher number of sensitive bacterial isolate from patients have five, six and seven day's meropenem treatment duration. In the current study, the results showed that the *Acinetobacter baumannii* showed that isolate have blaNDM resistance gene. This result agrees with the study which about 6% of our *A. baumannii* isolates tested positive for the class B carbapenemase NDM-1. This is consistent with regional and global results as there are reports in India, China, United Kingdom, Canada, France, Sweden, Morocco, South Africa, United Arab Emirates, and Iran among many others²². Also, the results showed that the *Acinetobacter baumannii* isolates have negative result for blaIMP, blaVIM, blaKPC and blaOXA-48 resistance gene. Although carbapenemases were reported to be species-specific in the 1990s, the identification and spread of plasmid-encoded carbapenemases has recently changed our view of the magnitude of the problem in Gram-negative bacteria, especially the rapid and extensive worldwide dissemination of

carbapenem-resistant bacteria carrying the KPC-type, VIM-type, NDM-type and OXA-type carbapenemases^{23,24}.

Conclusion

Acinetobacter baumannii, *Staphylococcus aureus* and *E. coli* were the most resistant microorganism to meropenem drug in Baquba Teaching Hospital. The gene resistant of *Acinetobacter baumannii* to meropenem treatment was (blaNDM).The post-operative wound was more susceptible to infection than other types of surgery. *Acinetobacter baumannii* was more resistant in male than female with wound infection.

References

- 1- Jasim, A. H.; Nabeel, A. D. and Sulaiman, T. I. (2013). The Effect of Perioperative Use of Prophylactic Antibiotics on Surgical Wound Infection. Iraqi Academic Scientific Journal. 12(2): 181-5.
- 2- Rao, B. H. and Chakravarthy, K. S. (2016). The study on post-operative wound infections at Vizianagaram in Andhra Pradesh, India. International Journal of Research in Medical Sciences. 4(12): 5351-5.
- 3- Al-Abbas, A. K. (2017). Aerobic bacteria isolation from post-caesarean surgical site and their antimicrobial sensitivity pattern in Karbala city, Iraq. Iraq Medical Journal. 1(4).
- 4- Burooj M. R. Al-aajem (2017). Assessment of bacterial contamination and antibiotics susceptibility of pathogenic Bacteria in the operating theaters in AL-Batool teaching hospital. Diyala journal for pure sciences. 13(2) ,174-183 .
- 5- Burooj M. R. Al-aajem (2018).Urinary Tract Infection among Diabetics and Non-Diabetics Post-Menopausal women and Estrogen levels compare with Healthy Women in Baquba City .*Research J. Pharm. and Tech.* 11(7),3063-3066.
- 6- Ameh, E. A.; Mshelbwala, P. M.; Nasir, A. A. *et al.* (2009). Surgical site infection in children: prospective analysis of the burden and risk factors in a sub-Saharan African setting. *Surgical infections.* 10(2): 105-9.
- 7- Kapischke, M.; Caliebe, A.; Tepel, J.; Schulz, T. and Hedderich, J. (2006). Open versus laparoscopic appendectomy. *Surgical Endoscopy And Other Interventional Techniques.* 20(7): 1060.
- 8- Garcell, H. G.; Arias, A. V.; Sandoval, C. A. P.; García, E. G.; Gamboa, M. E. V.; Sado, A. B. and Serrano, R. N. A. (2017). Incidence and etiology of surgical site infections in appendectomies: a 3-year prospective study. *Oman medical journal,* 32(1), 31.
- 9- Hodiwala, A. B. and Anuradha. M. (2018). SURGICAL SITE INFECTIONS (SSI). *Int J Pharm Biol Sci.* 8(2): 316-329.
- 10- Mahmood, A. (2000). Bacteriology of surgical site infections and antibiotic susceptibility pattern of the isolates at a tertiary care hospital in Karachi. *Infection.* 3, 4.
- 11- Weigelt, J. A.; Lipsky, B. A.; Tabak, Y. P. *et al.* (2010). Surgical site infections: causative pathogens and associated outcomes. *American journal of infection control.* 38(2):112-20.
- 12- Khorvash, F.; Mostafavizadeh, K.; Mobasherizadeh, S. *et al.* (2008). (SSI); A 1 year of surveillance. *Pak J Biol Sci.* 11(15): 1940-4.
- 13- Ulldemolins, M.; Soy, D.; Llaurodo-Serra, M. *et al.* (2015). Meropenem population pharmacokinetics in critically ill patients with septic shock and continuous renal replacement therapy: influence of residual diuresis on dose requirements. *Antimicrobial agents and chemotherapy.* AAC-00712.

- 14- Zeana, C.; Larson, E.; Sahni, J.; Baguya, S.J.; Wu, F. and Della-Latta, P. (2003). The epidemiology of multidrug-resistant *Acinetobacter baumannii*: does the community represent a reservoir. *Infect Control Hosp Epidemiol.* 24: 275–279.
- 15- Lowe, M. N. and Lamb, H. M. (2000). Meropenem. *Drugs*, 60(3), 619-646.
- 16- Roberts, J. A.; Uldemolins, M. and Lipman, J. (2010). Meropenem: focus on its use in serious bacterial infections. *Clinical Medicine Reviews in Therapeutics*, 2010(2), 145-158.
- 17- Maier, S.; Körner, P.; Diedrich, S.; Kramer, A. and Heidecke, C.D. [Definition and management of wound infections]. *Chirurg.* 2011;82:235–241. German.
- 18- Duque-Estrada, E.O.; Duarte, M.R.; Rodrigues, D.M.; and Raphael, M.D. (2003). Wound infections in pediatric surgery: a study of 575 patients in a university hospital. *Pediatr Surg Int.* 19:436–438
- 19- Mawalla, B.; Mshana, S. E.; Chalya, P. L. *et al.* (2011). Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. *BMC surgery.* 11(1): 21.
- 20- Sule, A. M.; Thanni, L. O. A.; Odu, O. S.; and Olusanya, O. (2002). Bacterial pathogens associated with infected wounds in Ogun state University Teaching Hospital, Sagamu, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 3(1), 13-16.
- 21- Cisneros, J. M. and Rodríguez-Baño, J. (2002). Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clinical Microbiology and Infection.* 8(11), 687-693.
- 22- Zeana, C.; Larson, E.; Sahni, J.; Baguya, S.J.; Wu, F. and Della-Latta, P. (2003). The epidemiology of multidrug-resistant *Acinetobacter baumannii*: does the community represent a reservoir. *Infect Control Hosp Epidemiol.* 24: 275–279.
- 23- Bercion, R.; Gaudeville, A.; Mapouka, P. A.; Behounde, T. and Guetahoun, Y. (2007). Surgical site infection survey in the orthopaedic surgery department of the "Hôpital communautaire de Bangui," Central African Republic. *Bulletin de la Société de pathologie exotique* (1990). 100(3): 197-200.
- 24- Queenan, A. M. and Bush, K. (2007). Carbapenemases: the versatile β -lactamases. *Clinical microbiology reviews*, 20(3), 440-458.

SURVIVIN TARGETING; AN *IN SILICO* APPROACH FOR ANTICANCER DESIGN

Aymen F. Al-Mulla ¹, Entissar F. Al-Mulla ²

1. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Univ. of Baghdad, Iraq.
2. College of Science, Biology Dep., Univ. of Baghdad, Iraq.

ABSTRACT

Survivin, a member of inhibitor of apoptosis family is increasingly used as a target for cancer therapy design because it has a key role in cell growth and inhibition of cell apoptosis. Also it can be used as a biomarker for targeting cancer because it is found in almost all cancer but not normal cells. Our strategy was to design (computationally) a molecule to be used as survivin inhibitor. This molecule was named lead10 and was used furthermore to find (virtually) existing drugs with a good survivin inhibition activity.

Key Words: Survivin, Drug Design, Cancer Therapy, MOE, Docking, Drug Repositioning, ADMET

How to cite this article: Al-Mulla AF, Al-Mulla EF (2019): Survivin targeting: an in silicon approach for anticancer design, Ann of Trop Med & Pub Health; 21: SP2045-19

INTRODUCTION

Survivin is the smallest member of the Inhibitor of apoptosis protein (IAPs) family, has a molecular weight of 16.5 kDa, and contains 142 amino acid residues (1). Inhibitor of apoptosis protein family (IAPs) is an important group of proteins involved in regulation of apoptosis. IAPs also have an important role in regulation of T cell responses in antitumor immunity. Survivin occupies a key position because of its overexpression in cancer cells. It is speculated that Survivin overexpression in tumor cells promotes tumor progression by multiple pathways such as dysregulation of apoptosis and cell division, altered sensitivity to antitumor drugs or promoting survival of cancer stem cells (2). Survivin has been proposed as an attractive target for selective cancer treatment because of its unique attributes (3). First, the expression of survivin is typically very high in tumor cells but very low or even undetectable in normal adult tissues (4). This feature is highly significant, because it provides selectivity, a major problem for current cancer drug development (5). Second, the functions of survivin contribute to the formation and maintenance of tumors (6), so targeting survivin will be very effective for treating cancer (5). Furthermore, survivin has been shown to be associated with resistance to multiple types of cancer therapies (7–9). Although survivin is a very promising target for cancer treatment and many studies have been carried out to develop survivin inhibitors, the pool of existing survivin inhibitors is still relatively small. Several problems limit the development of effective survivin inhibitors. Unlike an enzyme or a receptor molecule located on the cell surface, survivin is not a traditional target.

Survivin is a nodal protein involved in multiple processes of cancer cell proliferation, including mitosis, apoptosis and autophagy. It also interferes with various molecules such as Hsp90, Borealin, INCENP, aurora B kinase and caspases. Therefore, it is a significant challenge and is very critical to unambiguously confirm that the drug action is resulted from survivin-specific inhibition. Because of such challenges, currently it is difficult to evaluate the effectiveness and target specificity of survivin inhibitors accurately via high throughput assays. The lack of suitable assays to identify survivin inhibition by high throughput screenings is one main factor to impede the development of potent and selective survivin inhibitors (10). In view of the above problems in finding new drugs by high throughput screening; cost effective, reliable *in Silico* screening procedures are in practice (11). Therefore, new approaches of molecular modelling and virtual screening have been used in our study which we believe it would be beneficial to overcome these challenges and discover new small molecules line targeting survivin as anticancer agents.

MATERIALS AND METHODS

1. Protein Data Bank (PDB):

<http://www.rcsb.org/> a protein information platform used to retrieve the 3D crystallographic structure of the target protein(12).

2. Molecular Operating Environment (MOE):

https://www.chemcomp.com/MOE-Molecular_Operating_Environment.html/ is a drug discovery software platform that integrates visualization, modelling and simulations, as well as methodology development, in one package(13).

3. SwissSimilarity:

<http://www.swisssimilarity.ch/> a website allows you to perform ligand-based virtual screening of several libraries of small molecules. using different approaches (14).

4. PreADMET:

<https://preadmet.bmdrc.kr/> a web-based application for predicting ADME data and building drug-like library using in silico methods (15).

5. Mcule:

<https://mcule.com/> Mcule.com is an online drug discovery platform. It offers a unique solution for pharma and biotech companies by providing the highest quality purchasable compound database and molecular modelling tools (16).

6. Chemspace:

<https://chem-space.com> one of the largest database of small molecules: Building Blocks, Fragments, and Screening Compounds (19).

7. MolView:

<http://molview.org> is an Open-Source web-application to make science and education more awesome. MolView is mainly intended as web-based data visualization platform (20).

RESULTS

Protein retrieval:

The BIR domain of survivin functions as a mediator for protein-protein interactions, through utilization of a deep surface peptide-binding groove to recognize the Nterminal conserved Ala-containing IAP-binding motif (IBM) of the target protein (17). During apoptosis Survivin binds to Smac, which is released from induced mitochondria. Therefore, by reducing Smac/DIABLO antagonism to IAPs, such as XIAP, the free XIAP directly interacts with caspases and cell death is blocked (18).

The crystal structure of human Survivin in complex with Smac/DIABLO (1-15) peptide (3UIH) was retrieved from Protein Data Bank (Figure1).

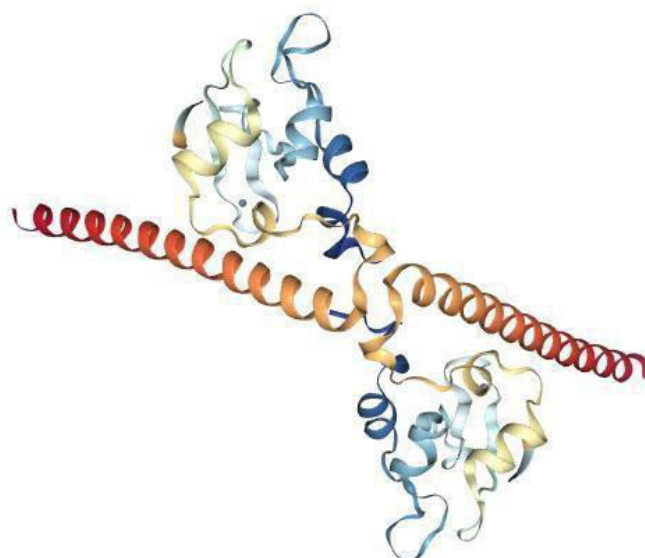


Figure1: (3UIH) Crystal structure of human survivin complexed with Smac/DIABLO binding motif

Protein preparation:

The protein was prepared for docking research using Molecular Operating Environment (MOE) package (version 2014), where correction, 3d protonation and energy minimization were done to prepare the protein (Figure2).

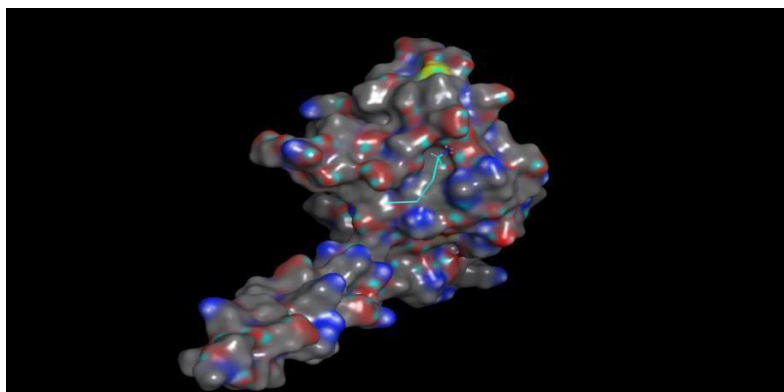


Figure2: The prepared protein complexed with its ligand (blue colour)

Molecular Design:

The ligand molecule (Smac/DIABLO peptide) was used to design a leading molecule. The building tool of (MOE) was used to design drug like molecules with more binding activity and low side effects, so ten leading molecules were designed (Figure3).

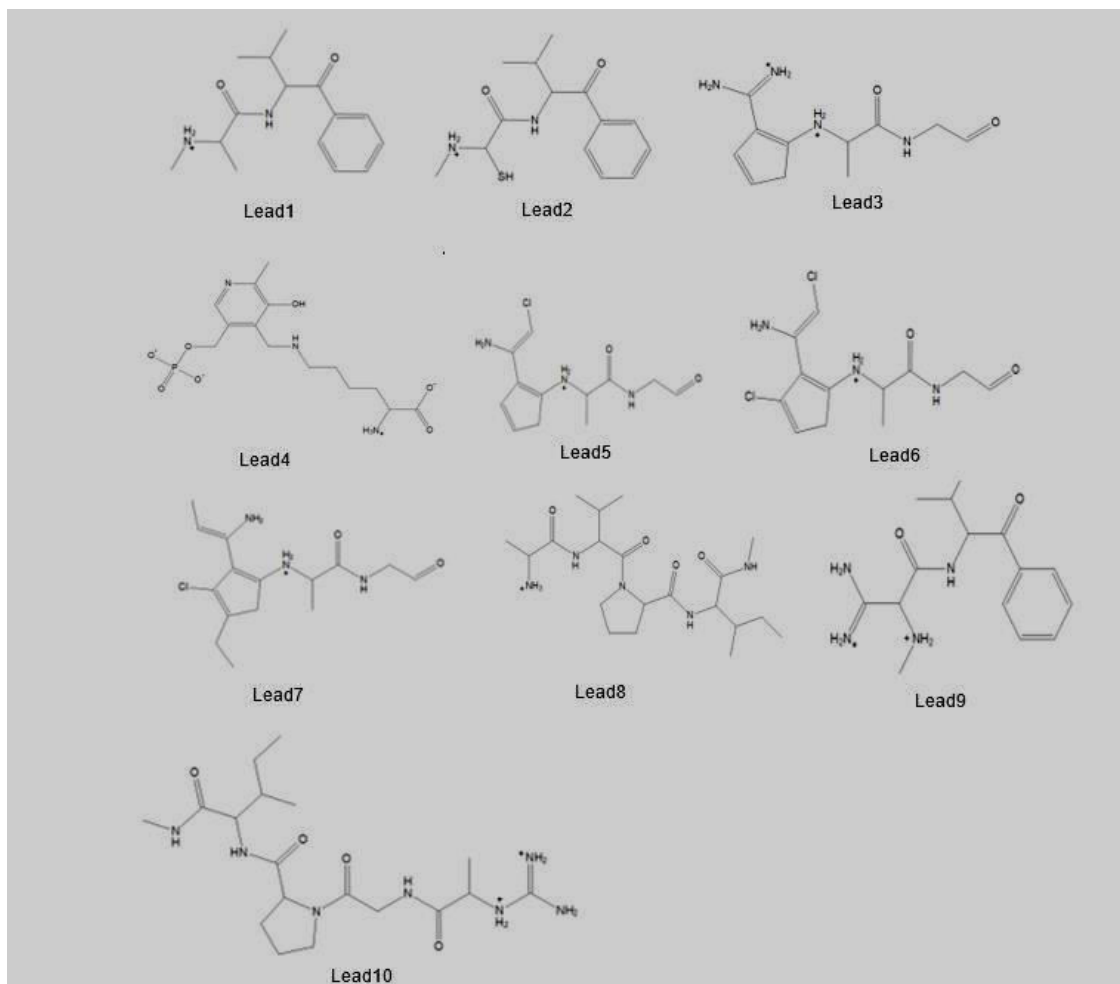


Figure3: the designed leading molecules

Docking Results:

The leading molecules were arranged in a library of dataset and by using docking algorithms of (MOE) with amber12:EHT forcefield, docking was done to measure the binding score for the most appropriate molecule.

Only one of these molecules (lead10) have succeeded and passed the ligand binding energy cutoff (-8.5 kcal/mol) with (-10.2 kcal/mol) binding energy value.

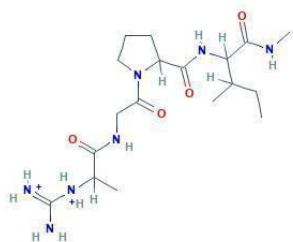
The New Born Molecule

The (Lead10) molecule have been studied furthermore virtually for its chemical and pharmaceutical properties.

1. Name: it was named using Chemspace website as:

[amino(iminiumyl)methyl](1-[[2-(2-[[2-methyl-1(methylcarbamoyl)butyl]carbamoyl]pyrrolidin-1-yl)-2oxoethyl]carbamoyl]ethyl)azanium

2. Chemical properties: its chemical properties were retrieved from molview website (Figure4)



Formula	C ₁₈ H ₃₅ N ₇ O ₄
Molecular weight	413.519 u
Hydrogen bond donors	6
Hydrogen bond acceptors	4

Percent composition

C	12.0107 u × 18	52.282 %
H	1.00794 u × 35	8.5312 %
N	14.0067 u × 7	23.711 %
O	15.9994 u × 4	15.476 %

Canonical SMILES

CCC(C)C(NC(=O)C1CCCN1C(=O)CNC(=O)C(C)[NH2+])C(N)=[NH2+])C(=O)NC

Isomeric SMILES

O=C(C1CCCN1C(CN([H]))C(C(C)[N+](//C(/N([H]))[H])=[N+](/[H])\[H])([H])([H])=O)N([H])C(C(N([H])C)=O)C(C)CC

InChIKey

PLMDMVFLNFIHEX-UHFFFAOYSA-P

InChI

InChI=1S/C18H33N7O4/c1-5-10(2)14(17(29)21-4)24-16(28)12-7-6-8-25(12)13(26)9-22-15(27)11(3)23-18(19)20/h10-12,14H,5-9H2,1-4H3,(H,21,29)(H,22,27)(H,24,28)(H4,19,20

Figure4: the chemical properties of Lead10 from molview website

3. ADMET Results:

The pharmacokinetic properties were obtained using PreADMET and Mcule toxicity checker (Figure5,6)

ID	Value
BBB	0.0283959
Buffer_solubility_mg_L	38720
Caco2	20.1414
CYP_2C19_inhibition	Non
CYP_2C9_inhibition	Non
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Weakly
CYP_3A4_inhibition	Non
CYP_3A4_substrate	Weakly
HIA	12.833988
MDCK	0.480134
Pgp_inhibition	Non
Plasma_Protein_Binding	36.123257
Pure_water_solubility_mg_L	46992.2
Skin_Permability	-4.75082
SKlogD_value	-5.051280
SKlogP_value	-5.051280
SKlogS_buffer	-1.028560
SKlogS_pure	-0.944470

Figure5: ADME for Lead10 using PreADMET website

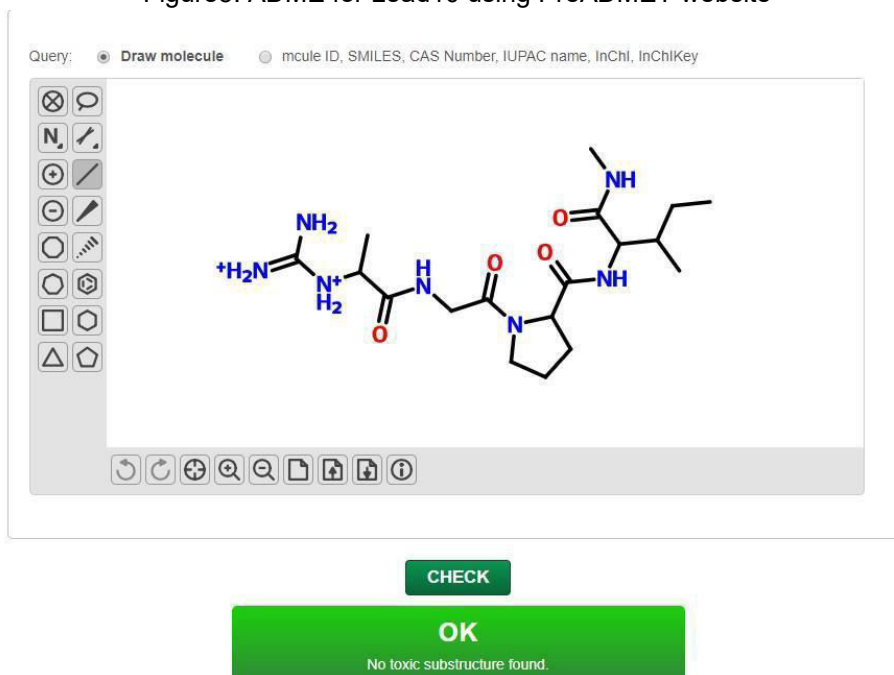


Figure6: Toxicity test for Lead10 using Mcule website

Drug Repositioning Research

Drug repositioning, also known as old drugs for new uses, is an effective strategy to find new indications for existing drugs and is highly efficient, low-cost and riskless (21).

Our strategy in drug repositioning was to find drug molecules similar to our designed molecule and have a good binding affinity to the target (survivin).

We used SwissSimilarity website to find similar drug molecules, chose from thousands of them 450 molecules for best similarity, designed a library of these molecules and started docking research using (MOE) to check for the best binding energy molecules.

The result was 4 drugs:

1. Telaprevir (antiviral)
2. Telmisartan (antihypertensive)
3. Ritonavir (antiviral)
4. Chlorhexidine (antiseptic)

They had binding affinity greater than the ligand molecule (Figure7).

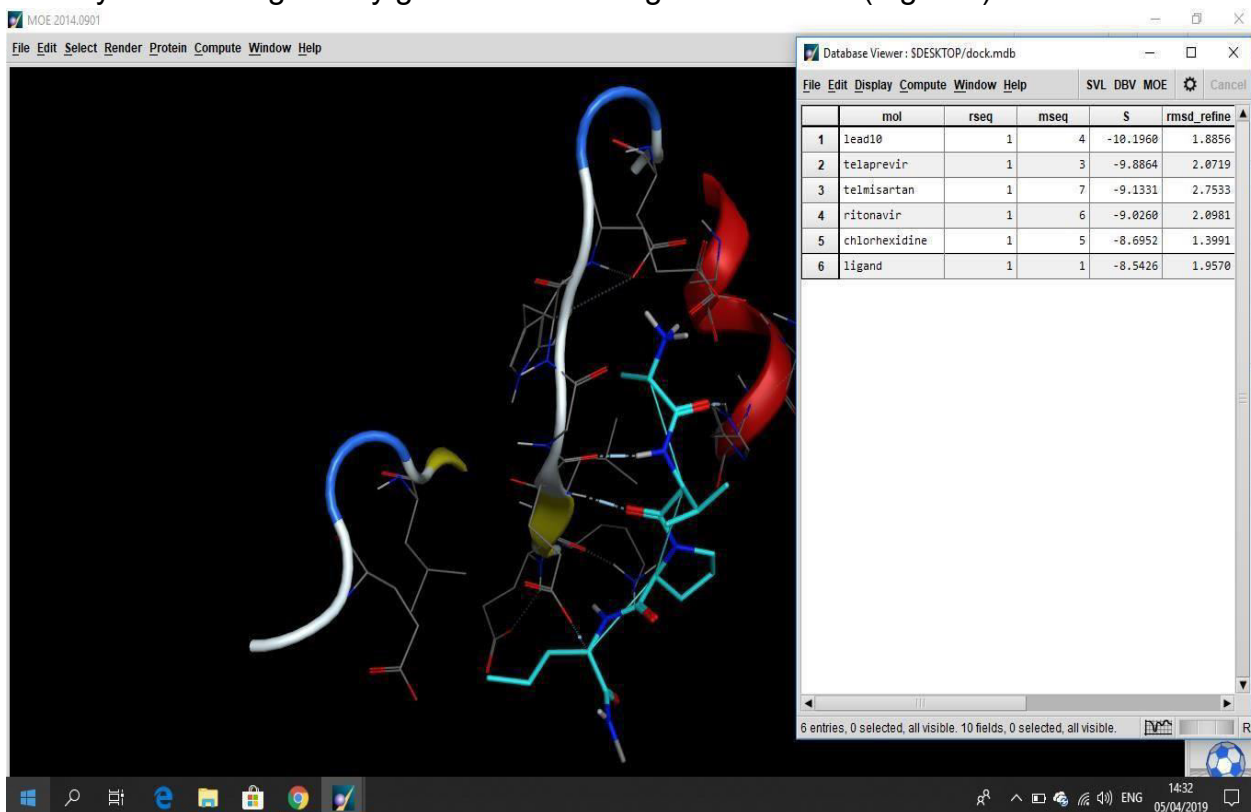


Figure7: Lead10, Telaprevir, Telmisartan, Ritonavir and Chlorhexidine docking results with survivin

Binding Pattern:

To see whether these molecules have activation or inhibition effect on the target we displayed the binding network for all these molecules in a comparison with the ligand molecule (Figure8).

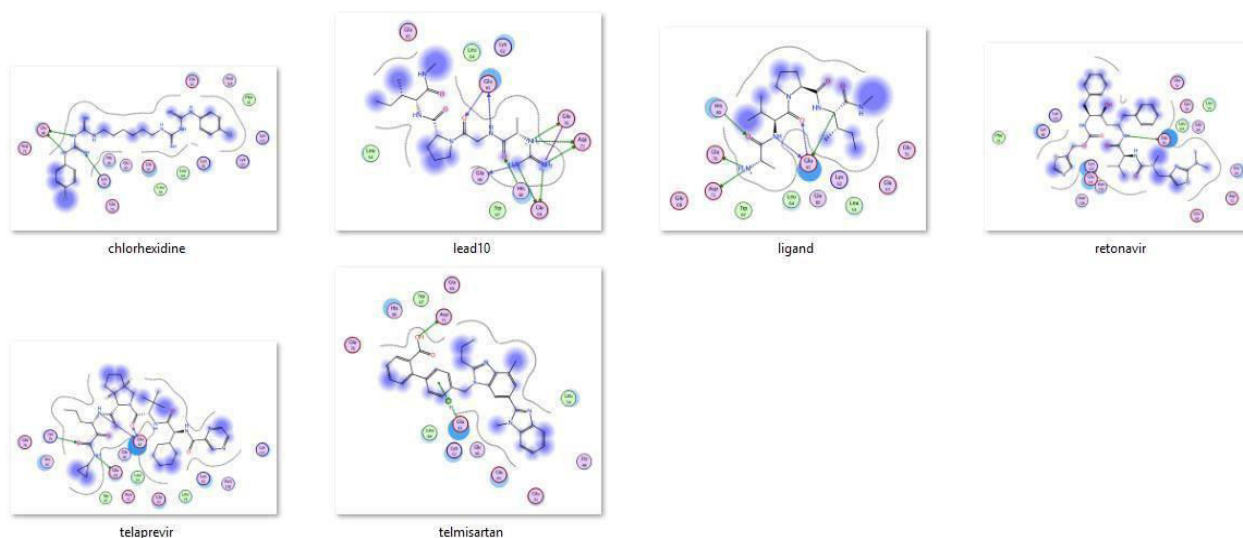


Figure8: comparison of the binding network for the final molecules with the ligand molecule

DISCUSSION AND CONCLUSION

Survivin is a key point in studying carcinogenesis. It can be used as a biomarker for cancer targeting because it is found almost in all types of cancer but not in most normal cells. In this study we used survivin as a target to design anticancer molecules. Our strategy divided into two lines; first by designing new inhibitor molecules and second by finding already existing drug molecules but with a good inhibition activity.

In the design step we used (MOE) package for preparing the protein for molecular docking. Also (MOE) builder was used to design a bunch of molecules as inhibitors for the protein. Ten molecules were arranged in a dataset and docked with survivin. The most appropriate one with highest binding energy was (Lead10), a molecule with

molecular weight (413.52 g/mol), logp (-4.6), binding affinity (-10.2 kcal/mol) and not toxic.

The addition of guanidine moiety at the terminal N of the first amino acid (Alanine) on the ligand molecule increased its polarity and made it more potent in binding at the active site. Also the deletion of isopropyl moiety at the second amino acid (valine) decreased its molecular weight and didn't influence its binding activity meaning that it's not a part of its pharmacophore.

This molecule is highly water soluble, nontoxic and can be used as a competitive inhibitor for survivin as its binding network fingerprint differ from the ligand molecule binding pattern and its binding score passed the ligand cutoff (-8.5 kcal/mol).

In the second step we used the newly designed molecule as a templet for searching similar drug molecules. SwissSimilarity website was used to search these similar molecules and 450 drug molecules have been chosen from thousands.

A library of these molecules was designed and molecular docking with survivin was operated. Four drug molecules were selected as having best binding score as following (Table1):

	Drug	Binding score (kcal/mol)
1.	Telaprevir (antiviral)	-9.88
2.	Telmisartan (antihypertensive)	-9.13
3.	Ritonavir (antiviral)	-9.02
4.	Chlorhexidine (antiseptic)	-8.69

Table1: similar drugs to Lead10 and their docking score with survivin

These molecules can be used as good inhibitors for survivin because their binding patterns differ from the ligand pattern.

Statistically **all these molecules have an acceptable RMSD values (<3°) as shown** in (figure 7).

At the end this is an *in silico* study and need to be completed with *in vivo* and *in vitro* studies. But, even though, Lead10 and the four other drug molecules can be considered as a promising weapons in the battle against cancer.

REFERENCES

1. Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anti-cancer therapy. Cancer Treat Rev. 2009; 35(7):553–562. [PubMed: 19559538].

2. Garg H, Suri P, Gupta JC, Talwar GP, Dubey S. Survivin: a unique target for tumor therapy. *Cancer Cell Int.* 2016;16:49. Published 2016 Jun 23. doi:10.1186/s12935-016-0326-1
3. Mobahat M, Narendran A, Riabowol K. Survivin as a preferential target for cancer therapy. *Int J Mol Sci.* 2014; 15(2):2494–2516. [PubMed: 24531137]
4. Altieri DC. Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer.* 2008; 8(1):61–70. [PubMed: 18075512]
5. Xiao M, Li W. Recent Advances on Small-Molecule Survivin Inhibitors. *Curr Med Chem.* 2015;22(9):1136–1146.
6. Athanasoula K, Gogas H, Polonifi K, Vaiopoulos AG, Polyzos A, Mantzourani M. Survivin beyond physiology: orchestration of multistep carcinogenesis and therapeutic potentials. *Cancer Lett.* 2014; 347(2):175–182. [PubMed: 24560928]
7. Ghanbari P, Mohseni M, Tabasinezhad M, Yousefi B, Saei AA, Sharifi S, Rashidi MR, Samadi N. Inhibition of Survivin Restores the Sensitivity of Breast Cancer Cells to Docetaxel and Vinblastine. *Appl Biochem Biotechnol.* 2014
8. Wang R, Chen DQ, Huang JY, Zhang K, Feng B, Pan BZ, Chen J, De W, Chen LB. Acquisition of radioresistance in docetaxel-resistant human lung adenocarcinoma cells is linked with dysregulation of miR-451/c-Myc-survivin/rad-51 signaling. *Oncotarget.* 2014
9. Sun XP, Dong X, Lin L, Jiang X, Wei Z, Zhai B, Sun B, Zhang Q, Wang X, Jiang H, Krissansen GW, Qiao H, Sun X. Up-regulation of survivin by AKT and hypoxia-inducible factor 1alpha contributes to cisplatin resistance in gastric cancer. *FEBS J.* 2014; 281(1):115–128. [PubMed: 24165223]
10. Xiao M, Li W. Recent Advances on Small-Molecule Survivin Inhibitors. *Curr Med Chem.* 2015;22(9):1136–1146.
11. Young D. Computational Drug Design. John Wiley& Sons, New Jersey, USA. 2009.
12. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. (2000) The Protein Data Bank *Nucleic Acids Research*, 28: 235-242.
13. Reynolds CH, Merz KM, Ringe D, eds. (2010). *Drug Design: Structure- and Ligand-Based Approaches* (1 ed.). Cambridge, UK: Cambridge University Press. ISBN 978-0521887236.
14. Zoete, V., Daina, A., Bovigny, C., & Michielin, O. SwissSimilarity: A Web Tool for Low to Ultra High Throughput Ligand-Based Virtual Screening., *J. Chem. Inf. Model.*, 2016, 56(8), 1399-1404.
15. Shakeel, Eram et al. “Molecular docking analysis of aplysin analogs targeting survivin protein.” *Bioinformation* vol. 13,9 293-300. 30 Sep. 2017, doi:10.6026/97320630013293.
16. Kiss, Robert & Sandor, Mark & Szalai, Ferenc. (2012). [Http://McuLe.com](http://McuLe.com): A public web service for drug discovery. *Journal of Cheminformatics.* 4. 10.1186/1758-2946-4-S1-P17.

17. Du J, Kelly AE, Funabiki H, Patel DJ. Structural basis for recognition of H3T3ph and Smac/DIABLO N-terminal peptides by human Survivin. *Structure*. 2012;20(1):185–195. doi:10.1016/j.str.2011.12.001.
18. Song, Zhiyin et al. “Direct interaction between survivin and Smac/DIABLO is essential for the antiapoptotic activity of survivin during taxol-induced apoptosis.” *The Journal of biological chemistry* 278 25 (2003): 23130-40.
19. Expanding Synthesizable Space of Disubstituted 1,2,4-Oxadiazoles;ACS Comb. Sci., 2016, 18 (10), pp 616–624 DOI: 10.1021/acscombsci.6b00103.
20. Smith, Thomas. (1995). MOLView: A program for analyzing and displaying atomic structures on the Macintosh personal computer. *Journal of molecular graphics*. 13. 122-5, 115. 10.1016/02637855(94)00019-O.
21. Xue, H., Li, J., Xie, H., & Wang, Y. (2018). Review of Drug Repositioning Approaches and Resources. *International journal of biological sciences*, 14(10), 1232–1244. doi:10.7150/ijbs.24612.

CYTOGENETIC STUDY OF AUTISM IN IRAQ

Methaq Hadi Lafta¹

1. Directorate of Education Diwaniyah / Ministry of Education, Iraq

Contact info: mithaqiraqi@gmail.com

Abstract

Structural variation (CNV) including deletion, duplication, displacement and reflection of chromosomes was identified in some individuals with autism spectrum disorder (ASD), but the full moral role is unknown. We evaluated the genome extensively for structural abnormalities in 427 unrelated ASD cases through monoclonal arrays and a nuclear pattern. With precise matrices, we discovered 277 of unbalanced CNVs in 44% of ASD families not present in 500 controls (and re-examined in 1152 other controls). The nuclear pattern revealed additional balanced changes. Although most variables were inherited, we found a total of 27 cases with de novo modifications, and in three (11%) of these individuals, two or more new variables were observed. De Novo CNVs were found in about 7% and about 2% of idiopathic families had one child, or two or more ASD siblings, respectively. We also discovered 13 sites with repeated / overlapping CNVs in unrelated cases. In these sites, deletions and repetitions affecting the same gene were found in different individuals and sometimes in symptom-free vectors. Despite complications, our results include more SHANK3-NLGN4-NRXN1 involvement in post-clamp density genes as well as new positions in DPP6-DPP10-PCDH9 (compound clamp), ANKRD11, DPYD, PTCHD1, 15q24, among others, for the role In ASD sensitivity. Our most convincing results revealed CNV at 16p11.2 ($p = 0.002$) (with characteristics of genetic disturbance) at a frequency of about 1%. Some areas of ASD were also common in places of mental retardation. Structural variables were found in the high-frequency effect sufficiently ASD suggests that cellular genetic analyzes and microarray are considered in the clinical workup routine.

Key words: autism, microarray, gene, family, Iraq, Autism spectrum disorders (ASD)

How to cite this article: Lafta MH (2019): CYTOGENETIC STUDY OF AUTISM IN IRAQ, Ann Trop Med & Pub Health; SP2046-19

Introduction

The contribution of genetic factors to autism has been demonstrated well, but the method of genetic transport is unclear. However, it is clear that autism is a complex disorder other than Mendelian, and is expected to share genetic heterogeneity and neonatal / genetic inheritance. (Richard et al., 2016)

There are several lines of evidence such as sexual bias, positive correlation results, and the role of Xchromosomal genes in perceptual and oblique impairment suggesting the potential involvement of X-chromosomal loci in autistic pathogens. Although the X chromosome is usually a weak genetic chromosome, the majority of known identity genes plot the X chromosome. To date, more than 80 are associated with X in different forms of identity(Luo et al., 2017). Because of the monochromatic status of most male sex chromosome genes, X or Y chromosome mutations are more severe in males, which may partially explain sexual bias in autism and identity. Malignant chromosomal abnormalities can be identified for detection in up to 7.4% of ASD cases. Higher genomic imbalances occur in syndromes of ASD. Transformation and balanced balancing represent 17% of the genome rearrangement. (Worachotekamjorn et al., 2017)

The most common chromosomal abnormality is the similarity of the mothers of chromosome 15q11-q13 in 1-3% of cases. Using chromosomal abnormalities to identify candidate ASD genes, mutations in SHANK3 were determined on chromosome 22q13, NLGN3, NLGN4 genes on X-chromosome, and gene neurexin 1 (NRXN1) on chromosome 2p16(Alvarez-Mora et al., 2016). Recently, CNVs have been shown to contribute to autism, and CNVs appear to be a more important risk factor in sporadic cases than in family forms of ASD. Epidemiological studies have shown gender bias in autism as well as in identity, where the number of autistic affected males is 3-4 times greater than females, and for identity, the ratio is close to 1.3: 1, although this is believed to decrease with low IQ and some

Studies indicate that severe ID may be more prevalent among females,(Mak, 2015). Also, a form of identity has been reported in up to 70% of children with autism. This overlap between autism and identity justifies the search for common causative factors and sex ratios in these disorders make the X chromosome an excellent candidate. (Do et al., 2017)

Thus, the present study hypothesized that a number of candidate genes are present on the X chromosome, some of which can be identified by chromosome X chromosome analysis and by direct sequencing of candidate genes between autistic variables. In this study we took advantage of technological advances in the field of microarrays DNA to determine the relationship between CNV on X chromosome and autism in Iraq. The genome survey was performed for more than 400 hundred sequences with autism using an Affymetrix 500k SNP array. We attempted to identify the candidate genes for autism by mapping CNV on the X chromosome, along with the sequence of the relevant candidate genes that map the CNVs, in order to identify additional patients with consecutive mutations.(Demily et al., 2018)

Materials and methods

DNA samples

The study included 25 families recruited in northern Iraq with ASD, with the total number of cases of 50, 30 with ASD and 20 of not ASD children with age limitation of 3-10 years .(Demily et al., 2018) The ADOS and ADI-R tools were used for assessments and all updates met DSM-IV diagnostic criteria for autism.. Siblings of proband were also evaluated for ASD. Twenty families had one child (ASD) and 5 families with more than one child (ASD). This high ratio of simple to multiplex is likely due to biases in household verification. Approximately 75% of cases of FRX mutations were examined, and households with FRX mutations were excluded from the study. Most experiments were performed on DNA extracted from lymphocytes (80%), and for the rest DNA was extracted from lymphocyte lines.

DNA was isolated from peripheral blood samples, according to the manufacturer's protocol using the Qiagen Mini Kit (Qiagen, Valencia, CA). DNA quality and concentration parameters were measured on the NanoDrop 2000c

Thermo Fisher Scientific Inc. and Qubit 2.0 (Life Technologies Inc.) After extracting the sample, the DNA was processed according to the Agilent protocol (March 7.3, 2014)

Microarray and karyotyping experiments

For each sample, approximately 500,000 SNPs were used in a genetic manner using the Arsenity GeneChip Human Mapping 500K Array(Yingjun et al., 2017). The microarrays are composed of 500K human mapping of the two stories, Affymetrix array NspI and Stery group. Also, some samples were analyzed on the early 500K human access map. All matrices were processed at the Microarray facility at the Applied Genome Center (TCAG) according to the manufacturer's instructions.

CNV analysis

CNVs, gain or loss of genomic material, were initially derived through comparative analysis of hybridization density using a dChip analyzer. After normalization, we used the Hidden Markov model (HMM) to infer the number of DNA copies of raw signal data. In addition, the data were also analyzed using the CNAG program, which is an improved algorithm for analyzing the number of copies of the human genome . The advantages of this algorithm are to improve signal-to-noise ratios (S / N) and to use an improved reference. To make the most of CNV detection and data validation, microarray data were also analyzed using another program called GEMCA. The NspI and StyI chips data analysis was performed separately as well as by combining both chips together in the analysis.

Analysis of the CNVs

CNV calls using real-time SYR-Green PCR (qPCR) were verified using FOXP2 as an amplicon control, where CNVs are not seen in this region in the general population. At least two independent assays were used to confirm the CNV. For hemizygous deletion of the loci X chromosome in males, standard PCR interactions were performed using primers within the deletion region and the products were analyzed on the agarose gel.

Selection of the Loci

In order to determine the gene / locus susceptibility to autism, 500K SNP microarray data were used to infer CNVs on the X chromosome in autism. To exclude polymorphism number of copies (CNPs), the presence of these CNVs was examined in the 1652 controls and the Genomic Variables Database (DGV). Existing CNVs were ignored in controls or in DGV. The rest of CNVs were classified as autism-specific events and were marked for validation using SYR Green-I PCR Quantum (qPCR), in probands and their family members. Further CNV studies that were either de novo or moved from unaffected mothers to the affected children were followed up. No deletion was observed in male patients who had known overlap with genetic variables in controls, under the hypothesis that CNV in the X-linked recessive gene may be present (and harmless) in female control to date psychologically relevant in hemizygous males . In specific areas of CNV, candidate genes were selected based on the function or profile of the gene expression or physical state.

Results

X chromosome CNVs in ASD cases

Affymetrix GeneChip® Human Mapping 500K SNP (NspI) and StyI Chips were used to examine ASD 50 genomes to assess the genomic abnormalities that may be associated with the disease. Using high-density SNP microarray ~ 10,000 SNP markers across the X chromosome, with an average marker density of ~ 15 kB, the genotyping that makes us able to summon variations and set the limits of these variations with relatively high accuracy. In order to select appropriate controls, we used SNP to classify the strain from samples and showed that more than 90% of samples had European origins. A total of 22 chromosome X chromosome chromosomes were detected among the ASD 50 samples (Table 1). Among these, 20 CNVs were gain (duplication) and 2 loss (deletion). These CNV studies have been further analyzed for interfering with multivariate CNVs in 500 PopGen controls, 1152 controls for Ontario and CNVs annotated in DGV. It was observed that among these variables, 16 CNVs were specific to autism, as they were not identified in any of the controls . Autonomous CNVs were further classified as rigid autistic, especially if CNV was selected by more than one of the three algorithms (DCHIP, CNAG or GEMCA), or on the NspI and StyI arrays. With these criteria, the number of specific = 12 specific CNVs was determined .

The variations in the number of copies identified are set from a few kilobases (KB) to several megabytes (MB) in size, some of which include functionally assigned candidate genes such as IL1RAPL1, TSPAN7, NLGN4 and FMR1, or genes unknown to date function of interest due to homogeneity with genes The function is known, for example PTCHD1 and IL1RAPL2. Two autism-specific CNVs, deleting 104 kB in Xq21.31 and 121 kB deletion in Xq27.3 do not involve any known RefSeq genes. However, these areas may contain some new or unusual genes or regulatory regions that may play a role in autism. Other areas of interest include Xq28, Xq25 and Xp22.31, which have CNVs in unrelated situations. Duplication in Xq28 was found in N = 4 autistic probes - one of these gains involves the genes of FMR1 and FMR1NB. This region has been reported by a modest association with autism, and the hypermethylation of the FMR1 gene is a known cause of mental retardation and autism at times. Therefore, our results in the CNV at this site reinforce the importance of detailed analysis of this CNV.

Table 1 Chromosome X CNVs identified in 50 ASD cases.

Type of CNVs	Analysis Results		
	Total CNVs	Gain	Loss
All Chromosome X CNVs	22	20	2
Autism Specific	16	13	3
Autism Specific Stringent	12	7	5

Validation of interesting sites and identification of autistic candidate genes was confirmed.

N = CNN interest areas that extend and / or candidate candidate genes to follow . Among these, six CNVs were gains and five CNVs were losses. We have tried to validate these CNV files by standard calls qPCR, SNP and PCR (for deletions in male probes). 5out 6CNVs have been successfully confirmed; however, the gain of 741 kB at Xp27.3-q28 (when FMR1) is not validated by qPCR. Maybe this CNV call was wrong. We discovered 167 KB (chrX: 23,114,179-23,281,723; NCBI Build 36) running on exon 1 and the upstream zone in the corrected region containing one gene (PTCHD1, NM_ 173495.2) in Xp22.11).

The deletion also extends to a pre-encoded small RNA (NC-RNA), DA355362 (PTCHD1AS2). Initially, the deletion was validated by amplifying the PCR from the primers that extend to the exon sequence 1. Failure of the PCR reaction in amplification in case 11 as well as in the infected twin brother. To check for this deletion in other family members, qPCR tests were performed. Chapter analysis revealed that the CNV was inherited from the carrier

mother, resulting in the abolition of PTCHD1 in case 11 and his infected twin brother, while their sister was not affected by the carrier (Figure 1). In silico analysis, PTCHD1 888 is a protein of long acidic membranes. The amino acid contains an incomplete region containing twelve heels across the membrane, strongly associated with the hedgehog receptors (Hh) PATCHED1 (PTCH1) and PTCH2, as well as N1 (H1), one of the main signaling pathways involved in the membrane formation of the neural tube and brain, Distinguish between internal and autonomic motor neurons. Mutations were reported in SHH (MIM 600725), in patients with developmental abnormalities, delayed speech acquisition and learning difficulties. Niemann-Pick type C1 is a disorder in the transfer and estimation of cholesterol, and involves a neurological and intellectual deficit (MIM 257220). This led us to investigate the potential role of PTCHD1 as a candidate candidate for ASD and ID.

Figure 1 The genomic area shows the deletion of 167 kB (blue line) at Xp22.11 which includes exon 1 of the PTCHD1 gene.

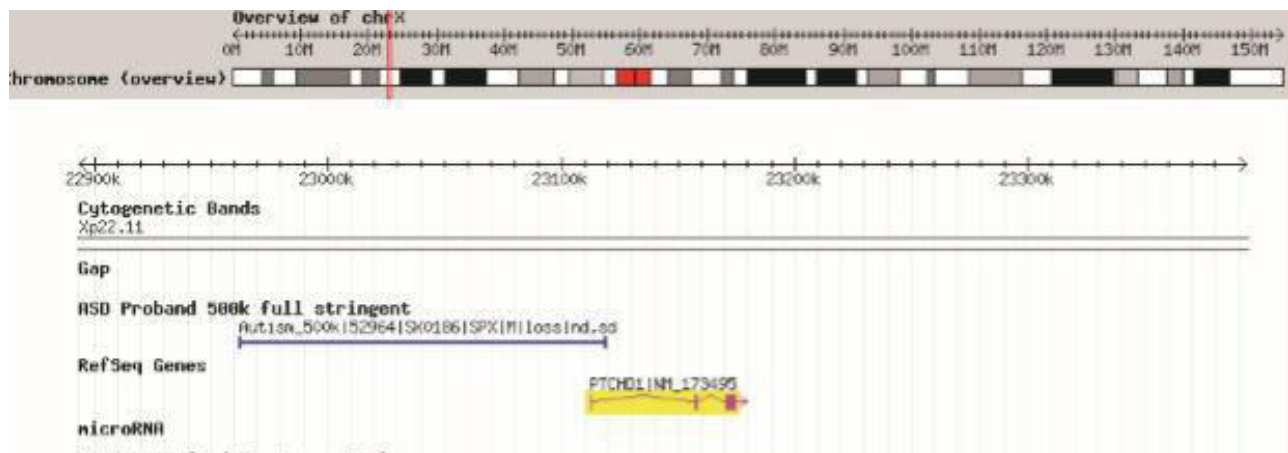


Figure 2. The genomic zone shows the deletion of 82 kB (blue line) at Xp21.3 in the intron 5 gene IL1RAPL1.

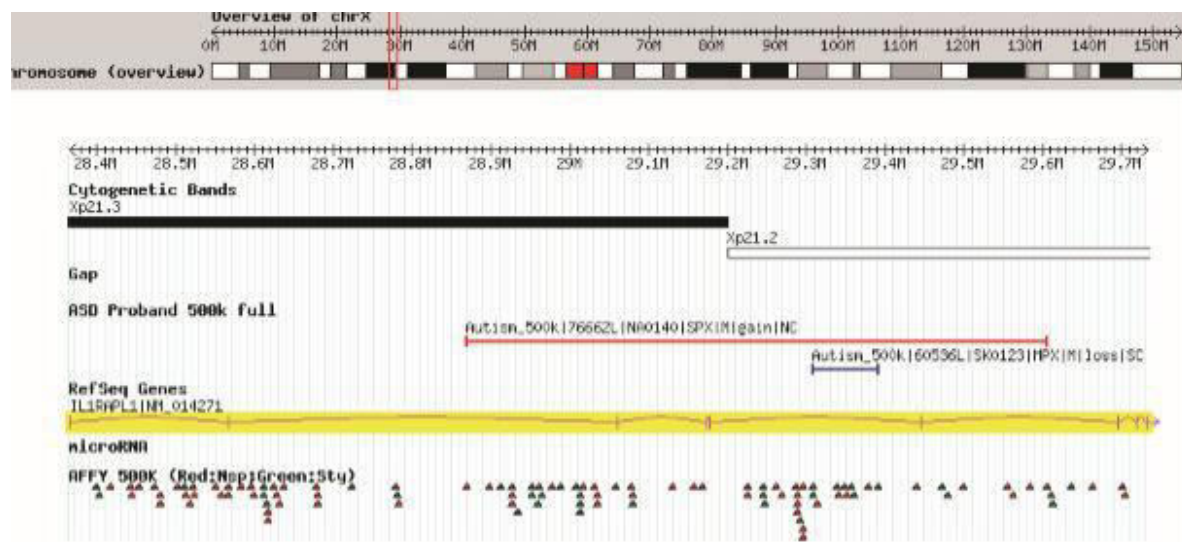


Figure 3. The genomic region shows a duality of 485 kb (red line) at Xq22.3 containing IL1RAPL2 gene.

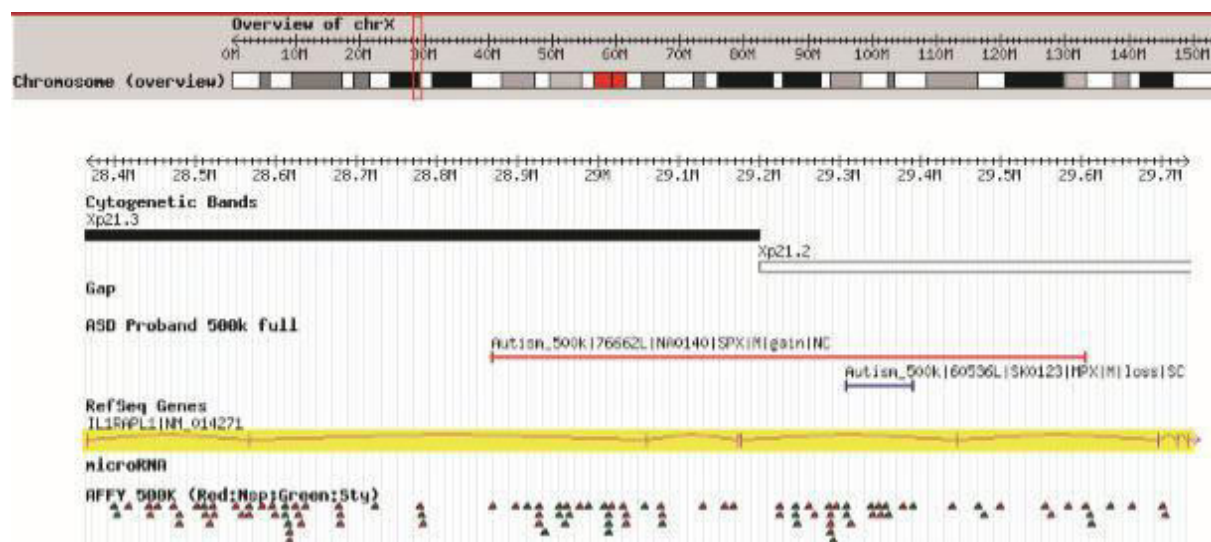
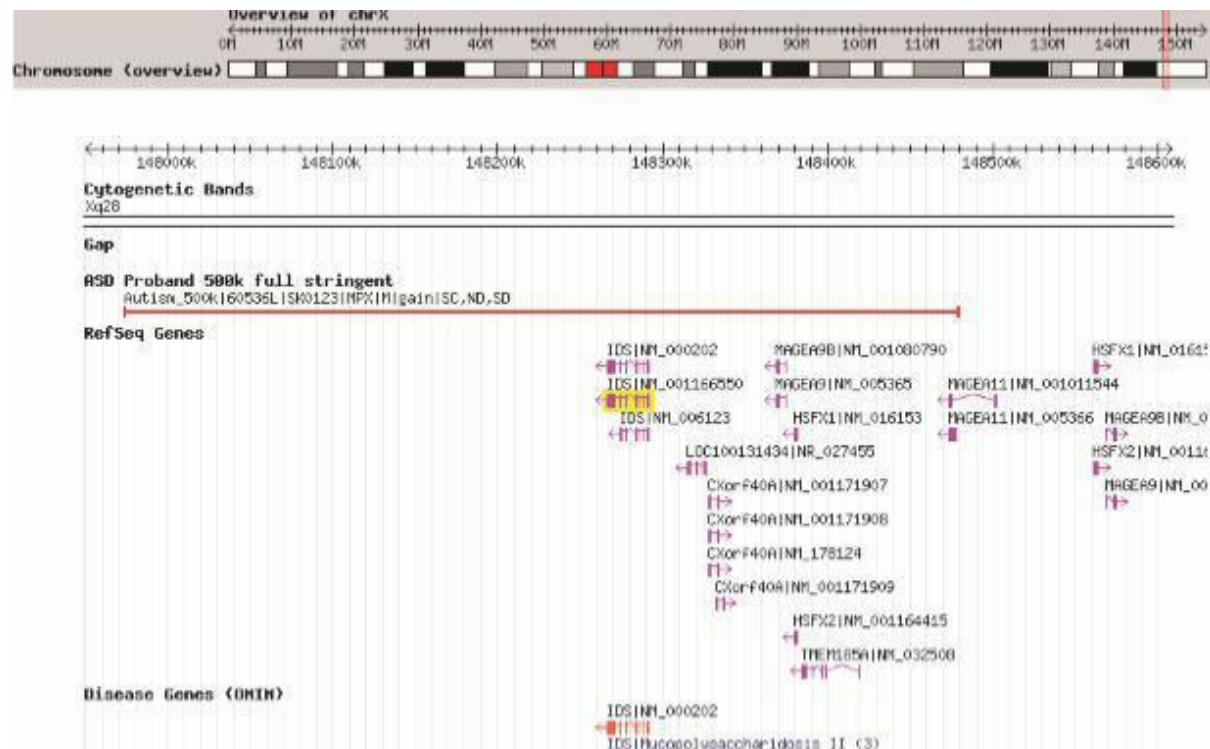


Figure 4. The genomic region shows a 505 kb (red line) duplication in the IDS position (Xq28)



Discussion

In the present study, we distinguished the CNV on the X chromosome to identify the autistic genes. X chromosome is particularly important in mapping genes involved in the causes of autism and identity. Using high-density SNP arrays, we identified 50 X chromosomes in CNVs in non-autistic pathogens. In contrast to CNVs at the genome level, where the number of deletions and duplicity was approximately equal, on the X chromosome, the deletion number was much lower than the frequency. This may be due to the fact that the males are hemizygous for the X chromosome and deletions can probably not be tolerated. After filtering the CNVs in the controls, N = 16 CNV was found to be autistic. Between autism and CNV, N = 12 was derived by more than one algorithm.

We attempted to identify the candidate genes for autism by characterizing regions associated with CNV. One of our findings was convincing identifying several CNVs that directly involved ID syndrome or previously unknown syndrome. For example, we identified the deletion of 85 kilobytes (KB) of IL1RAPL1, which is a member of the interleukin-1 receptor family, which was previously involved in a non-inflammatory identifier. We also identified 485 kb of duplication in a closely related gene, IL1RAPL2. (Lovrečić et al., 2018) 175 KB was identified in IL13RA2, another member of the interleukin family. Based on these findings, we suggested the possibility of these genes being involved in autism. Subsequent studies supported our findings by reporting IL1RAPL1 mutations in autism. A study by Bhatt et al. Reported a basal reflection, which led to the disruption of the IL1RAPL1 gene in an autistic and specific patient. Piton et al arranged the IL1RAPL1 and IL1RAPL2 encoding regions in autistic patients and determined the height of the frames in IL1RAPL1 in autism. However, they failed to identify any coding mutations in IL1RAPL2. (Siu et al., 2016)

We also identified an increase of 121 kb that extends to more than 5 exons from the TSPAN7 gene. The protein encoded by this gene is the cell surface protein that may control neuronal growth. This gene has already been implicated in non-XLID syndrome. First, al-Obeidi and others reported the deletion of two key points in this gene in

a specific patient. Second, mutations in this gene were reported in 4 male patients with unspecified XLID. Thus, TSP7 is another participant in XLID, and in our study we found a disease associated with CNV that also included this gene. Based on our findings, we proposed TSP7 as a candidate for autism. Recently, a large-scale serial study was conducted by Petton et al. The wrong mutation has been identified in two autistic patients in this gene, further supporting our findings. The authors also serialized 190 ethnically identical controls and could not determine this variable in any of the controls. Interestingly, a similar error variable was also reported by another group.(Rollins, 2017; Velinov, 2019).

References

- Alvarez-Mora, M. I., Escalona, R. C., Navarro, O. P., Madrigal, I., Quintela, I., Amigo, J., . . . Carracedo, A. (2016). Comprehensive molecular testing in patients with high functioning autism spectrum disorder. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 784, 46-52.
- Demily, C., Lesca, G., Poisson, A., Till, M., Barcia, G., Chatron, N., . . . Munnich, A. (2018). Additive Effect of Variably Penetrant 22q11. 2 Duplication and Pathogenic Mutations in Autism Spectrum Disorder: To Which Extent Does the Tree Hide He Forest? *Journal of autism and developmental disorders*, 1-4.
- Do, H. D., Bui, T. P. H., Ly, T. T. H., Pham, T. D. L., Tran, T. K., Le, T. T. H., . . . Nguyen, T. L. (2017). AB090. Preliminary results of array CGH test in Vietnamese children with autism spectrum disorder. *Annals of Translational Medicine*, 5(Suppl 2).
- Lovrečić, L., Rajar, P., Volk, M., Bertok, S., Stražišar, B. G., Osredkar, D., . . . Peterlin, B. (2018). Diagnostic efficacy and new variants in isolated and complex autism spectrum disorder using molecular karyotyping. *Journal of applied genetics*, 59(2), 179-185.
- Luo, M., Fan, J., Wenger, T. L., Harr, M. H., Racobaldo, M., Mulchandani, S., . . . Conlin, L. K. (2017). CMIP haploinsufficiency in two patients with autism spectrum disorder and co-occurring gastrointestinal issues. *American Journal of Medical Genetics Part A*, 173(8), 2101-2107.
- Mak, S.-I. (2015). Copy number variation in Chinese patients with autism spectrum disorder. *HKU Theses Online (HKUTO)*.
- Richard, A. C., Rovelet-Lecrux, A., Delaby, E., Charbonnier, C., Thiruvahindrapuram, B., Hatchwell, E., . . . Scherer, S. W. (2016). The 22q11 PRODH/DGCR6 deletion is frequent in hyperprolinemic subjects but is not a strong risk factor for ASD. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 171(3), 377-382.
- Rollins, N. K. (2017). An Imaging Glimpse into the Autistic Brain. In: Radiological Society of North America.
- Siu, W.-K., Lam, C.-W., Mak, C. M., Lau, E. T.-K., Tang, M. H.-Y., Tang, W.-F., . . . Leung, P. W.-L. (2016). Diagnostic yield of array CGH in patients with autism spectrum disorder in Hong Kong. *Clinical and translational medicine*, 5(1), 18.
- Velinov, M. (2019). Genomic copy number variations in the autism clinic-work in progress. *Frontiers in cellular neuroscience*, 13, 57.
- Worachotekamjorn, J., Hansakunachai, T., Rojnueangnit, K., Roongpraiwan, R., Ruangdaraganon, N., Wattanasirichaigoon, D., . . . Limprasert, P. (2017). AB051. Chromosomal microarray analysis in a

large cohort of Thai patients with autism spectrum disorder. *Annals of Translational Medicine*, 5(Suppl 2).

Yingjun, X., Haiming, Y., Mingbang, W., Liangying, Z., Jiaxiu, Z., Bing, S., . . . Xiaofang, S. (2017). Copy number variations independently induce autism spectrum disorder. *Bioscience reports*, 37(4), BSR20160570.

Synthesized of Thermally Isolated Antibacterial (Silicone/TiO₂) Nano-composites Paint for Reservoirs

NOOR HADI AYSA¹

1. Dept. Clinical laboratory Sciences, college of Pharmacy, University of Babylon, Iraq.

E-mail: phar.noor.hadi@uobabylon.edu.iq, noorpharmacy83@gmail.com.

Abstract:

Developments in thermally insulated and antibacterial paints using antibacterial nanoparticles (ANC) can be a promising project for appropriate environmental applications. Here, we are photographing a tactic to create an antibacterial coating of metal nanoparticles (MNPO), in one step. The well-dispersed MNPO-in-liquid silicone can be used directly in almost all types of surfaces such as wood, glass, steel and plastic. The reservoirs surfaces coated with silicone / nanoparticle TiO₂ coatings showed superior properties by killing bacteria. Nanocomposites are created by adding MNPO with silicone rubber base and on the surface. Samples have been tested against bacteria that are transmitted due to water pollution and cause diseases that cause diarrhea and cholera. The electron microscope indicated any topographical damage after exposure to nanoparticles. The current work consists of silicone rubber nanocomposites as fixed anti-bacterial levels to prevent injury in high-risk environments and surrounding conditions. TiO₂ nanoparticles will be used for reflective infrared radiation, coated with a clear resin, coating for infrared absorption of solar ultraviolet radiation. It also has the advantage of not having great ultraviolet radiation, a property that can improve the weather conditions of any exposed material to the sun.

Keywords: Nanoparticles, antibacterial, paint, water tank, Cholera.

How to cite this article: Aysa NN (2019): Synthesized of thermally isolated antibacterial (Silicone/TiO₂) Nano-composite paint for reservoirs, *Ann Trop Med & Pub Health*; 21: SP2048-19

Introduction: Recently, serious health problems have been exacerbated by contamination of drinking water tanks [1-3]. Control and reduction of bacterial infections in high risk areas is a major challenge. Contamination of reservoirs is associated with more than one reason, the most important of which is the loss of chlorine during the storage period, and the effect of high temperature during the summer months, and therefore contain no reservoirs in any way to save people from harmful water pollution [4,5].

The main contaminants are sediments that coexist with microbial contamination either as a result of bacteria, viruses or parasites such as cholera, salmonella, skin swelling and many other diseases [6-8]. Sediment produces a source of nutrient feed, which multiplies and increases in water in the reservoir. The colonies form a sticky gelatinous layer on the walls of the reservoir and constitute a constant source of

pollution, posing a threat to the public health of humans. The use of high-power motors that pull liquids that surround the water networks in the case of cracks, which confirms water pollution of the aquatic organisms [9-12].

Over the past few years, vibrio has shown cholera resistant to many drugs that kill bacteria used to treat cholera, and remind specialists to look for more effective and safe alternatives to use as another option or as an adjuvant. Therefore, the nature of materials used to manufacture reservoirs plays a role in preventing water pollution [13, 14].

Corrosion of metals and plastics when exposed to weather factors deteriorates and produces units of plastic structure, which are carcinogenic [15-17].

The technological developments in the field of water and the need to use high-efficiency storage materials to solve problems and add an economic dimension that really affects the way of life and to cope with the sudden progress in industrial development, everyone should use the latest and best to cover his place and fulfill the best way because the old roads After the advent of solutions that are the speed of use and maximum protection in addition to the savings and long life and therefore the use of nanomaterials sterilization and thermal insulation of various enterprises is a very urgent need to maintain the degree of freedom and to prevent the growth of bacteria. The authors also report that free radicals are identified to reduce mineral salts to their unsaturated metal particles [18,19].

Because of this, we have taken advantage of the free radicals created through the natural drying process of drying to prepare TiO₂ nanoparticles. The TiO₂ NP compact paints are of particular benefit due to their microbial activity. Several methods for the preparation of inorganic-organic compounds have been reported; most of the techniques used to incorporate metals into silicon matrices include either chemical reactions such as reduction, [6] mixing of pre-formed metal nanoparticles with silicon [7] or complex physical technical methods [8]. TiO₂-based compounds are high antimicrobial agents because of their properties in killing germs for several types of small bacteria. Antimicrobial agents based on TiO₂ receive considerable notice, due to the low toxic quality of the active TiO₂ ion of human cells [20], as well as being a long-lasting chemical in life with high thermal stability and low-risk nature due to the larger space area per unit volume [4,1]. The catalytic activity of the reactive oxygen species produced by the catalytic reaction is the main cause of the antibacterial effect.

Materials and methods of work:

The research is based on the use of nanoparticles and their integration with liquid silicone rubber, which is used for medical purposes. It is chemically treated and stabilized rather than decomposed to produce heat-resistant paints and deadly bacteria.

TiO₂ nanoparticles were produced through individual ratios and observed to kill and inhibit the growth of cholera bacteria. The metal oxide (TiO₂) was dissolved in the chemical to remove the paint, and was added to the chloroform solution. The mixture was painted on glass slides and left in the air to dry, causing oxidation and subsequent formation of a coating free of silicone scratches embedded from the MNPO.

Sample preparation for germ-killing tests: Glass slides (2.5×7.5 cm) were coated with the TiO₂ NP-embedded paint by simple inundation into TiO₂ NP-embedded paint or spray coating with the TiO₂ NP-embedded paint followed by drying in air. Both

methods produced fully plated glass samples. The slides were left in one day or heated at 60 °C for 20 min to make sure that the drying process was fully complete, so that a scratch-free film was formed. Usually, a 100 µl suspension of *Vibrio cholera* in 0.1M PBS was added to 20 ml of the yeast–dextrose broth in a 50 ml sterile centrifuge tube, followed by shaking at 200 r.p.m and 37°C overnight. Bacterial solution was sprayed separately on available glass slides coated with TiO₂NPs. The glass slides (2.5 x 7.5 cm) were coated with TiO₂ NP coating by simple dumping in TiO₂ NP coating or sepray coating with TiO₂ NP coating followed by drying in the air. Both methods produced samples of fully coated glass. The slides were left in one day or heated at 60 °C for 20 minutes to ensure that the drying process was completely complete, so that the film was formed free from scratches. Typically, a 100 µl suspension of *Vibrio cholera* was added in 0.1M PBS to 20 mL of yeast-dextrose broth in a sterile 50 ml centrifuge tube, followed by shaking at 200 rpm and 37 °C overnight. The bacterial solution was sprayed separately on available TiO₂NPs coated glass slides.

Results and discussion: FTIR description of the prepared (silicone/TiO₂ nanocomposites paint surface: We record an FTIR spectrum of the TiO₂ sample, we water down nanocomposites material in KBR at maximum 1%wt. Of TiO₂

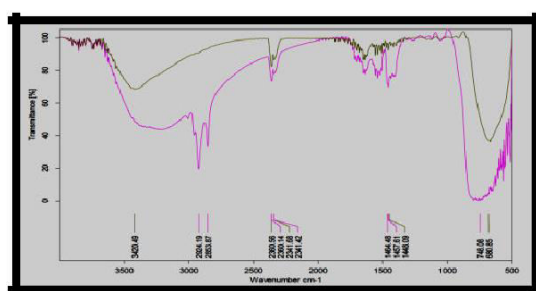


Fig. 1 FTIR for thermally isolated germ-killing (silicone / TiO₂) nanocomposites paint surface coated with TiO₂ NPs.

The infrared spectrum of the TiO₂ nanoparticles created was between 500-6000 cm⁻¹ and characterized the chemical bonds as well as the functional group in the compound. The strong absorption corresponds to the frequency zone ranging from 400-1000 cm⁻¹ with Ti-O-Ti bonds and refers to the formation of titanium oxide coating. The wide and intensive range of less than 1200 cm⁻¹ is due to Ti-O-Ti vibrations.

X-ray for nanocomposites paint surface.

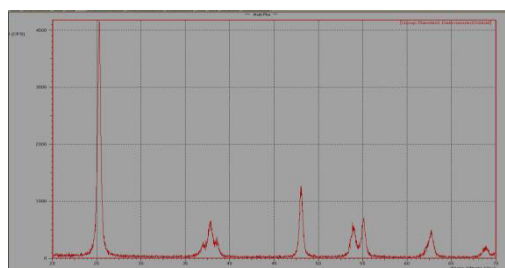


Fig.2 X-ray for thermally isolated antibacterial (silicone / TiO₂) nanocomposites paint surface coated with TiO₂ NPs

Two diffraction peaks were found at $2\theta = 25.28^\circ$ and $2\theta = 37.8^\circ$, which belong to anatase peak became more pronounced, suggesting that with absolutely nothing else TiO_2 mixed films are received directed toward C [5,6]. The above XRD results indicate that the structure of films has changed from an amorphous phase to an anatase phase with an increase in temperature (T_S) at 20°C

Scanning Electron Microscopy Results

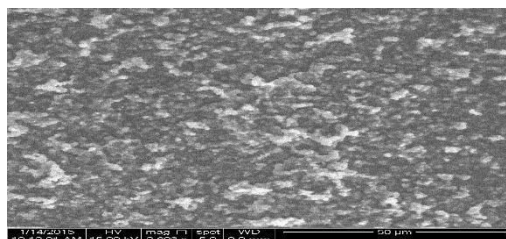


Fig.3 Scanning Electron Microscopy Results for thermally isolated antibacterial (silicone / TiO_2) nanocomposites paint surface coated with TiO_2 NPs

The SEM test did not indicate any topographical damage after exposure to MNPO and a homogeneous distribution within the silicon base and surface blocker that ensures that all liquid is exposed to antibacterial effects.

The inhibitory activity of TNPs By Agar Well-Diffusion:

The inhibitory activity of TNPs was tested by Agar Well-Diffusion: 50 isolates of *Vibrio cholera*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and DMSO was used as a negative control. TiO_2 solution at its three concentrations showed high and clear inhibitory efficiency for all isolates studied and for all concentrations (Table 1). The diameter of the inhibitor ranges from 12-25 mm for 20 g / ml, (15-35) mm for 30 g / ml and (22-40) mm for 40 g / ml of TiO_2 nanoparticles.

Table 1: Inhibition zone diameters (mm) of different concentrations of TiO_2 NPs against bacterial growth.

TiO_2 concent ration $\mu\text{g/ml}$	Inhibition zone diameters (mm)		
	<i>Cholera</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
20	12	19	24
30	15	20	33
40	35	22	38

Conclusion:

TiO_2 NPs / silicon coating was created. The electron microscope showed that TiO_2 NPs was integrated throughout the silicon matrix, but mostly lived on the silicon surface and confirmed that MONPs were also incorporated into all silicon bulk parts and incorporated on the surface. The catalytic activity of the reactive oxygen species induced by the catalytic reaction in the MNPO molecules (TiO_2) is the main cause of the observed killing effect of microbes.

At some concentrations, TiO_2 is not toxic to mammalian cells and is widely used in medical care and dental care. It has antimicrobial and antifungal properties and was previously used as a topical disinfectant. Are still on the WHO list despite being replaced with new drugs in medical treatment. For the first time, silicone and TiO_2 NPs were tested for bacterial killing activity against cholera and *S. aureus*. Results showed a clear bacterial response. In order to get a better view of the mechanism that works inside the silicone coating / TiO_2 . This may be due to any energy stored inside the TiO_2 lattice structure that allows the electrons and holes on the surface to interact with the light sensor, which may improve the effect of the bactericidal agent.

These antibacterial surfaces have the potential to maintain low bacterial levels and reduce the risk of bacteria spreading around reservoirs. It can be applied to a wide range of medical devices and surfaces that are often touched such as phones, keyboards and iPad covers. There is a synergistic promotion of microbial activity when NPs and silicone are combined, compared to silicone alone, which is considered to be useful for health uses in a medical setting.

References

1. Amal Talib Al-Saady; Habeeb Sahib Naher "study of routes of etiologic bacteria causing neonatal infections in Al-Hilla city, Iraq", *Biochem. Cell. Arch.* Vol.18, No.1, pp.577-586.
2. Samah Ahmed Kadhum, "the effect of two types of Nano-particles (ZnO , SiO_2) on different types of bacterial growth", *Biomedical & pharmacology journal*, Vol.10 (4), pp-1701-1708, 2017.
3. Rasha Hadi Saleh, Nada Khazal Kadhim Hindi, Mohammed R. Abid Ali "Antibacterial Activity of Aquatic Zea Mays L. Hairs Extract against Different Bacteria in Babylon Province: An In-Vitro Study", *Journal of Global Pharma Technology*, ISSN 0975 -8542, 2017.
4. Noor Hadi Aysa, "Preparation and Surface Modification of Silica Nanoparticles for Superhydrophobic Coating", *Journal of Babylon University/Pure and Applied Sciences/ No.(1)/ Vol.(26):* 2018.
5. Noor Hadi Aysa, Halah Dawood Salman, "Antibacterial activity of modified zinc oxide nanoparticles against *Pseudomonas aeruginosa* isolates of burn infections" *WSN 33 1-14 EISSN 2392-2192*, (2016).
6. Noimark S, Allan E, Parkin IP. Light-activated antimicrobial surfaces with enhanced efficacy induced by a dark-activated mechanism. *Chemical Science*, Vol. 5 (6): pp 2216-2223, (2014).
7. Azam A, Ahmed AS, Oves M, Khan MS, Memic A. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and – negative bacterial strains. *International Journal of Nanomedicine*.;7:3527-3535, (2012).
8. Smijs TG, Pavel S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. *Nanotechnology, Science and Applications*, 4:95-112, (2011).
9. Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of cholera in endemic countries. *PLoS Negl Trop Dis*. 2015;9(6):e0003832.
10. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Control Release*. 2011;156(2):128–145

11. Chernousova S, Epple M. Silver as antibacterial agent: ion, nanoparticle, and metal. *Angew Chem Int Ed*. 2013;52(6):1636–1653.
12. Palza H. Antimicrobial polymers with metal nanoparticles. *Int J Mol Sci*. 2015;16(1):2099–2116.
13. Park CM, Chu KH, Heo J, et al. Environmental behavior of engineered nanomaterials in porous media: a review. *J Hazard Mater*. 2016;309:133–150.
14. Gao W, Fang RH, Thamphiwatana S, et al. Modulating antibacterial immunity via bacterial membrane-coated nanoparticles. *Nano Lett*. 2015;15(2):1403–1409.
15. Dumitriu C, Popescu M, Ungureanu C, Pirvu C. Antibacterial efficiencies of TiO₂ nanostructured layers prepared in organic viscous electrolytes. *Appl Surf Sci*. 2015;341:157–165.
16. Yan X, Dai H, Li S. Preparation of nano-TiO₂ by liquid hydrolysis and characterization of its antibacterial activity. *J Wuhan Univ Technol Mater Sci Ed*. 2014;29(2):407–409.
17. Ashkarran AA, Hamidinezhad H, Haddadi H, Mahmoudi M. Double-doped TiO₂ nanoparticles as an efficient visible-light-active photo-catalyst and antibacterial agent under solar simulated light. *Appl Surf Sci*. 2014;301:338–345.
18. Joost U, Juganson K, Visnapuu M, et al. Photocatalytic antibacterial activity of nano-TiO₂ (anatase)-based thin films: effects on *Escherichia coli* cells and fatty acids. *J Photochem Photobiol B*. 2015;142:178–185.
19. Charpentier PA, Burgess K, Wang L, Chowdhury RR, Lotus AF, Moula G. Nano-TiO₂/polyurethane composites for antibacterial and self-cleaning coatings. *Nanotechnology*. 2012;23(42):425606.
20. Gao W, Fang RH, Thamphiwatana S, et al. Modulating antibacterial immunity via bacterial membrane-coated nanoparticles. *Nano Lett*. 2015;15(2):1403–1409.

Corneal Epithelial and Stromal Thickness Profile in Keratoconus Using Anterior Segment Optical Coherence Tomography: A Comparative Study

Yousif Farhan Dawood¹, Ammar F. Issa², Laya K Omar³

1. Department of Ophthalmology, College of Medicine, University of Anbar, Anbar, Iraq & Department of Ophthalmology, Ibn Al Haitham Teaching Eye Hospital, Baghdad, Iraq
2. Department of Ophthalmology, Ibn Al Haitham Teaching Eye Hospital, Baghdad, Iraq & Jenna Ophthalmic Center, Baghdad, Iraq
3. Department of Ophthalmology, Ibn Al Haitham Teaching Eye Hospital, Baghdad, Iraq

Abstract

Aim of the study

To assess the spatial of thickness profile of epithelium and stroma of the cornea in keratoconus and healthy eyes using anterior segment spectral-domain optical coherence tomography (SD-OCT).

Patients & method

Corneal pachymetry scans using SD-OCT were analyzed in 120 eyes, including 80 eyes from 46 keratoconus patients and 40 eyes from 20 healthy subjects.

Epithelial and stromal thickness was measured in 9 points, 1.25 millimeter between each point, through the central 6 millimeter of the cornea in both 90 and 180 meridians by using RTVue-100 SD-OCT (Optovue, California, USA).

Results

The mean maximum keratometry measurements in keratoconus and healthy eyes were 47.8 ± 3.1 D (range, 42.9–52.8) and 44.8 ± 2.1 D (range, 41.5–47.3), respectively, with mean corneal astigmatism of 4.6 ± 2.4 D (range, 0.75–8.5) and 2.75 ± 2.1 D (range, 2.0–5.75), respectively. The mean epithelial thickness was $52.81 \pm 4.31 \mu\text{m}$ in keratoconic eyes and $54.43 \pm 1.18 \mu\text{m}$ for controls ($p=0.046$). Epithelial thickness was significantly thinner ($p<0.05$) in keratoconus at the corneal center, 1.25mm on each side of the center on the horizontal axis, and at 1.25mm below the center. Epithelial thickness fluctuated considerably in keratoconus group, in some cases there was a distinction of up to $40 \mu\text{m}$ among various points of the same eye. The overall pattern of epithelial thickness for keratoconus was irregular across the cornea compared to a regular uniform pattern for the controls. The mean stromal thickness was $445.48 \pm 35.01 \mu\text{m}$ in keratoconic eyes and $507.84 \pm 34.87 \mu\text{m}$ for controls ($p<.001$). Stromal thickness was significantly thinner ($p<.001$) in keratoconus at all points compared to control eyes. Central corneal epithelial and stromal thickness was significantly thinner as the keratoconus stage progresses.

Conclusion

Spectral domain optical coherence tomography revealed significant distinctions in spatial of thickness profile of epithelium and stroma of the cornea (central and regional) between keratoconus and healthy eyes. These distinctions may be valuable in providing a new parameter for early discovery of keratoconus.

Keywords: Corneal epithelia, stromal, keratoconu, anterior segment, optical coherence

How to cite this articles: Dawood YF, Issa AF, Omar LK (2019): Corneal Epithelial and Stromal Thickness Profile in Keratoconus Using Anterior Segment Optical Coherence Tomography: A Comparative Study, Ann of Trop Med & Pub Health; 21: SP2049-19

INTRODUCTION

The most common corneal is ectasia is keratoconus. It usually affects both eyes in asymmetric severity [1, 2, 3, 4] and characterized by progressive localized corneal thinning and protrusion. Normally corneal thinning occurs in the central as well as inferotemporal cornea, [5] also superior regions have been mentioned. [6, 7] Keratoconus has a great effect on the visual quality of the subject due to myopia and irregular astigmatism as a result of protrusion of the thinned cornea. It usually appears during the second decade of the life, i.e. during puberty, [2, 8] although it has also been noticed to occur earlier, and later in life and it usually progresses until the fourth decade of life, when it becomes stable. [9] A recent study has found that fifty percent of subjects with unilateral keratoconus will develop the disease in their other eyes in 16 years. [10]

It is known from earlier study with the very high frequency arc-scanning ultrasound (VHF Artemis) system that the corneal epithelium has a remodeling or compensation property, in which epithelium tries to compensate topographic abnormalities of the underlying stromal layer. One of the studies reveals that epithelium becomes thin over steep areas of the cornea as at the cone of keratoconic eyes and becomes thick in the other flat areas. [11]

Scanning high frequency ultrasound (HF-UBM, Artemis), confocal microscopy and anterior segment optical coherence tomography (AS-OCT) are examination devices for studying corneal epithelial thickness. The latter provides both optical corneal layers and also thickness data. Spectral-domain Fourier analysis (SD-OCT) is the most developed AS-OCTs actually use, with compared to older, time-domain tomographers (TD-OCT), Spectral-domain Fourier analysis (SD-OCT) provides significant higher speed and better resolution. The anterior segment optical coherence tomography (AS-OCT) system has another important feature in which the 3D imaging of the cornea can be provided and this gives us a practical and clinical device for mapping of corneal epithelium, with the advantage of accurate, rapid data gaining. [12]

This study evaluated the thickness profile of both epithelial and stromal layers assessed using SD-OCT in eyes with keratoconus and in healthy eyes. Corneal astigmatism ≥ 2.5 D of healthy eyes was chosen in this study to better simulate the possible astigmatic effect on the epithelial/stromal profile and also to decrease the effects of possible differences due to the astigmatism itself.

PATIENTS AND METHOD

A retrospective review of 46 medical records of keratoconus patients who presented to Jenna Ophthalmic Center from December 2016 to April 2018 was conducted. Twenty profiles of healthy eyes of those who were candidates of refractive surgery were taken as the control group provided they have at least 2D of regular astigmatism.

Patients were examined using AS-OCT by RTVue 100 (Optovue, CA, USA), Scheimpflug imaging using Galilei (Zeimer Ophthalmic Systems AG, Switzerland) in addition to subjective refraction, and complete ocular assessment by slit-lamp examination. Keratoconus severity was classified using Amsler-Krumeich classification (Table 1). Exclusion criteria included:

- 1) Eyes with advanced keratoconus changes such as scars or hydrops.
- 2) Scarred corneas from any other cause.
- 3) Operated or traumatized eyes.
- 4) Diabetic eyes.
- 5) Ocular surface disease e.g. dry eyes, allergy, chronic topical drug use and contact lens wear.

Optical Coherence Tomography (OCT) exams

The RTVue 100 Fourier domain OCT system (software version 5.1.0.90) with a corneal-adaptor module (CAM) was used in this study. The system works at 830nm wavelength and has a speed of 26000 axial scans per second. The depth resolution of the system is 5µm (full-width, half-maximum) in tissue. The CAM produces telecentric scanning for anterior segment imaging by using a wide-angle or high-magnification adaptor lens. This study used the wide-angle lens, which provides a scan width of 6.0mm and a transverse resolution of 15µm (focused spot size). Scans were acquired using line scan module centered on the entrance pupil as seen on the real-time OCT image. Then the epithelial and stromal thickness measurements were done with the “Flap Tool” in the RTVue-CAM software. The “Flap Tool” calculation framing is vertical to either the anterior or posterior layers of the cornea and 4 µm was the least thickness difference between each mouse clicks. The user only has to put the center caliper mark at the interface. The software automatically identifies both anterior and posterior corneal boundaries and the results of the measurements (Figure 1).

The studied target for epithelial measurement was from the line representing the epithelial surface to the most anterior portion of the solid white line representing increased reflection that corresponds to epithelium-Bowman interface. Epithelial and stromal thickness were measured in 9 points for each eye including the central point, 4 points on horizontal and 4 points on vertical meridians, 1.25 mm apart across the central 6 mm diameter zone as shown in (Figure 2).

To obtain data from symmetrical points in right versus left eyes, measurements taken from temporal side of each eye were denoted with the negative sign as -1.25 and -2.5 points and those with the positive sign 1.25 and 2.5 represent the nasal points on horizontal axis.

Statistical analysis

Statistical Package for the Social Sciences (SPSS version 20) was used for statistical analysis. Analysis of variance (ANOVA) test was used to compare epithelial thickness measurements of the cornea in keratoconus to normal group, and was used to compare among stages of keratoconus group. P values less than 0.05 were considered statistically significant.

Table 1: Amsler-Krumeich keratoconus classification

Stage 1 -Myopia and or astigmatism <5 D -Mean central keratometry reading <48 D -Eccentric corneal bulging -Vogt's striae, no corneal opacities
Stage 2 -Myopia and or astigmatism 5 -8 D -Mean central keratometry reading 48-53 D -No central opacities -Pachymetry > or = 400 µm
Stage 3 -Myopia and or astigmatism 8 - 10 D -Mean central keratometry reading > 53 D -No central opacities -Pachymetry 200-400 µm
Stage 4 -Refraction impossible to determine -Mean central keratometry reading > 55 D -Corneal scars -Pachymetry < 200 µm

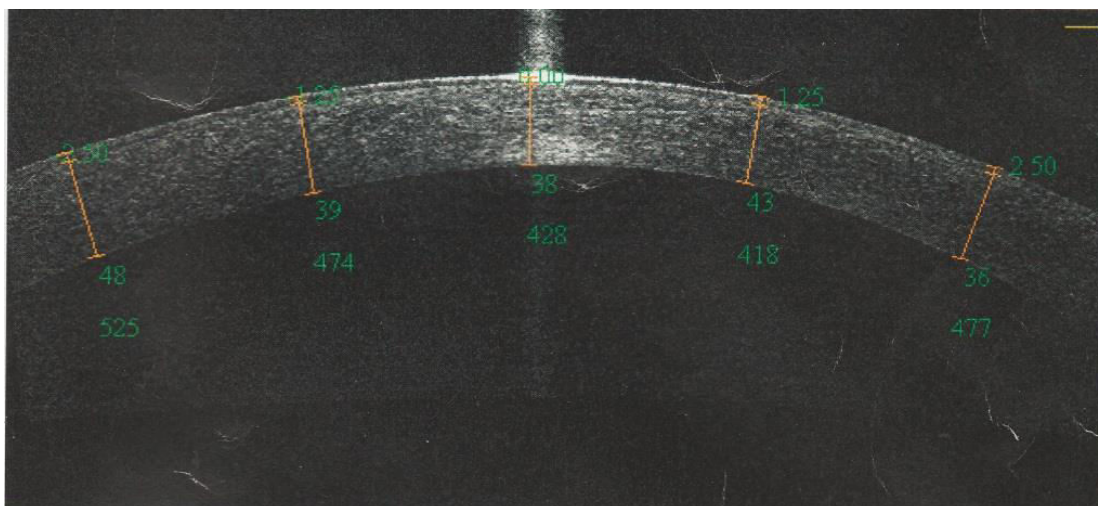


Figure 1: demonstrates the identification of epithelium and Bowman layers by flap tool method in OCT test. The numbers represent position, epithelial thickness and stromal corneal thickness respectively from above to downwards.

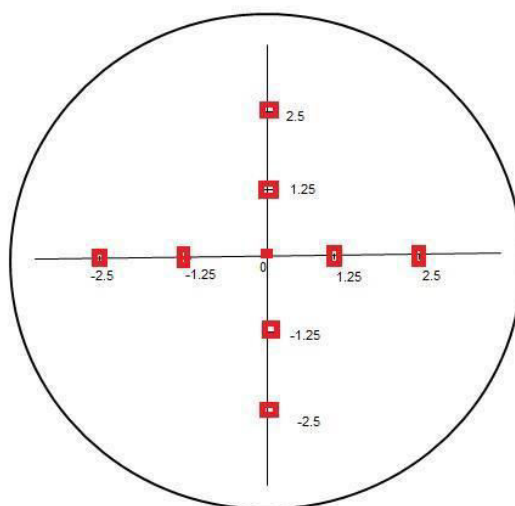


Figure 2: shows points chosen in the right eye to measure epithelium/stromal thickness in both keratoconus and normal eyes

RESULTS

Data analyzed for a total of 120 eyes (66 patients), including 80 eyes from 46 patients with keratoconus and 40 eyes from 20 healthy participants showed that the mean maximum keratometry measurements in keratoconus and healthy eyes were 47.8 ± 3.1 D (range, 42.9–52.8) and 44.8 ± 2.1 D (range, 41.5–47.3), respectively. The mean corneal astigmatism was 4.6 ± 2.4 D (range, 0.75–8.5) and 2.75 ± 2.1 D (range, 2.0–5.75), respectively.

For the keratoconus group, cases were classified into four stages (1, 2, 3, and 4) as shown in (table 2).

Table 2. Stages of keratoconus group according to Amsler-Krumeich classification.

Stage	Number	Percent
1	22	27.5
2	30	37.5
3	9	11.3
4	19	23.8
Total	80	100%

Epithelial thickness comparison

The mean epithelial thickness in keratoconus ($52.81 \pm 4.31 \mu\text{m}$) was slightly less than that of normal eyes ($54.43 \pm 1.176 \mu\text{m}$), ($p=0.046$) as shown in (Figure 3). Statistically, the mean of thickness of epithelium was significantly lower thickness in keratoconus ($P<0.05$) than in normal eyes at multiple points including corneal center, 1.25mm below it and 1.25mm on each side of the center on horizontal axis. There was also statistically significant thickening at 2.5-mm above the center ($p=0.01$) as shown in (Table 3).

Figure 4 shows epithelial pattern in both normal and keratoconus eyes. The epithelium in normal eyes showed relatively uniform pattern across the cornea while in keratoconus there was significant zonal epithelial thickness profile inconsistency with some focal areas of thinning and thickening in irregular manner.

Table 4 shows mean epithelial thickness profiles in multiple points across the 6-mm of the center of the cornea on the 90 and 180 axes in keratoconus eyes according to stages. There was a statistically significant thinning of the epithelium at the corneal center as the keratoconus stage progresses.

Stromal thickness comparison

The mean stromal thickness was (445.48 ± 35.01) μm in keratoconic eyes and (507.84 ± 34.87) μm for controls ($p<.001$) as shown in (Figure 3). Stromal thickness was significantly thinner ($p<.001$) in keratoconus at all points compared to control eyes as shown in (Table 3) and (Figure 5). There was a statistically significant thinning of the stroma at the corneal center as the keratoconus stage progresses shown in (Table 5).

TABLE

Mean epithelial and stromal thickness through the 6-mm of the corneal center in the 90 and180 axes in normal and keratoconuseyes

Horizontal Meridian				Corneal Center	Vertical Meridian				Groups
-2.5 mm	-1.25 mm	1.25 mm	2.5 mm		-2.5 mm	-1.25 mm	1.25 mm	2.5 mm	
53.25±5.37	53.50±4.72	54.40±4.79	56.30±4.72	52.80±6.17	55.45±4.79	53.95±5.04	54.60±4.77	55.60±5.25	Normal
40-66	47-69	47-73	48-70	42-72	50-74	47-73	47-69	48-72	Range (µm)
53.23±6.72	49.31±7.92	51.35±8.28	58.53±9.43	46.95±7.14	54.76±8.44	48.33±6.64	53.54±8.91	59.34±8.20	KC
40-70	30-69	30-73	36-84	30-64	35-76	26-61	30-77	40-80	Range (µm)
0.984	0.003	0.033	0.163	0.000	0.634	0.000	0.483	0.010	P*
519.2±40.7	498.3±36.6	497.4±34.9	513.6±37.4	494.2±34.0	513.1±38.3	493.7±33.4	510.7±43.5	530.5±44.1	Stroma Normal
465.0±45.0	430.5±41.8	428.5±40.4	458.3±43.5	420.2±43.9	458.8±41.4	414.6±40.7	449.6±35.0	483.8±37.6	Stroma KC
.000	.000	.000	.000	.000	.000	.000	.000	.000	P*

* Using t-test
KC = keratoconus

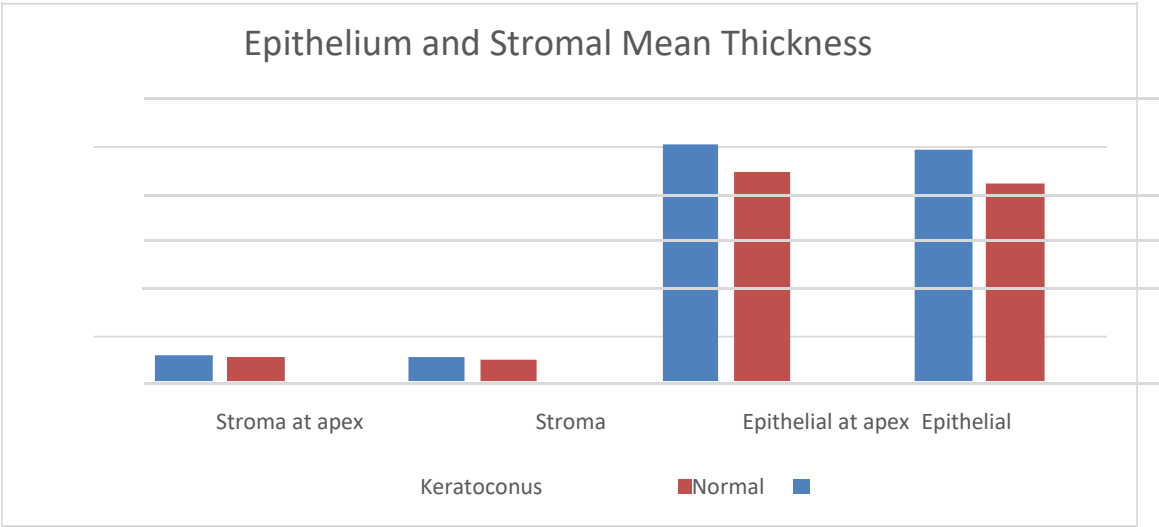


Figure 3: Comparison between keratoconus eyes and controls in overall mean epithelial and stromal thickness and mean thickness at corneal center.

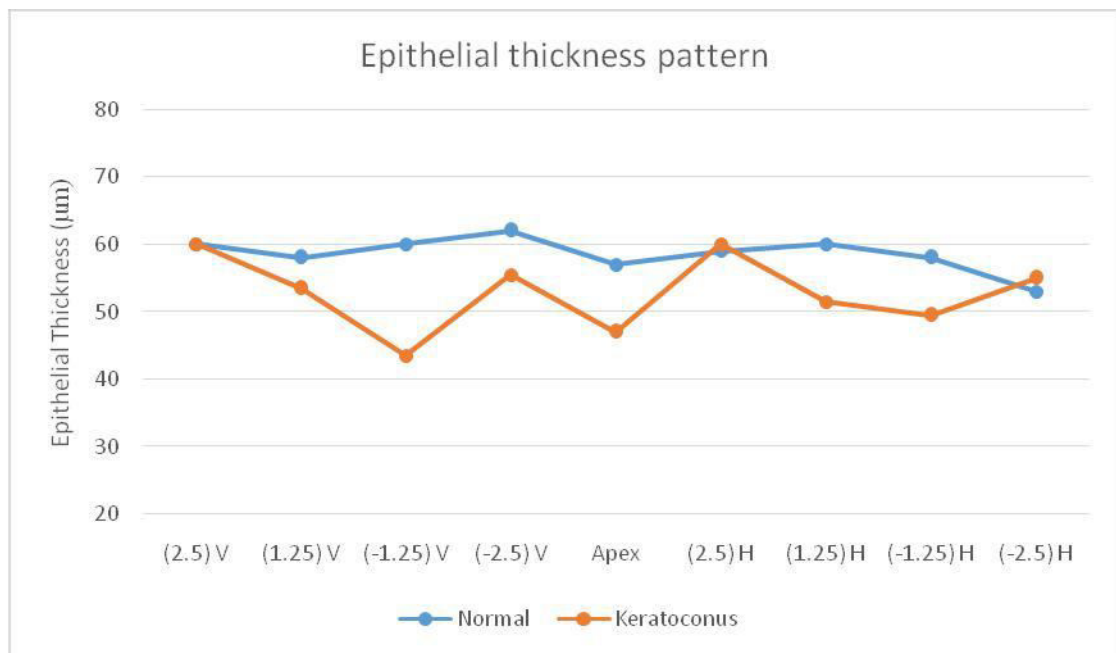


Figure 4: epithelial pattern in normal and keratoconus eyes.

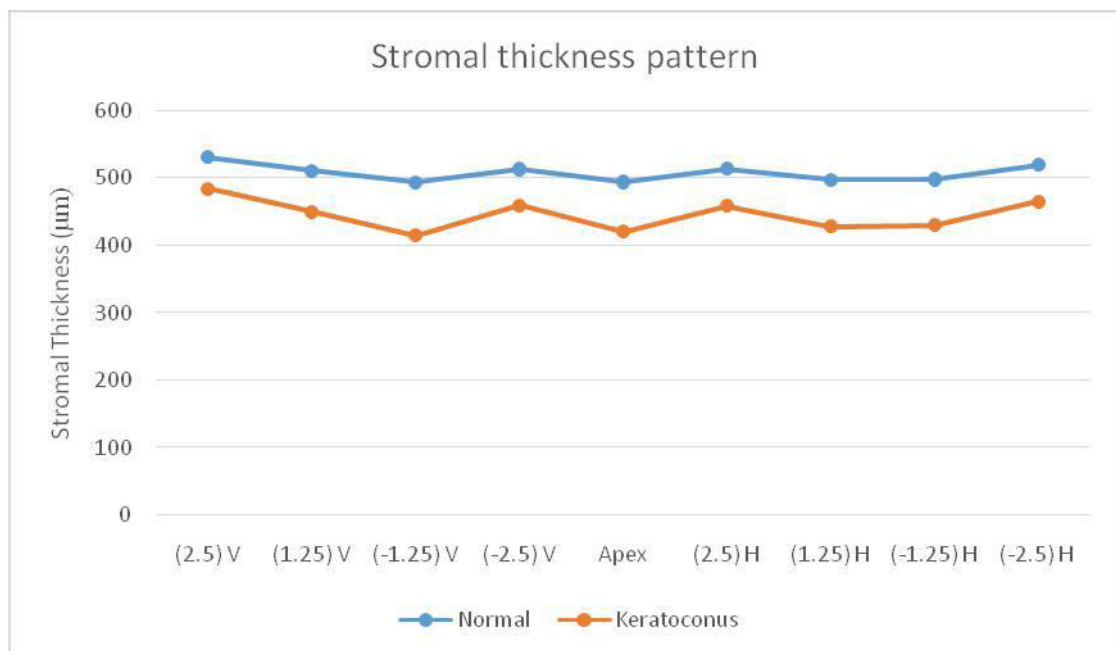


Figure 5: Stromal thickness profile in normal and keratoconic eyes.

Table 4

Mean epithelial thickness through the 6-mm of the corneal center in 90 and 180 axes in keratoconus eyes according to four stages

Stage	Vertical				Corneal Center	Horizontal			
	2.5	1.25	-1.25	-2.5		2.5	1.25	-1.25	-2.5
1	56.64±9.38	54.09±8.34	50.09±5.85	55.09±7.92	49.18±6.80	58.45±9.83	53.82±6.59	51.14±7.69	53.45±7.69
2	60.87±7.60	54.90±8.45	47.37±5.33	52.80±8.33	48.07±5.77	57.73±7.89	51.37±7.82	50.83±6.56	52.87±5.77
3	59.67±6.63	57.78±10.17	52.33±5.02	60.44±10.28	44.78±8.99	58.00±12.08	52.56±10.99	47.56±7.28	53.33±4.77
4	59.89±8.15	48.74±8.33	45.89±8.74	54.79±7.62	43.63±7.58	60.11±10.42	47.89±8.78	45.63±9.46	53.47±8.15
p *	0.321	0.036	0.042	0.124	0.046	0.861	0.140	0.075	0.987

* using ANOVA testing

Table 5

Mean stromal thickness through the 6-mm of the corneal center in 90 and 180 axes in keratoconus eyes according to four stages

Stage	Vertical				Corneal center	Horizontal			
	2.5	1.25	-1.25	-2.5		2.5	1.25	-1.25	-2.5
1	490.0±45.1	462.0±41.9	433.8±43.7	466.0±47.2	440.6±41.6	472.1±47.8	447.3±41.6	445.7±44.4	472.6±48.9
2	480.0±34.9	451.5±32.2	419.8±38.5	466.6±37.7	423.6±47.0	459.8±39.2	428.7±38.3	433.4±37.5	470.5±39.1
3	491.0±30.0	456.2±27.4	409.1±30.1	446.1±43.7	425.7±26.5	458.2±35.4	427.3±23.9	444.4±32.5	483.0±37.6
4	479.2±36.8	429.2±25.8	386.9±30.3	444.3±36.2	388.7±31.5	440.0±44.9	406.8±40.2	401.7±36.8	438.9±44.8
p *	.681	.019	.002	.178	.001	.131	.014	.003	.028

* using ANOVA testing

DISCUSSION

Optical coherence tomography (OCT) has confirmed to be a useful device in diagnosing, managing and follow up of retinal and optic nerve diseases. New technology has improved to include examining the anterior segment. Anterior segment OCT (AS-OCT) provides cross-sectional scans of the tissue to help in examining the cornea, anterior chamber angle, iris and lens.^[13]

In the current study, SD-OCT was capable of picturing the epithelium and Bowman's layer in both normal and keratoconus eyes across the central 6mm area of the cornea, which enabled us to measure the epithelium and stromal thickness and study the distinctions in both groups.

According to the current study findings, OCT indicated significant irregularity differences in epithelial thickness profiles in different corneal regions in eyes with keratoconus compared to healthy eyes. The epithelium was statistically significantly of lower thickness in keratoconus at the center and 1.25mm on each side of the center horizontally and 1.25mm below the center. The epithelial thinning may be due to epithelial modification or remodeling to maintain a more spherical anterior corneal surface, the fact of epithelial remodeling or modification has been mentioned in eyes with keratoconus, wearing rigid contact lenses for long periods and in eyes underwent to refractive surgeries by excimer

laser ablations^[14–19] Also, epithelial thinning may be due to constant proportion of epithelium to stromal thickness so that the epithelium mirrors the underlying stromal irregularities. In this study, the epithelium was distributed in the manner that appeared to remodel or compensate for anterior surface irregularities of corneal stroma.

Mean epithelial thickness at the corneal center was significantly reduced for keratoconus, which is consistent with the previous histopathological viewing of central epithelial thinning,^[20,21] very high frequency digital ultrasound arc scanner^[19] and OCT.^[22,23]

Reinstien et al.^[24] studied the profile thickness of epithelium of 56 healthy eyes by a VHF ultrasound. The mean of central corneal epithelial thickness was $(53.4 \pm 4.6 \mu\text{m})$, which is close to the finding in healthy eyes in our study $(52.8 \pm 6.17 \mu\text{m})$. He also studied the profile of thickness of 54 eyes with keratoconus by Artemis VHF ultrasound.^[19, 25] The mean of central corneal epithelial thickness was $(45.7 \pm 5.9 \mu\text{m})$ which is also close to the central epithelial measurement in our study for the keratoconus eyes $(46.95 \pm 7.14 \mu\text{m})$.

Kanellopoulos et al.^[26] observed generalized thickening of the epithelium particularly at the center of the cornea using a VHF digital ultrasound (Artemis II), which we could not demonstrate in the current study which could be attributed to different measurement principles.

Rocha et al.^[27] used SD-OCT, in which 39 normal eyes and 49 keratoconus eyes were studied. The thickness of epithelial was measured in 21 locations of 0.5 mm apart on horizontal and vertical axes. The mean of the central epithelial thickness was $(50.45 \pm 3.92 \mu\text{m})$ in normal eyes, while its thickness at the same point in keratoconus eyes was $41.18 \pm 6.47 \mu\text{m}$. Mean epithelial thickness was significantly reduced in keratoconus at multiple points on horizontal and vertical axes which is close to the findings in our study.

However, in previous studies, they used healthy participant eyes without concern to the corneal astigmatism and their comparison of the thickness of epithelium was only between two major comparative groups; while in our study, we took corneal astigmatism into consideration in healthy eyes (≥ 2.5 D) and the thickness of epithelium was compared not only between two major comparative groups, but also between different regions inside each group.

Zhou W et al.^[28] also used SD-OCT, in which epithelial and stromal thicknesses of 20 proved eyes of keratoconus and in 20 control participants with corneal cylinder >2 D were assessed at 11 regions across higher steep and flat meridians and conclude that in keratoconic eyes both epithelium and stroma become thin inferiorly and become thick superiorly as compared with control healthy eyes.

Regarding stroma of keratoconus, the thickness of collagen lamellae is unaltered, but the number of lamellae and keratocytes decreased, in addition to degeneration and disintegration of fibroblasts, alterations in the arrangement of the lamellae, and abnormal collagen fibril distribution both inter- and intra-lamellae, mostly in the area that surrounds the

apex of the cone have been noticed^[29–31], the changes being greater with more advanced disease^[32, 33]. In the current study there was a significant thinning of the corneal stroma ($p < .001$) in keratoconus at all points compared to control eyes.

Clinical interest in developing new methods for detection of subclinical keratoconus continuously improving as this ability is so important in corneal refractive surgery to avoid post-operative ectatic corneal disorders.^[34,35] In our study,

we further investigated the influence of disease severity on the corneal epithelium and stroma; we found a correlation between disease severity and epithelial thickness, in stage 4 the mean epithelial thickness at the center was $(43.63 \pm 7.58 \mu\text{m})$ while in stage 1 the mean thickness at same location was $(49.18 \pm 6.8 \mu\text{m})$. This may also give the chance for at least stop disease progression using therapeutic intervention such as corneal collagen cross linking in early disease,^[36] and also we found a trend in epithelial thickness pattern. Therefore, it would be so vital to investigate whether such findings in corneal epithelium could be used to screen keratoconus suspects. Also we found a correlation

between disease severity and stromal thickness, the reduction of central thickness being greater with more advanced disease. In other studies^[16,26, 28, 37] that studied the profile thickness of epithelium and stroma in keratoconus, there was no

concern regarding the classification of the grade of the keratoconus eye group and they depended on nonselected healthy eyes as controls.

The OCT measurements show a high degree of repeatability and reproducibility.^[38] especially at the central and paracentral zones up to 5 mm diameter for both corneal epithelial thickness and corneal thickness measurements.^[39]

Comparing OCT to other methods of measuring epithelial thickness such as Scheimpflug imaging, both techniques offer highly repeatable and reproducible measurements of central and mid-peripheral epithelial thickness.^[40]

The drawback in this study is that only 9 points were measured on horizontal and vertical axes. No attempt was made to correlate thickness findings with the topographical findings also. Further studies with consideration of taking more points on more radial axes with the correlation of the topography findings may be warranted. Also, in this study, there was no concern to assessment of the degree of opening of eyelid and the period of the exposure and dryness of corneal through eye test, this point may lead to variations in the evaporation and movement of the tear film between eyes and may lead also to some errors in the definitive thickness calculations of epithelium.^[41]

In recent years, the assessment of corneal thickness has become an important step in the preoperative assessment of refractive surgery cases. More recently, analysis of the regional corneal epithelial thickness is becoming an additional valuable parameter in practical, clinical and in research purposes. Localized or diffuse topographic abnormalities of epithelial thickness, can highly affect the total corneal refractive power. Also, corneal epithelium in many cases may be masking underlying stromal abnormalities,^[12] and this may indicate underlying ectasia, so that corneal epithelial map evaluation can help the ophthalmologists in decision making for refractive surgery candidates.

In conclusion, spectral domain optical coherence tomography exhibited significant central and zonal epithelial and stromal thickness profile distinctions between keratoconus and controls. These distinctions can be valuable in providing a new parameter for early diagnosis of keratoconus.

Further studies considering both corneal topography and corneal epithelial thickness evaluation are thus required for a multidisciplinary classification of keratoconus.

References

1. Zadnik K, Barr JT, Gordon MO, Edrington TB, CLEK Study Group. Biomicroscopic signs and disease severity in keratoconus. *Cornea* 1996;15:139–46.
2. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. *Am J Ophthalmol* 1986;101:267–73.
3. Zadnik K, Steger-May K, Fink BA, Joslin CE, Nichols JJ, Rosenstiel CE, et al. Between-eye asymmetry in keratoconus. *Cornea* 2002;21:671–9.
4. Chopra I, Jain AK. Between eye asymmetry in keratoconus in an Indian population. *Clin Exp Optom* 2005; 88:146–52.
5. Auffarth GU, Wang L, Völcker HE. Keratoconus evaluation using the Orbscan topography system. *J Cataract Refract Surg* 2000; 26:222–8.
6. Prisant O, Legeais, Renard G. Superior keratoconus. *Cornea* 1997;16:693–4.
7. Weed KH, McGhee CN, MacEwen CJ. Atypical unilateral superior keratoconus in young males. *Contact Lens Anterior Eye* 2005;28:177–9.
8. Rabinowitz YS. Keratoconus. *Surv Ophthalmol* 1998;42:297–319.
9. Rahmen W, Anwar S. An unusual case of keratoconus. *J Pediatr Ophthalmol Strabismus* 2006;43:373–5.
10. Rabinowitz YS, Yang H, Rasheed K, Li X. Longitudinal analysis of the fellow eyes in unilateral keratoconus. *Invest Ophthalmol Vis Sci* 2003;44
11. Kanellopoulos AJ, Asimellis G (2013) Anterior segment optical coherence tomography - assisted topographic corneal epithelial thickness distribution imaging of a keratoconus patient. *Case Rep Ophthalmol* 4(1): 74–78.

12. Karabatsas CH (2016) Studying Corneal Epithelium In Vivo: A New Tool in Clinical Practice. *Adv Ophthalmol Vis Syst* 4(2): 00098. DOI: 10.15406/aovs.2016.04.00098.
13. Ramos JL, Li Y, Huang D. Clinical and research applications of anterior segment optical coherence tomography-a review. *Clin Experiment Ophthalmol*. 2009 Jan;37(1):81-9.
14. Reinstein DZ, Gobbe M, Archer TJ, Silverman RH, Coleman DJ. Epithelial, stromal, and total corneal thickness in keratoconus: three-dimensional display with artemis very-high frequency digital ultrasound. *J Refract Surg* 2010; 26:259–271.
15. Reinstein DZ, Gobbe M, Archer TJ, Couch D. Epithelial thickness profile as a method to evaluate the effectiveness of collagen cross-linking treatment after corneal ectasia. *J Refract Surg* 2011; 27: 356–363.
16. Li HF, Petroll WM, Moller-Pedersen T. Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). *Current eye research* 1997; 16: 214–221.
17. Haque S, Jones L, Simpson T. Thickness mapping of the cornea and epithelium using optical coherence tomography. *Optometry and vision science* 2008; 85: E963–976.
18. Correa-Perez ME, Lopez-Miguel A, Miranda-Anta S, Iglesias-Cortinas D, Alio JL, et al. Precision of high definition spectral-domain optical coherence tomography for measuring central corneal thickness. *Invest Ophthalmol Vis Sci* 2012; 53: 1752–1757.
19. Reinstein DZ, Archer TJ, Gobbe M. Corneal epithelial thickness profile in the diagnosis of keratoconus. *Journal of refractive surgery* 2009; 25: 604–610.
20. Scroggs MW, Proia AD. Histopathological variation in keratoconic corneas. *Cornea*. 1992; 11:553-559.
21. Wygledowska-Promienska D, Rokita-Wala I, Gierek-Ciaciura S, Piatek-Koronowska G. The alterations in corneal structure at III/IV stage of keratoconus by means of confocal microscopy and ultrasound biomicroscopy before penetrating keratoplasty. *Klinika Oczna*. 1999; 101:427-432.
22. S. Haque, T. Simpson and L. Jones, Corneal and epithelial thickness in keratoconus: a comparison of ultrasonic pachymetry, Orbscan II, and optical coherence tomography. *J. Refract. Surg.* 22(5), 486–493 (2006).
23. Y. Li et al. Corneal epithelial thickness mapping by Fourier-domain optical coherence tomography in normal and keratoconic eyes. *Ophthalmology* (e-published ahead of print) (2012). 659468.
24. Reinstein DZ, Archer TJ, Gobbe M, Silverman RH, Coleman DJ. Epithelial thickness in the normal cornea: three-dimensional display with Artemis very high-frequency digital ultrasound. *J Refract Surg*. 2008;24:571-581.
25. Reinstein DZ, Gobbe M, Archer TJ, Silverman RH, Coleman DJ. Epithelial, stromal, and total corneal thickness in keratoconus: three-dimensional display with Artemis very-high frequency digital ultrasound. *J Refract Surg* 2010; 26:259–271.
26. Kanellopoulos AJ, Aslanides IM, Asimellis G. Correlation between epithelial thickness in normal corneas, untreated ectatic corneas, and ectatic corneas previously treated with CXL: is overall epithelial thickness a very early ectasia prognostic factor? *Clin Ophthalmol*. 2012;6:789-800.
27. Rocha KM, Perez-Straziota CE, Stulting RD, Randleman JB. SD-OCT analysis of regional epithelial thickness profiles in keratoconus, postoperative corneal ectasia, and normal eyes. *J Refract Surg* 2013; 29: 173–179.

28. Zhou W, Stojanovic A (2014) Comparison of Corneal Epithelial and Stromal Thickness Distributions between Eyes with Keratoconus and Healthy Eyes with Corneal Astigmatism ≥ 2.0 D. PLoS ONE 9(1): e85994. doi:10.1371/journal.pone.0085994.
29. Sherwin T, Brookes NH. Morphological changes in keratoconus: pathology or pathogenesis. Clin Experiment Ophthalmol 2004;32:211–7
30. Takahashi A, Nakayasu K, Okisaka S, Kanai A. Quantitative analysis of collagen fibres in keratoconus. Acta Soc Ophthalmol Jap. 1990; 90:1068-73.
31. Meek KM, Tuft SJ, Huang Y, Gill PS, Hayes S, Newton RH, et al. Changes in collagen orientation and distribution in keratoconus corneas. Invest Ophthalmol Vis Sci 2005;46:1948–56.
32. Ku JY, Niederer RL, Patel DV, Sherwin T, McGhee CN. Laser scanning in vivo confocal analysis of keratocyte density in keratoconus. Ophthalmology 2008;115:845–50.
33. Fullwood NJ, Tuft SJ, Malik NS, Meek KM, Ridgway AEA, Harrison RJ. Synchrotron Xray diffraction studies of keratoconus corneal stroma. Invest Ophthalmol Vis Sci. 1992; 33:1734-41.
34. Seiler T., Quurke A. W., “Iatrogenic keratectasia after LASIK in a case of forme fruste keratoconus,” J. Cataract Refract. Surg. 1998; 24(7), 1007–1009.
35. Randleman J. B. et al., “Risk assessment for ectasia after corneal refractive surgery,” Ophthalmol. 2008; 115(1), 37–50.
36. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr. Opin. Ophthalmol. 2006; 17(4), 356–360.
37. Li Y, Meisler, D M., Tang M, Lu AT, Thakrar V, Reiser BJ, Huang D. Keratoconus diagnosis with optical coherence tomography pachymetry mapping. Ophthalmology 2008; 115: 2159–2166.
38. Sarah Muscat; Nicole McKay; Stuart Parks; Ewan Kemp; David Keating. Repeatability and Reproducibility of Corneal Thickness Measurements by Optical Coherence Tomography. Investigative Ophthalmology & Visual Science June 2002, Vol.43, 1791-1795.
39. Ma, Xingxuan Jack, Li Wang, and Douglas D. Koch. Repeatability of Corneal Epithelial Thickness Measurements Using Fourier-Domain Optical Coherence Tomography in Normal and Post-LASIK Eyes. Cornea 2013; 32.12: 1544–1548.
40. Jinhai Huanget al. A Comparison between Scheimpflug Imaging and Optical Coherence Tomography in Measuring Corneal Thickness J. Ophtha. 2013. 02. 0.22.
41. Du C, Wang J, Cui L, Shen M, Yuan Y. Vertical and horizontal corneal epithelial thickness profiles determined by ultrahigh resolution optical coherence tomography. Cornea 2012; 31: 1036–1043.

Application: Coating of Nanoparticles to Minimizing Harmful Environmental Pollution by Chemical Fungicide

Rana Ibrahim Khaleel*

**College of Engineering, University of Samarra, Samarra, Iraq*

ranagh83@yahoo.com

Abstract

Field experiment carried out to evaluate use of nano-silver solution to reduce environment pollution by fungicides. Four concentrations of AgNPs, (100 ppm, 50 ppm, 25 ppm, and 12.5 ppm) were prepared by diluting the original stock solution with distilled water. The concentrations was used in coated orang fruit compared with fungicide and uncoated fruit as control treatment. The fruits were kept in box carton (40x40x30cm) and incubated under room conditions for 28 days to study the formation to Green mold and Black fungal and shelf life for fruit stored. The result showed high infection recorded in uncoated fruit compared with other treatments with values reached (86.66 and 91.1) for Green mold and Black rot respectively, while ppm recorded completely inhibition infections (0%) for fungal decay index. Also coating fruit with 100 and 50 ppm recorded significant different (3.8 and 3.9%) from other concentrations and fungicide that, recorded high weight loss with mean reached (13.66%) after four weeks from storage. Nanotechnology when applied could play a fundamental role for this purpose and research in packaging fresh fruit and minimize environmental pollution by chemical fungicides.

Keywords: Silver-Nanoparticles, Potential Alternative, Orange, Fungicide.

How to cite this article: Khaleel RI (2019): Application: Coating of Nanoparticles to minimizing harmful environmental pollution by chemical fungicide, Ann Trop Med & Pub Health; 21: SP2050-19

1. Introduction

Nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells (Nejad et al., 2014). Also, nanotechnology has amplified the effectiveness of silver particles as antimicrobial agents. Nanoparticles such as silver is known to attack a broad range of biological processes in microorganisms including cell membrane structure and functions (Lamsal et al., 2012). Nanoparticles also inhibit the expression of proteins associated with ATP production, although its specific antimicrobial mechanisms are still not completely understood. The use of nano-sized particles as antimicrobial agents has become more common as technological advances make their production more economical. One of the potential applications of Nanoparticles is to manage plant disease. Since silver displays multiple modes of inhibitory action to microorganisms (Kamran et al., 2011), it may be used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides. Until now, reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science (Gavanji et al., 2013). Furthermore, nano-materials can be modified for better efficiency to facilitate their applications in different fields such as bioscience and medicine. This simple size comparison gives an idea of using nanoparticles as very small probes that would allow

the elucidation of the cellular machinery without introducing too much interference (Lamsal et al., 2012). Understanding of biological processes on the nano scale level is a strong driving force behind development of nanotechnology (Salata et al., 2004). This study aim to investigate in activity of silver nanoparticles as alternative of fungicides to decrease environmental pollution.

2. Methodology

2.1 AgNPs Solation Preparation

AgNPs, WA-CV-WA13B (CV) were used in the experiment that classified and using in different manufacturing processes. Four concentrations: 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm were prepared by diluting the original stock solution with distilled water and stored at 4°C until further use.

2.2 Fruit collection of Fungal Pollination

Citrus fruit (orange) of medium size and in good physical conditions such as being insect and disease and damage free were purchased from local market in Samarra, Iraq. The fruits were visually inspected for any damage or disease during collection. They were brought to the enlivenment lab and kept in a cool room at 4°C for 24hrs to be used the next day for the experiment.

2.3 Preparation Fruit for Inoculation

The current research in cooperated a modification of this range to selected 100, 50, 25 and 12.5ppm. Fruits (oranges) washed with tap water, air-dried and then sterilized by immersion in 70% ethanol for 1 min. The fruits were then randomly divided into six equal groups (Four nano-treatment + two controls). Fruits were injected to a depth of 5.0mm with a 1.25mm diameter needle at the equator for all the treatments and inoculated by spraying with suspension of fungi. The treated fruit were left for an hour after spraying to stabilize the spores on the wound.

2.4 Preparation Fruit of Coating Treatment

From the concentration prepared previously, the homogenous solutions (100, 50, 25 and 12.5/ppm) used in coated fruit using a clean and soft brush. Coated fruits were allowed to dry at ambient temperature about 30 minutes, after that transfer to package it.

2.5 Packaging Treated Fruit

The fruits were kept in box carton (40x40x30cm) and incubated under room conditions (25°C±2 and 65-75 % RH) for 28 days to study the formation of fungal rot and shelf life for fruit stored. There were three replicate trials of five fruits per replicate using the Completely Randomized Design (RBD).

2.6 Fungal decay

Fungal decay in stored fruit was evaluated after 21 days under storage conditions. The percentage of infection was determined using scale of 1-5 (Scale modified from Tzortzakakis, 2006) as shown in Table 1.

Table .1 Percentage of infection fungal decay index

Percentage infection	Remarks
0	Clean
1-5	Trace
5-15	Slight
15-30	Moderate
>30	High

2.7 Shelf Life Fruit

The percentage of shelf life fruit was calculated by determining the progressive reduction in fruit weight during storage after a period of 21 days in relation to the original fresh weight at the beginning of storage (Tune et al., 2007):

$$\text{WL (\%)} = \frac{\text{WL before storage} - \text{WL after storage}}{\text{WL before storage}} \times 100 \quad (\text{Eq 1})$$

2.8 Statistical Analysis

The experiment was laid out in the Completely Randomized Design (CRD) with three replicate. Data collected and analysed using analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan's Multiple Range test ($P \leq 0.05$). Following ANOVA statistical analysis, which were performed using SPSS version 19- 2012 (SPSS Inc., Chicago, USA).

3. Result and Discussion

3.1 Fungal Decay Index

The statistical analysis (ANOVA) as reported in Table.2 shows statistical different between the treated and untreated fruit. High infection recorded in uncoated fruit compared with other treatments with values reached (86.66 and 91.1) for Green mold and Black rot respectively. Also not statistical difference ($P \leq 0.05$) between 25, 50 and 100 ppm treatments which exhibited significantly decrease in disease severity trace degree to completely inhibition infections (0%), compared with 12.5ppm and fungicide which recorded (6.66 and 11.10) fungal decay index .

Table. 2 Effect of coating fruit with different concentrations of Silver (12.5 to 100 ppm) on the development of mycelium growth *Penicillium digitatum* and *Aspergillus niger*(colony diameter/cms) on the orange surface during storage for 21 days at $25^{\circ}\text{C} \pm 2$ and 65-75% RH.

Treatments (ppm)	<i>Penicillium digitatum</i>	<i>Aspergillus Niger</i>	**Infection
---------------------	----------------------------------	------------------------------	-------------

12.5	6.66 ^b	11.10 ^c	Slight
25	0 ^a	2.22 ^b	Trace
50	0 ^a	0 ^a	Trace
1000	0 ^a	0 ^a	Trace
Water	86.66 ^c	91.10 ^d	High
Fungicides	6.66 ^b	11.10 ^c	Slight

* Alphabets different in the same columns shows significant difference at ($P \leq 0.05$) between concentrations and average was calculated from three replicates.

The current study is consistent with previous studies that demonstrated that nanoparticles with antimicrobial properties can be employed for safer food packaging. Nanoparticles such as titanium dioxide, zinc oxide and magnesium oxide, as well as a combination of them, once functionalized can be efficient in killing micro-organisms which is cheaper and safer instead of metal based nanoparticles (Barik *et al.* 2008; Cui *et al.* 2010). The study of Sasson *et al.* (2007) reported that Nanotechnology has the potential for efficient delivery of chemical and biological pesticides using nanosized preparations or nanomaterial based agrochemical formulations. The benefits of nanomaterial based formulations are the improvement of efficacy due to higher surface area, higher solubility, induction of systemic activity due to smaller particle size and higher mobility and lower toxicity due to elimination of organic solvents in comparison to conventionally used pesticides and their formulation. Also the findings of Abdelmalek and Salaheldin (2016) are consistent with the result current study that: Silver nanoparticle, 150 ppm, showed potent antifungal activity against the isolated fungi from fresh fruit. Also the current result is consistent with study of Lucimeire *et al.*, which demonstrated that coatings chitosan nanoparticles of 110 nm showed higher antimicrobial activity against mold and yeasts, and mesophilic and psychrotrophic bacteria than the other treatments. Silver nanoparticles may directly attach to and penetrate the cell membrane to kill spores, although penetration of silver nanoparticles into microbial cell membranes is not completely understood (Morones and Elechiguerra, 2005).

3.2 Shelf life Fruit

Effects of different coatings on percentage of weight loss are shown in Table.3. The result showed difference between the effects of the coating treatments on weight loss at different concentrations. Coating fruit with 100 and 50 ppm recorded significant different (3.8 and 3.9%) from other concentrations and fungicide after four weeks from storage. Also statistical analysis ($P \leq 0.05$) did not showed significant difference between concentration 25 and 12.5ppm (5.42 and 5.5) which difference from fungicide that, recorded high weight loss with mean reached (13.66%) after four weeks from storage.

Table.3 Effect of coating fruit using nano-silver solution on weight loss (%) that storage at $25 \pm 2^\circ\text{C}$ and 65- 75 % RH for four weeks(28S) days.

Treatments	W1	W2	W3	W4	Mean
100ppm	2.9 ^a	3.5 ^a	3.9 ^a	4.9 ^b	3.8 ^a
50ppm	3.5 ^b	3.5 ^a	4 ^b	4.63 ^a	3.9 ^a
25ppm	5 ^c	5.5 ^{cb}	5.5 ^c	5.70 ^c	5.42 ^b
12.5ppm	5 ^c	5 ^b	6 ^d	6 ^d	5.5 ^b
Uncoated fruit	10.3 ^e	11.9 ^d	13.7 ^e	18.76 ^e	13.66 ^c

*Alphabets different in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$) and average was calculated from three replicates.

The treatment of 100ppm of silver showed significant decrease in weight loss compared with uncoated fruit and other treatments Table. 3 Active coating using nano-silver may help improve and modify the atmosphere around the fruits, thereby preventing extremely low levels of O_2 and/or high levels of CO_2 , which could produce anaerobic metabolism with possibility of off-flavour generation (Serrano *et al.* 2008; Valero *et al.* 2006 ; Valverde *et al.* 2005). Results of current study recorded clear increase in weight loss ranged (5.7- 6%) with treated 25 and 12.5 ppm respectably.

Current finding is consistent with other studies (Bautisty *et al.* 2003; Ali *et al.* . 2011) that, demonstrated that coating fruit by some materials such as chitosan concentration of 2-5% with crude plant extract of custard apple leaves, papaya leaves and papaya seeds 1000-3000 did not influence the percentage weight loss during the storage fruit but there was inhibition of fruit decay percentage. The result of this study are in agreement with findings of other studies (Li and Wang, 2006; Li *et al.* 2009) that, demonstrated that nano-packing technology applied on fruit and vegetables exhibited better physico-chemical, sensory physiological and preservation properties compared to normal packaging. Also with the study of Lopez-Rubio *et al* and Jimenez *et al* that, demonstrated that Nano encapsulation of active compounds used in edible coating control rates and conditions and thus protect the coated items from moisture, heat or other extreme conditions while enhancing their stability and viability.

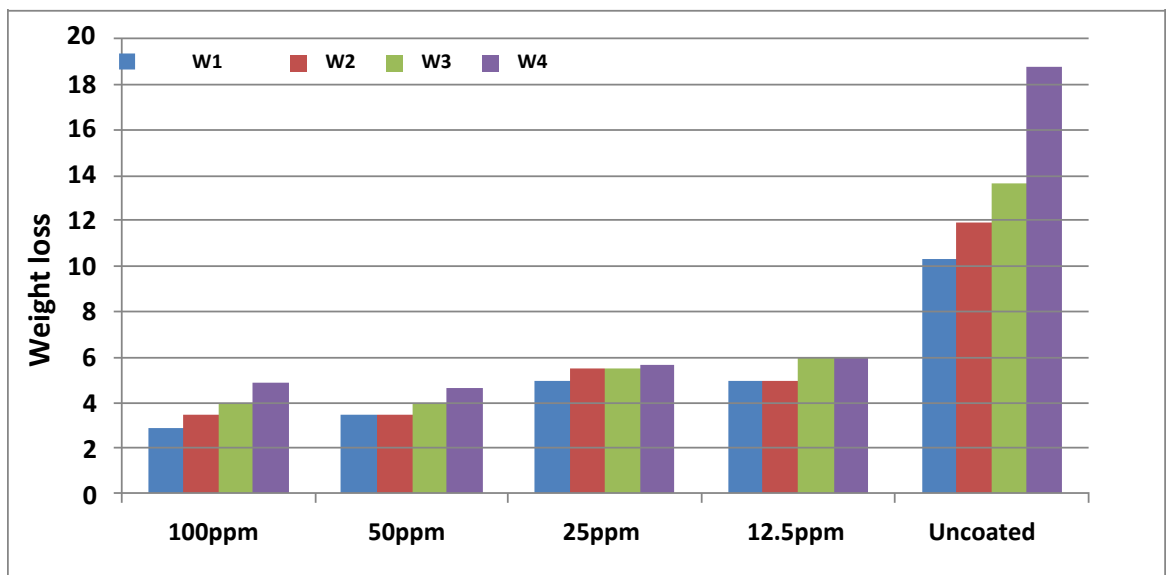


Figure .1 Effect of coating fruit using nano silver on weight loss (%) at 25±2°C and 65- 75% RH storage condition for 28 days.

The experimental data by Arnon *et al.* 2015; Ouzounidou *et al.* 2013 confirmed that -Ag coatings markedly reduced the weight loss in those kinnow mandarin treatments. Weight loss in fresh fruits and vegetables has not only been associated with vapor pressure at various locations, but also occurs through respiration phenomenon. Result current study confirmed these findings (Zambrano-Zaragza *et al.* 2013) that, Nano-emulsions and Nanoparticles can improve barrier properties and increase functionality of coating because submicronic systems allow for more efficient control of coating properties with better distribution and homogeneity on the coated fruit skin. Also support study (Hazirah and Armylisas, 2016) that reported, Nano-particles are recognized as micro additives and submicroscopic particles which enhance the physical properties of food packaging such as edible films and coating.

Conclusion

Application of coating nano-materials as further proved the quality of fresh fruits by decline fungal decay index and increasing lengthen fruit shelf life. Using of the silver nanoparticles as alternative fungicides for crop protection is emerging technology in food packaging but still need more studies to assesses nano-silver to avoid side negative and their possible negative and toxicological effects on environmental and human health.

Acknowledgements

Acknowledgements and Reference heading should be left justified, bold, with the first letter capitalized but have no numbers. Text below continues as normal.

References

- Alavi .F. Nejad, R. Saberi Riseh, P. Khodaygan and R. Ranjbar-karimi.2014. Biological control of take-all disease by isolates of *Pseudomonas fluorescens* and biosynthesis of silver nanoparticles by the culture supernatant of *Pseudomonas fluorescens* CHA0. Archives of Phytopathology and Plant Protection. 47, (14): 1752-1763.
- Abdelmalek GAM, Salaheldin TA (2016) Silver Nanoparticles as a Potent Fungicide for Citrus Phytopathogenic Fungi. J Nanomed Res 3(5): 00065. DOI: 10.15406/jnmr.2016.03.00065.
- Ali .A., Maqbool .M., Alderson .P. and Zahid. N. (2011). Efficacy of biodegradable novel edible coatings to control postharvest Anthracnose and maintain quality of fresh horticultural produce. Proc. 4th International Conference Postharvest Unlimited 2011.pp39.44.
- Arnon, H.; Rina, G.; Ron, P.; Elena, P. Development of polysaccharides-based edible coatings for citrus fruits:A layer-by-layer approach. Food Chem. 2015, 166, 465-472.
- Barik, T. K., Sahu, B. and Swain, V., 2008, Nanosilica-from medicine to pest control. Parasitol Res., 103: 253-258.
- Bautisty. S., Hernreández-López. M., Bosquez-Molina.E., Wilson. C.(2003). Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit,22: (9), 1087–1092.
- Cui, H. X., Sun, C. J., Liu, Q., Jiang, J. and Gu, W., 2010, Applications of nanotechnology in agrochemical formulation, perspectives, challenges and strategies. International conference on Nanoagri, Sao pedro, Brazil, June, 20-25.
- Gavanji S, Larki B, Mehrasa M (2013) A review of effects of molecular mechanism of silver nanoparticles on some microorganism and eukaryotic cells. Tech J Eng App Sci 3: 48-58.

Jimenes,M; Garcia, H, Beristain, C .2004. Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. European food research and technology, 219(6): 588-592.

Kamran. S., Forogh .M, Mahtab. E and Mohammad. (2011). *In Vitro* Antibacterial Activity of Nanomaterials for using in Tobacco Plants Tissue Culture. World Academy of Science, Engineering and Technology 79:372-37.

Lopez-Rubio, A; Gavara .R and Lagaron, J.2006. Bioactive packaging turning foods into healthier foods through biomaterials *Trend in food science and technology*, 17(10): 567-575.

Lucimeire Pilon, Poliana C. Spricigo, Marcela Miranda, Marcia R. de Moura, Odilio Benedito and et al.(2014). Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while not affecting quality attributes. *International Journal of Food Science and Technology*.doi:10.1111/ijfs.12616.

Li, H., Li, F., Wang, L., Sheng, J., Xin, Z., Zhao, L., et al., 2009. Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujuba* Mill. var. *inermis* (Bunge) Rehd). *Food Chem.* 114,547–552.

Li, Z and Wang, M.(2014). Research on nano-SiO₂/chitosan keeping fresh agent and application to Fuji apples. *Journal of Guizhou University of Technology*.35:99-102.

Lamsal, K., S.W. Kim, J.H. Jung, Y.S. Kim, K.S. Kim and Y.S. Lee, 2011. Application of silver nanoparticles for the control of *Colletotrichum* species in vitro and pepper anthracnose disease in field. *Mycobiology*, 39: 194-199.

- Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., and Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* 16:2346-2353.
- Ouzounidou, G.; Papadopoulou, K.; Asfi, M.; Mirtziou, I.; Gaitis, F. Efficacy of different chemicals on shelf-life extension of parsley stored at two temperatures. *Int. J. Food Sci. Technol.* 2013, 48, 1610–1617.
- Sasson, Y., Levy-Ruso, G., Toledano, O., Ishaaya, I., Nanosuspensions: emerging novel agrochemical formulations., 2007, In: Ishaaya I, Nauen R, Horowitz AR, editors. *Insecticides design using advanced technologies* Netherlands: Springer-Verlag. p. 1-32. Schaad NW, Opgenn.
- Salata, O.V., 2004. Applications of nanoparticles in biology and medicine. *J. Nanobiotechnol.* 30;2(1):3.
- Serrano, M.D., Martinez-Romero, S., and Valero, D. (2008). The use of the natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food and Emerging Technologies*, 6:115-123.
- Siti-Hazirah.M; Noor-Armylisas.A.2016. Nanomaterials-Recent advancement in edible coating technology .*Plam oil developments*.(66):1-4.
- Tzortzakis. (2006). Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative Food Science and Emerging Technologies*, 8:111-116.
- Tune, S., Chollet, E., Harlier, C P., Preziosi-Belloy L., and Gontard. (2007). Combined effect of volatile antimicrobial agents on the growth of *Penicillium notatum*. *Int. J. Food Microbiol.* 113: 263-270.

Valero, J.M., Valverde, D., Martínez-Romero, F., Guillén, S., and Castillo, M. (2006). The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety, and functional properties of table grapes. *Postharvest Biology and Technology*, 41: 317-327.

Valverde, J. M. (2005). Nuevas tecnologías no contaminantes para preservar la calidad de la uva de mesa Durante su conservación post-recolección. PhD thesis, University Miguel Hernández.

Zambrano-Zaragoza,M; Mercado-Silva.E; Ramirez-Zamorano.P; Corneja-Villegas.A; Gutierrez-Cortez.D.2013.Use of solid lipid nanoparticles (SLNs) in edible coating to increase guava(*Psidium guajava* L.) shelf-life *food research international*, 51(2): 946-953.