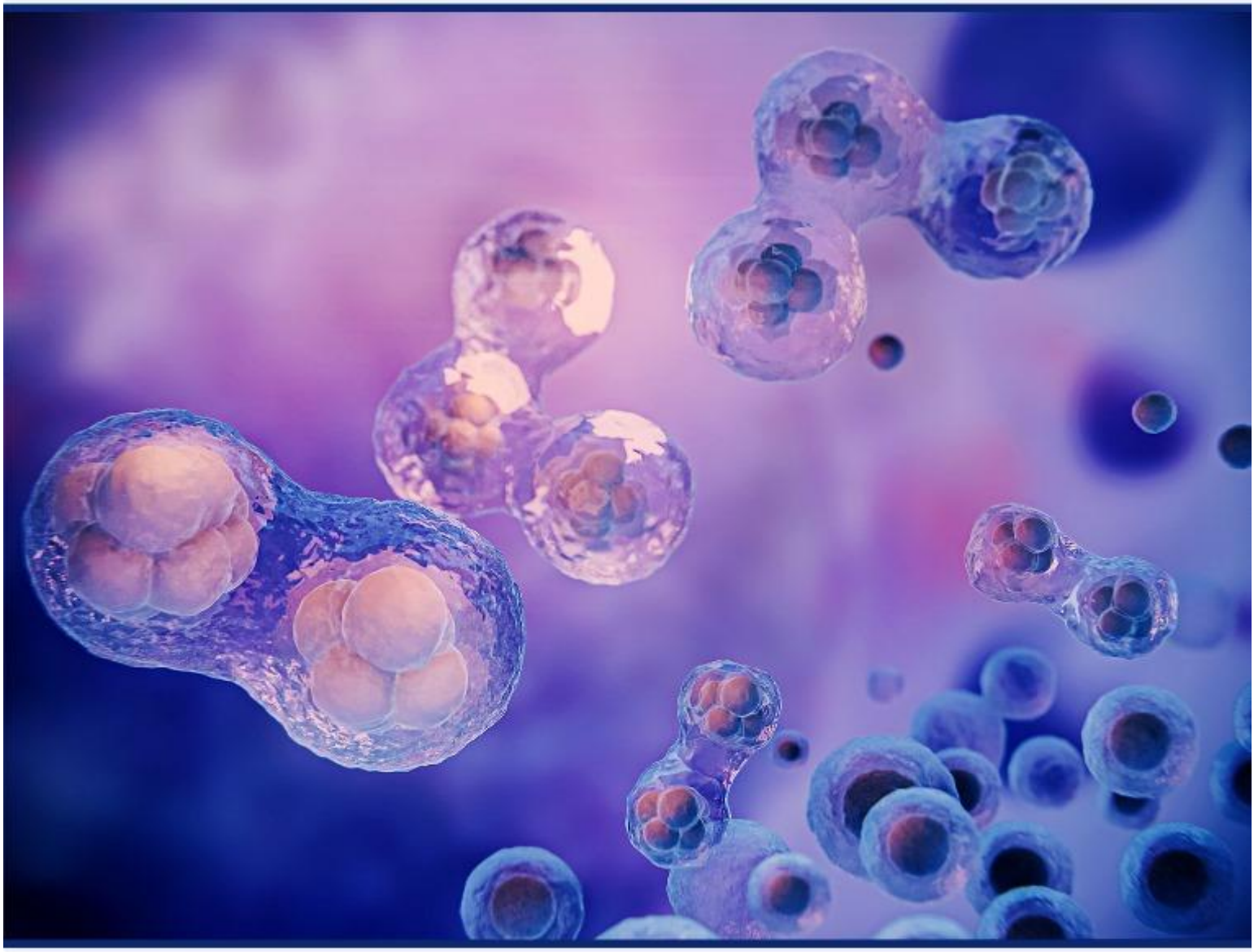
Al-Mukhtar
Journal of Sciences

Volume 38 | Issue 4 DEC 2023



ISSN:26-17-2186 (Online)
ISSN:26-17-2178 (Print)
NL - LDN 280-2013

Published by
OMU

Al-Mukhtar Journal of Sciences

Peer-reviewed scientific journal, Volume Thirty-Eight, Issue Four, 2023

Published by Omar Al-Mukhtar University, Al-Bayda, Libya

Al-Mukhtar Journal of Sciences

NL – LDN 208-2013

A peer-reviewed journal published by Omar Al-Mukhtar University, Al Bayda, Libya
Peer-reviewed scientific journal, Volume Thirty-Eight, Issue Four, 2023
Email: omu.j.sci@omu.edu.ly
To pour. Box 919 Al Bayda - Libya, Fax: +218 69 463 7053

EDITORS & STAFF

Advisory Committee

Al-Mukhtar Journal of Sciences 38: (4), 2023

Al-Mukhtar Journal of Sciences 38 (4): 326-335, 2023
Doi: https://doi.org/10.54172/g2n6te13

Research Article **Open Access**

Electron Beams Commissioning and Initial Measurements on an Elekta Synergy Platform Linear Accelerator

Abdurraouf. M. Aghila[1*], Saad. S. Saad[2], Faraj A. Elmasrub[3]

***Corresponding author:** ab.aghila@uot.edu.ly, Department of Physics, Faculty of Education, University of Tripoli, Libya.

Second Author: saadmaddi@yahoo.com, Higher Institute of Medical Sciences and Technology, Abu-Salim, Tripoli, Libya.

Third Author: f_masrub@hotmail.com , National Cancer Institute of Sabratha, Sabratha, Libya

Received:
03 February 2023

Accepted:
26 April 2023

Publish online:
31 December 2023

Abstract

A linear accelerator (Linac) must be commissioned for use in treating cancer patients. This research work aims to analyze part of the electron beam data produced by the Elekta Synergy Platform Linac at the radiotherapy department at Tripoli University Hospital, Tripoli, Libya. In thispaper, percentage depth dose (PDD), beam profile and applicators factor were studied at five different electron beam energies (4, 6, 8, 10 and 12 MeV). The relative measurement part of this study was carried out using a PTW MP3-M 3D water scanning system, and the absolute measurement was taken using a plane parallel ionization chamber and a unidose E electrometer. The analysis for these measurements has yielded the following results: a) The Penetrative qualities of all the electron beam energies were within the manufacturer's tolerance limits of ±1%; b) the maximum values of beam flatness of 2.94 and beam symmetry of 1.93 are within the accepted limits of International Electrotechnical Commission criteria; c) A penumbra maximum value of 1.30 was measured at the 4-MeV electron beam energy and using a 14×14 cm^2 applicator size. All the obtained parameters were within the permissible limits. Therefore, the electron beams can be safely used forclinical purposes.

Keywords: Elekta Synergy Platform; Electron Beam Commissioning; PDD; Beam Profile; Applicator Factor.

INTRODUCTION

One common treatment method for cancer disease is radiotherapy. It uses high-energy radiation of different ionization types to destroy tumor cells. The type of treatment radiation depends on tumor size and location, tumor type, and tumor stage. For superficial tumors, electron beams are widely used due to their sharp dose fall-off and relatively short range of particles in tissue (Arunkumar et al., 2010). A linear accelerator is the most common and most sophisticated machine used for external beam radiotherapy made by different manufacturers. Linacs of different manufacturers have different structure order and design especially in Linacs treatment head. The differences in the treatment head affect the characteristics of electron and photon beams. Moreover, Linacs of the same manufacturer are slightly different in their beam characteristics (Brahme et al., 1976; Khan, 1991; IEC, 1984; Varatharaj et al., 2016).

Accurate data measurements of photon and electron beam characteristics produced by Linac, which need to be transferred into the Treatment Planning System (TPS) to predicate the radiation dose distribution inside the patient, are important factors that affect the success of radiation therapy (Acqah *et al*., 2014). According to the recommendations of the American Association of Physicists in Medicine (AAPM) in the Task Group 40, and International Commission on Radiation Units and Measurements in report 50 (Kutcher et al., 1994; ICRU, 1993), the dose delivery to tumor should be within ±5% of a prescribed dose. Therefore, commissioning and acceptance tests of the linear accelerator are required before setting the machine in clinical-use (Khan, 2010; Sahool et al., 2012).

Due to the lack of local standards of measurement, this paper aims to present and analyze part of electron beams commissioning to allow physicists of other institutes to compare their results with those of this study. The measurements include the percentage depth dose curves, beam profiles parameters and applicators factor of an Elekta Synergy platform linear accelerator released by Elekta Oncology Systems, Crawley, UK.

MATERIALS AND METHODS

This work was carried out at Radiotherapy Department, Tripoli University Hospital in Tripoli, Libya. Measurements of percentage depth dose (PDD), beam profile and applicators factor were carried out for electron beam of nominal energies 4, 6, 8, 10 and 12 MeV generated by Elekta Synergy platform linear accelerator. The dosimetry system for measurements of PDD and beam profile was a PTW MP3-M 3D water scanning system (PTW, Freiburg, Germany). The system consists of a water tank of inner size 59.6×59.4×50.25 cm^3, a TANDEM electrometer, a TBA control unit and two 0.125 cm^3semiflex ionization chambers for both reference and ionization field. The measured PDD and beam profile were collected using PTW MEPHYSTO mc^2 navigation software (PTW, Freiburg) version 1.6. Applicators factors were measured in polystyrene slab phantom at depth of the maximum dose using a plane parallel ionization chamber connected to PTW Unidose E electrometer at a source to surface distance (SSD) of 100 cm.

PDD and beam profiles were measured at (SSD) of 100 cm setup and at gantry and collimator angles of 0^o. The applicators used for these measurements were of size 6×6 cm^2, 10×10 cm^2, 14×14 cm^2 and 20×20 cm^2 in different depths according to the electron beam applied and taking into account the bremsstrahlung contamination in the beam. All measurements were made according to TRS-277 and TRS-398 (IAEA, 1987; IAEA, 2000).

Parameters calculated from the central axis PDD according to AAPM Task Group 25 (Gerbi et al., 2009) are the mean energy $\bar{E}_0$and the most probable energy $E_{p,0}$ at the water phantom surface using equations (1) and (2) respectively:

$$\bar{E}_0 = 2.33\ R_{50} \quad (1)$$

$$E_{p,0} = 0.22 + 1.98R_p + 0.0025\ R_p^2 \quad (2)$$

Where R_{50} is the depth at which the dose is 50% of the maximum, and R_p is the practical range.

Another important parameter is dose gradient (G), which measures how quickly the dose decreases beyond the therapeutic range and is given by equation (3) (Gerbi et al., 2006; Podgorsak ,2003)

$$G = \frac{R_p}{R_p - R_q} \quad (3)$$

Where R_q is the depth at which the tangent through the dose inflection point intersects the maximum dose level. For electron beams with mean energy of 5 MeV – 30 MeV, the lower limit of G is 2.3 (Kirby et al., 1985; Jamshidi et al., 1987).

Beam flatness, beam symmetry and penumbra width were calculated based on measurements of beam profiles. The IEC 60976 (IEC, 2007) criteria were followed for flatness, symmetry and penumbra width calculation.

RESULTS

Penetrative quality: The penetrative quality of the electron beam is defined as the depth of the 80% point to the maximum absorbed dose on the central beam axis. Table 1 shows the results of declared and measured electron beam penetrative quality which measured at $SSD = 100\ cm$ for the applicator of size 14×14 cm.

Table (1). Declared and measured values of penetrative quality of electron beams.

Nominal energy (MeV)	Penetrative quality		Difference mm
	Declared Mm	measured mm	
4	13.30	13.55	-0.25
6	20.00	19.82	+ 0.18
8	26.70	26.32	+ 0.38
10	33.30	33.64	- 0.34
12	40.00	40.20	- 0.20

Percentage depth dose: Figure 1 illustrates the percentage depth dose curves of 4, 6, 8, 10 and 12 MeV electron beam energies for the applicator of size 10×10 cm^2at $SSD = 100\ cm$.

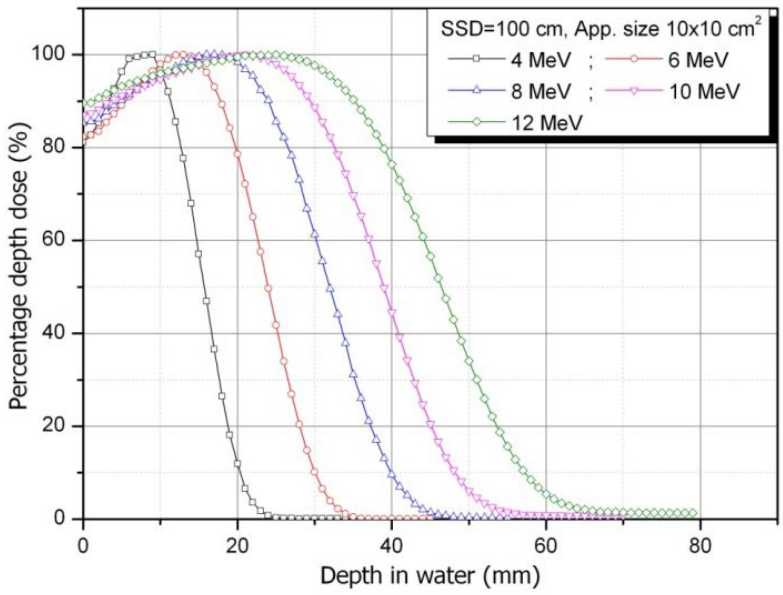


Figure (1). PDD curves of 4, 6, 8, 10 and 12 MeV electron energies for the applicator of size 10×10 cm2 at SSD 100 cm

Electron beams parameters R_{50}, R_{80}, R_{100}, R_p, R_q, percentage surface dose D_s and bremsstrahlung contamination D_x obtained from the measured PDD at SSD of 100 cm are presented in Table 2.

Table 3 presents the calculated parameters from the measured (PDD) data for the electron beam of energies 4, 6, 8, 10 and 12 MeV and applicators of size $6 \times 6\ cm^2$, $10 \times 10\ cm^2$, $14 \times 14\ cm^2$ and $20 \times 20\ cm^2$.

Table (2). Electron beam parameters obtained from PDD at $\mathbf{SSD = 100\ cm}$.

App. Size cm^2	R_{50} mm	R_{80} mm	R_{90} mm	R_{100} mm	R_p mm	R_q mm	$\mathbb{D}_x$ %	$\mathbb{D}_x$ %
Electron nominal energy : 4 MeV								
6×6	16.00	12.98	11.56	8.00	20.53	11.91	79.32	0.21
10×10	15.69	12.70	11.31	8.00	19.87	11.22	81.25	0.19
14×14	16.61	13.55	12.16	9.00	20.67	12.00	80.78	0.17
20×20	16.87	13.81	12.39	9.00	21.16	12.57	81.43	0.11
Electron nominal energy : 6 MeV								
6×6	23.43	19.27	17.32	12.00	29.35	17.47	81.50	0.27
10×10	23.93	19.75	17.80	12.00	30.29	17.62	82.32	0.35
14×14	24.02	19.82	17.87	12.00	30.29	17.56	82.95	0.34
20×20	24.22	20.00	17.92	12.00	30.53	17.83	84.30	0.52
Electron nominal energy : 8 MeV								
6×6	30.69	25.82	23.24	17.00	37.63	24.09	84. 62	0.61
10×10	31.91	26.54	24.03	17.00	38.57	23.77	85.27	0.38
14×14	31.73	26.32	23.74	17.00	39.86	24.35	85.49	0.71
20×20	31.72	26.33	23.76	17.00	39.48	24.48	87.04	0.52
Electron nominal energy : 10 MeV								
6×6	39.09	32.64	29.63	21.00	47.55	30.57	86.55	0.63
10×10	38.95	32.61	29.47	21.00	46.29	30.58	87.03	0.84
14×14	40.03	33.64	30.50	21.00	47.71	31.38	87.17	0.66
20×20	39.94	33.54	30.40	21.00	47.32	31.07	88.22	0.47
Electron nominal energy : 12 MeV								
6×6	46.28	38.62	34.75	25.00	56.69	35.43	89.55	1.11
10×10	46.43	38.93	35.15	25.00	56.42	35.20	89.61	1.44
14×14	47.77	40.20	36.40	25.00	56.57	36.25	89.13	1.28
20×20	47.50	39.98	36.26	25.00	55.90	36.59	90.38	1.03

Table (3). Electron beam parameters calculated by PDD at $\mathbf{SSD = 100\ cm}$.

App. Size (cm^2)	$E_{p,0}$ (MeV)	$\bar{E}_0$ (MeV)	G	%error$E_{p,0}$& Nominal Energy
Electron nominal energy : 4 MeV				
6×6	4.19	3.73	2.38	-4.81
10×10	4.05	3.66	2.30	-1.37
14×14	4.22	3.87	2.38	-5.54
20×20	4.32	3.93	2.46	-8.09
Electron nominal energy : 6 MeV				
6×6	6.05	5.46	2.47	-0.81
10×10	6.25	5.57	2.39	-4.15
14×14	6.25	5.60	2.38	-4.15
20×20	6.30	5.64	2.40	-4.99
Electron nominal energy : 8 MeV				
6×6	7.83	7.15	2.78	2.17
10×10	8.03	7.44	2.61	-0.38
14×14	8.31	7.39	2.57	-3.89
20×20	8.23	7.39	2.63	-2.85
Electron nominal energy : 10 MeV				
6×6	10.00	9.11	2.80	-0.02
10×10	9.72	9.08	2.95	2.77
14×14	10.04	9.33	2.92	-0.38
20×20	9.95	9.31	2.91	0.49
Electron nominal energy : 12 MeV				
6×6	12.05	10.78	2.67	-0.42
10×10	11.99	10.82	2.66	0.09
14×14	12.02	11.13	2.78	-0.19
20×20	11.87	11.07	2.89	1.07

The relation between the mean energy $\bar{E}_0$ and R_{50} is illustrated in Figure 2.

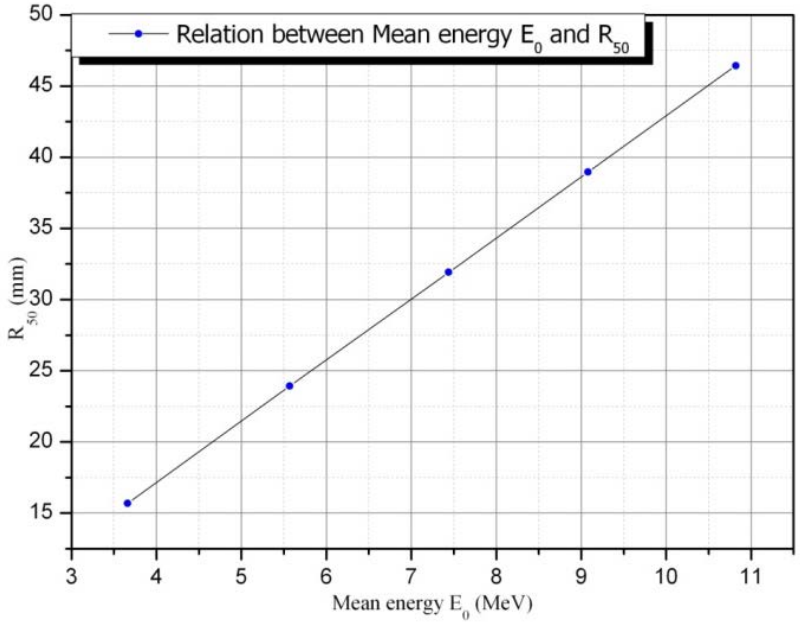


Figure (2). The relation between $\bar{E}_0$ and R_{50}

Beam profiles:

Figures 3, 4, 5 and 6 illustrate selected beam profiles for energies 4 MeV and applicator size 6×6 cm^2, 6 MeV and applicator size 10x10 cm^2, 10 MeV and applicator size 20×20 cm^2, and 12 MeV and applicator size 14×14 cm^2, respectively.

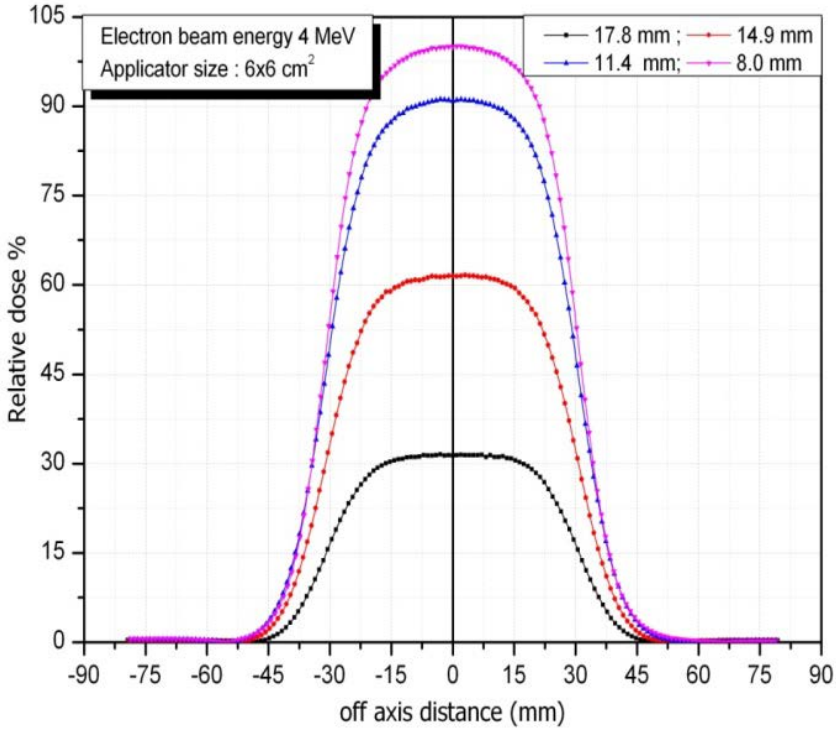


Figure (3). Beam profile for 4 MeV electron beam and applicator of size 6×6 cm^2

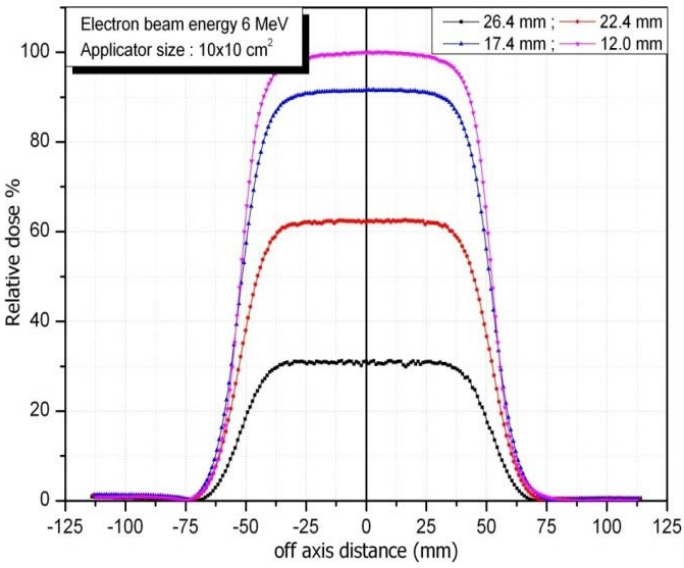


Figure (4). Beam profile for 6 MeV electron beam and applicator of size 10×10 cm^2.

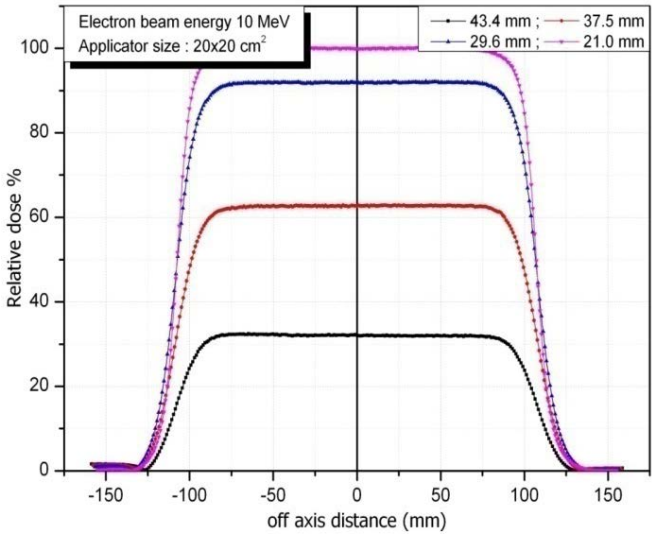


Figure (5): Beam profile for 10 MeV electron beam and applicator of size 20×20 cm^2.

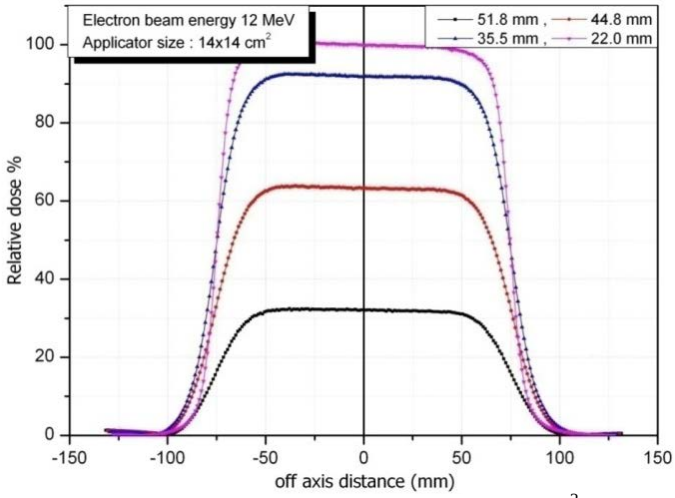


Figure (6). Beam profile for 12 MeV electron beam and applicator size 14×14 cm^2.

The characteristics of beam profiles (beam flatness, beam symmetry and penumbra) for electron beam energies 4, 6, 8, 10 and 12 MeV using two applicators of size 14×14 cm^2 and 20×20 cm^2 are tabulated in Table 4.

Table (4). Beam profiles characteristics of the electron beam

App. size (cm^2)	Flatness %	Symmetry %	Penumbra width (cm)	
			L	R
Electron beam energy: 4 MeV				
14×14	2.94	1.93	1.28	1.30
20×20	2.23	0.12	1.23	1.27
Electron beam energy: 6 MeV				
14×14	2.14	0.22	1.19	1.20
20×20	1.14	1.33	1.14	1.14
Electron beam energy: 8 MeV				
14×14	2.59	0.05	1.12	1.14
20×20	1.81	0.74	1.09	1.11
Electron beam energy: 10 MeV				
14×14	2.23	1.82	1.17	1.19
20×20	1.10	0.03	1.13	1.14
Electron beam energy: 12 MeV				
14×14	2.39	0.47	1.01	1.03
20×20	1.67	1.14	0.96	0.97

Applicators factors:

Applicator factor defines the ratio of output of the utilized applicator to the output of the reference applicator (10×10 cm^2). Table 5 represents the applicator factors measured at the depth of dose maximum and SSD=100 cm.

Table (5). Applicators factor of energies 4, 6, 8, 10 and 12 MeV electron beams.

Applicator size (cm^2)	Electron energy (MeV)				
	4	6	8	10	12
6×6	0.847	0.934	0.956	0.976	0.984
10×10	1.000	1.000	1.000	1.000	1.000
14×14	1.041	1.013	0.987	0.977	0.982
20×20	1.036	1.018	0.994	0.965	0.976

DISCUSSION

The maximum deviation of the measured value from the declared value of penetrative quality should be ±1 mm as recommended by manufacturer customer acceptance tests (Elekta, 2007). The results showed that the maximum deviation was 0.37 mm, which occurred for 8 MeV nominal energy. This is within the acceptable limit of ±1 mm. As shown in Figure 1, the measured PDD is increased with electron beam energy and is decreased with depth. The rapidness of the radiation dose is a decreasing function of electron beam energy while the magnitude of bremsstrahlung contamination is an increasing function of electron beam energy.

The bremsstrahlung contamination to the central axis percentage depth dose, $D_x\%$, was less than 0.55% of the maximum dose for electron beam of energies 4 MeV - 8 MeV, 0.84% for 10 MeV and

1.44% for electron beam of energy 12 MeV. These percentages are within the tolerance limits of 1% for electron beam energy 4 MeV, and 4% for electron beam energy 20 MeV (Podgorsak, 2003). Table 3 shows that the most probable energy $E_{p,0}$ is higher than the mean energy $\bar{E}_0$ due to the spread of the electron beam spectrum. The tolerance limit between calculated most probable energy and nominal energy is ±5% (Chosdu, et al., 1995). Thus, the calculated most probable energies are in agreement with the tolerance limit except electron beam of energy 4 MeV and applicator of sizes 14×14 $cm^{2,}$,and 20×20 cm^2 which exceeded the tolerance limit. Furthermore, the values of dose gradient G for all electron beam energies agree with the lower limit of 2.3.

The relation between mean energy $\bar{E}_0$ and R_{50} is linear and is in agreement with the theoretical results of Monte Carlo calculations (HPA, 1975).

The values of beam flatness and symmetry were calculated according to International Electrotechnical Commission (IEC) (IEC, 1997). The tolerance limits of flatness and symmetry are ±3% and ±2%, respectively. It is noticeable that the values of flatness decrease as applicator size increases which means that the values of flatness are field size dependent. The highest value of flatness was 2.94% for electron beam energy 4 MeV and applicator size 14×14 cm^2, whereas the lowest value was 1.10% for electron beam energy 10 MeV and applicator size 20×20 cm^2. The values of flatness meet the tolerance limit of ±3%. The values of symmetry range from 0.03% to 1.93%. This means that the values of symmetry for all energies and two applicators of size 14×14 cm^2 and 20×20 cm^2 are well in agreement with the manufacturer's specifications and IEC.

Table 4 also shows that the penumbra width is an electron energy and field size dependent. The penumbra width is a decreasing function of electron beam energy and field size. The maximum recorded value of the penumbra was 1.30 cm for electron beam energy 4 MeV and applicator size 14×14 cm^2, while the minimum recorded value of the penumbra was 0.96 cm for electron beam energy 12 MeV and applicator size 20×20 cm^2.

The results of applicator factor show that the applicator factor is increased as the applicator size increased for energies 4 and 6 MeV, whereas for energies 8, 10 and 12 MeV is decreased as the applicator size increased. The results are in agreement with Varian TrueBeam and Elekta Versa HD studies (Glide et al., 2013; Narayanasamy et al., 2016).

CONCLUSION

In this work, the electron beams commissioning of an Elekta Synergy platform linear accelerator were presented and analyzed. The results show that the penetrative quality for all electron beam energies were within the tolerance limit of ±1 mm. Bremsstrahlung contamination obtained from measured (PDD) was less than 1.5% for electron beam of nominal energy of 12 MeV which is within the tolerance limits. Values of dose gradient G meet the lower limit of 2.3. The relation between the mean energy $\bar{E}_0$ and R_{50} is linear, and the percentage error between the most probable energy $E_{p,0}$ and nominal energy fall within the ±5%limit. Beam flatness and beam symmetry, which characterized the beam profiles, meet the IEC 60731 tolerance. The penumbra width is a decreasing function of electron beam energy. The maximum value of the penumbra recorded was 1.30 cm for 4 MeV, whereas the minimum value was 0.96 cm. for 12 MeV. Finally, it is concluded that the measured data of all electron beam energies were found to be within permissible limits. Therefore, all energies of the electron beam can safely be used for clinical purposes.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: The first and the third authors did the measurements and collected all nec-

essary data needed. All authors were involved in the discussion of the results. First author wrote the paper with contributions from the second and third coauthors.

Funding: This work is not funded.

REFERENCES

Acquah, G. F., & Schiestl, B. (2014). Quality assurance and performance evaluation of an elekta linear accelerator: results from over a year QA experience in Ghana. *IJSBAR*, *14*, 356-363.

Arunkumar, T., Supe, S. S., Ravikumar, M., Sathiyan, S., & Ganesh, M. (2010). Electron beam characteristics at extended source-to-surface distances for irregular cut-outs. *Journal of Medical Physics/Association of Medical Physicists of India*, 35(4), 207.

Brahme, A., & Svensson, H. (1976). Specification of electron beam quality from the central-axis depth absorbed-dose distribution. *Medical physics*, 3(2), 95-102.

Chosdu, R., Hilmy, N., Tobing, R., Kicky, L. T. K., Razzak, M., Kovacs, A., & Miller, A. (1995). Dosimetry measurements during the commissioning of the GJ-2 electron accelerator. *Radiation Physics and Chemistry*, 46(4-6), 1175-1179.

Elekta customer acceptance test. (2007). Elekta Oncology Systems Ltd. Grawely: Document number 4513 370 1870 08

Gerbi, B. J. (2006). Clinical Applications of High-Energy Electrons. In Levitt, S. H., Purdy, J. A. editors. Technical Basis of Radiation Therapy. Medical Radiology (Radiation Oncology), Berlin, Springer.

Gerbi, B. J., Antolak, J. A., Deibel, F. C., Followill, D. S., Herman, M. G., Higgins, P. D., ... & Khan, F. M. (2009). Recommendations for clinical electron beam dosimetry: supplement to the recommendations of Task Group 25. *Medical physics*, 36(7), 3239-3279.

Glide-Hurst, C., Bellon, M., Foster, R., Altunbas, C., Speiser, M., Altman, M., ... & Solberg, T. (2013). Commissioning of the Varian TrueBeam linear accelerator: a multi-institutional study. *Medical physics*, 40 (3), 031719.

Hospital Physicist Association., (1975). A practical guide to selection dosimetry below 5 MeV for Radiotherapy purposes. London: HPA Report No. 13

International Atomic Energy Agency (IAEA). (1987). Absorbed Dose Determination in Photon and Electron Beams: An International Code of Practice for Dosimetry. Technical Report Series No. 277, Vienna, Austria

International Atomic Energy Agency (IAEA). (2000). Absorbed Dose Determination in External Beam Radiotherapy: An International Code of Practice for Dosimetry Based on Standards of Absorbed Dose to Water. Technical Report Series No. 398, Vienna, Austria

International Commission on Radiation Units and Measurements (ICRU). (1993). Prescribing, recording, and reporting photon beam therapy. ICRU report No. 50, Washington, DC, USA

International Electrotechnical Commission. (1984). Report 35-I and 35-II, Medical electrical equipment, Medical Electron Accelerator, Section IV: Functional performance characteristics and report, Geneva, Switzerland

International Electrotechnical Commission. (1997). Medical Electrical Equipment: Dosimeters with ionization chambers as used in radiotherapy standard. IEC-60731, Geneva, Switzerland

International Electrotechnical Commission. (2007). Medical electrical equipment, Medical Electron Accelerators: Functional performance characteristics. IEC-60976, Geneva, Switzerland

Jamshidi, A., Kuchnir, F. T., & Reft, C. S. (1987). Characteristic parameters of 6–22 MeV electron beams from a 25-MeV linear accelerator. *Medical physics*, 14(2), 282-288.

Khan, F. M. (2010). *Physics of Radiation Therapy*. 4th edition. Philadelphia, Lippincott Williams & Wilkins, USA.

Khan, F. M. (1991). Clinical electron-beam dosimetry. Report of AAPM Radiation Therapy Committee Task Group 25. *Med. Phys.*, *18*, 73-109.

Kirby, T. H., Gastorf, R. J., Hanson, W. F., Berkley, L. W., Gagnon, W. F., Hazle, J. D., & Shalek, R. J. (1985). Electron beam central axis depth dose measurements. Medical physics, 12(3), 357-361.

Kutcher, G. J., Coia, L., Gillin, M., Hanson, W. F., Leibel, S., Morton, R. J., ... & Wingfield, L. (1994). Comprehensive QA for radiation oncology: report of AAPM radiation therapy committee task group 40. *Medical Physics-Lancaster PA-*, *21*, 581-581.

Narayanasamy, G., Saenz, D., Cruz, W., Ha, C. S., Papanikolaou, N., & Stathakis, S. (2016). Commissioning an Elekta Versa HD linear accelerator. *Journal of applied clinical medical physics*, 17(1), 179-191.

Podgorsak, E. B. (2003). Review of radiation oncology physics: a handbook for teachers and students. *Vienna, Austria: IAE Agency*, 19, 133.

Sahool, S. K., Rath, A. K., Mukharjee, R. N., & Mallick, B. (2012). Commissioning of a Modern LINAC for Clinical Treatment and Material Research. *International Journal of Trends in Interdisciplinary Studies*, 1(10).

Varatharaj C, Shwetha B, Rekha Reddy B, Ravikumar M, S. Sathiyan, Ganesh K. M. (2016). Physical characteristics of photon and electron beams from a radiotherapy accelerator. *International Journal of Medical Research and Review Res Rev* .4 (7), 1178- 1188.

Al-Mukhtar Journal of Sciences 38 (4): 336-344, 2023
Doi: https://doi.org/10.54172/y4bfxs70

Research Article **Open Access**

Designing an Inhibitor Molecule to Combat Cancer through the Inhibition of Mutant PI3K (P110 α) Subunit Protein

Esmaiel I. F. Saad

***Corresponding author:**
Esmail.saad@omu.ed.ly
Department of Microbiology,
Omar Al Mukhtar University,
Libya

Received:
29 May 2023

Accepted:
01 December 2023

Publish online:
31 December 2023

Abstract

The PI3K pathway plays a crucial role in the development of various types of cancers. Specifically, the P110 α component is frequently modified in the formation of human cancer, leading to disruptions in cell growth regulation and the emergence of malignancies. This research paper delves into the increasing utilization of computational tools to forecast potential molecules capable of inhibiting cancer. To validate stable structures of PI3K (P110α) component protein models, the study employed Ramachandran map analysis, which revealed active regions in close proximity to the mutation site. Through the employment of PyRX software, molecular docking studies were conducted, resulting in the identification of four inhibitor derivatives that exhibited significant effectiveness with the lowest docking energies in preventing the mutated PI3K (P110 α) component protein structures. Furthermore, these inhibitors adhered to Lipinski's rule of five, further enhancing their potential.

Keywords: PI3k; Modeller; Molecular; Docking; PyRx

INTRODUCTION

The proto-oncogene PI3K (Phosphoinositid-3-Kinase) controls various essential cell signaling pathways that stimulate cellular replication and proliferation while repressing growth and apoptosis (Cantley 2002; Moens et al., 2013). The PI3K protein family comprises eight members categorized based on their sequence, domain structure, and mode of regulation into three groups: Class I PI3K, Class II PI3K, and Class III PI3K. Class I PI3K is composed of p110 α, p110 β, and p110γ subunit classes and plays a significant role in cancer biology. PI3K mutations are prevalent only in Class I PI3K, with the p110α subunit being the most commonly mutated protein in breast, endometrial, urinary tract, colorectal, and ovarian cancers. Mutations at codon 542, 545, and 1047 of PI3K (P110α) subunit protein are the most frequent mutational events in human carcinogenesis, resulting in the constitutive activation of downstream pathways and altered cellular proliferation and malignant transformation regulation. All this makes it an attractive drug target for treating cancer. Validation and identification of disease-associated targets are crucial for drug design and discovery. Mutations in the PIK3CA gene(s) that code PI3K (P110α) subunit promote uncontrolled cell growth and proliferation while inhibiting apoptosis. Chemotherapy, operative intervention, radiation treatment, and hormone therapy are conventional methods for managing cancer. However, they lack specificity and often damage healthy cells, resulting in side effects. Target-based drug design offers a promising approach to utilizing the PI3K (P110α) subunit as an anticancer protein (Jorgensen, 2004; Pearce

et al., 2008; Sliwoski et al., 2013; Bassani and Moro 2023). Recent studies have demonstrated the efficacy of targeting the PI3K (P110α) subunit in cancer therapy (Apel et al., 2019; Uniyal et al., 2022; Sen et al., 2022).

MATERIALS AND METHODS

A Boolean query was used to trace the protein structure of PI3K (P110 α) subunit in Homo sapiens and was analyzed against the UniProt database (http://www.uniprot.org). The protein sequence query (P42336) was obtained in fasta format from the UniProt database (http://www.uniprot.org/uniprot/). Modeller 9.17 software was utilized to predict the 3D structures of both wild-type and mutated proteins (Sali et al., 1995). Relevant mutations at codons 542, 545, and 1047 of the PI3K (P110 α) subunit were collected from literature sources (Engelman et al., 2006; Yuan and Cantley 2008; Janku et al., 2011). The quality of the structural models was assessed using the SAVESv6.0 tool, which employs the Ramachandran plot to evaluate the stereochemical characteristics of the protein structure (Laskowski et al., 1993). The predicted active site of the mutated conformations of the PI3K (P110 α) subunit was determined using PASS software and utilized for virtual screening. In silico virtual screening was performed using PyRx software to rank the library molecules based on their affinity towards the active site of the mutated protein conformations of the PI3K (P110 α) subunit. The top ten potential library molecules were further analyzed using Lipinski filter software to assess their suitability as drugs (Lipinski et al., 1997). Recent studies have demonstrated the effectiveness of in silico virtual screening in the field of drug discovery (Patra et al., 2014; Garner et al., 2017; An et al., 2020; Kim et al., 2022; Saad and Almabruk 2022).

RESULTS AND DISCUSSION

In this study, we utilized the homology modeling procedure to establish the arrangement of the PI3K (P110 α) subunit protein along with its three modified models. This allowed us to gain a better understanding of the structural organization of these proteins and their potential implications. The BLASTP search was performed against the PDB database using the Modeller 9.17 software to find suitable templates for homology modeling. The template with maximum identity, high score, and lower e-value was selected for homology modeling. The final stable structure of the PI3K (P110 α) subunit protein was visualized in Figure 1.

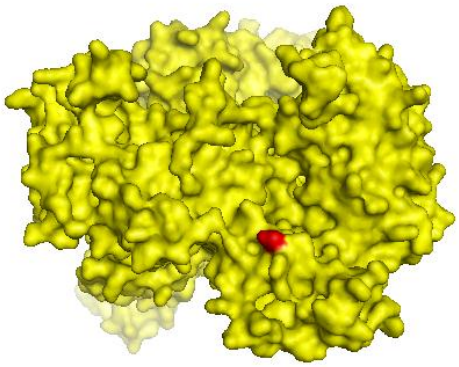


Figure (1). Protein models constructed for wild type with normal codon 545 (Glu) in red color.

The modifications were selected based on the findings of several prior studies, including those conducted by Samuels et al. in 2004, Lee et al. in 2005, Ikenoue et al. in 2005, Isakoff et al. in 2005, Kang et al. in 2005, Samuels and Ericson in 2006, and Zhao and Vogt in 2008. These findings provide valuable insights into the structural aspects of PI3K (P110 α) subunit protein and its mutations, which can be beneficial in drug discovery and design. Recent studies have demonstrated the potential of homology modeling in drug discovery (Saad 2017; Saad and Attialla 2017; Kim et al., 2022; Samad et al., 2023; Osweiher and Saad 2022). Additionally, homology modeling has been used to determine the structure of various proteins involved in diseases such as cancer and infection by SARS-COV-2 (Apel et al., 2019; Sen et al., 2022). The use of mutated models in homology modeling has also been shown to be an effective approach to drug discovery (Uniyal et al., 2022; Samad et al., 2023).

In this study, the homology modeling procedure was used to determine the arrangement of the PI3K (P110 α) subunit protein and its three modified forms. For each protein, three distinct models was created. Figures 1 and 2 showcase the selected models for the normal and mutated 545 codons of the PI3K (P110 α) subunit. These figures clearly demonstrate the significant alterations in amino acids caused by the mutations in codon 545.

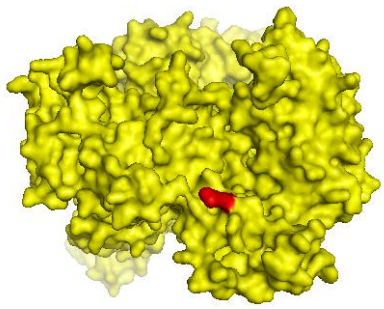


Figure: (2). Protein models constructed for mutated PI3K (P110 α) subunit of Codon 545 which mutated by Lys in Red color.

In this investigation, comparative modeling techniques were used to create protein structures of both the natural and mutated forms of the PI3K (P110 α) subunit. The resulting models were then evaluated using the SAVES server from the NIH MBI Laboratory for Structural Genomics and Proteomics. Two tools, PROCHECK and Verify_3D, were employed to assess the validity of the models. Interestingly, one of the models generated by Modeller was found to be more acceptable than the others, making it the most reliable choice for each protein. This selection was made based on Table 1.

When analyzing the protein models, it was found that the majority (85.1%) of the phi/psi angles of the residues fell within the most favored regions. This indicates that the models possess a high level of quality. To identify potential binding sites within the protein models, the researchers utilized PASS software. As a result, three potential binding sites were identified. To pinpoint the active site, the researchers considered the probe with the largest number of residues. These findings provide valuable insights into the structure of the PI3K (P110 α) subunit protein, as well as its mutation. Such information can prove to be highly advantageous in the field of drug discovery and design.

Table: (1). Validation of PI3K (P110 α) subunit structure

Selected PI3K (P110α) protein model	Ramachandran map analysis			
	phi/psi angles %	Additional allowed regions	Additional disallowed regions	Generously allowed regions
Model of PI3K (P110α)	85.1	11.7	0.8	2.4

In various studies, the quality of protein models has been assessed using validation tools like PROCHECK and Verify_3D (Colovos and Yeates, 1993; Berman et al., 2000; Lovell et al., 2003). These evaluations have shown that software such as PASS is effective in identifying binding sites in protein models for drug discovery (Saad and Attialla 2017; Apel et al., 2019; Sen et al., 2022; Osweiher and Saad 2022; Samad et al., 2023). The main aim of this investigation was to compare the binding sites detected by PASS software with the active site of the template protein in order to identify the active site of PI3K (P110 α) subunit protein models. The results revealed that the active site was located at residues 570, 596, 616, 631, 636, 676, 686, and 885, as shown in Figure 3.

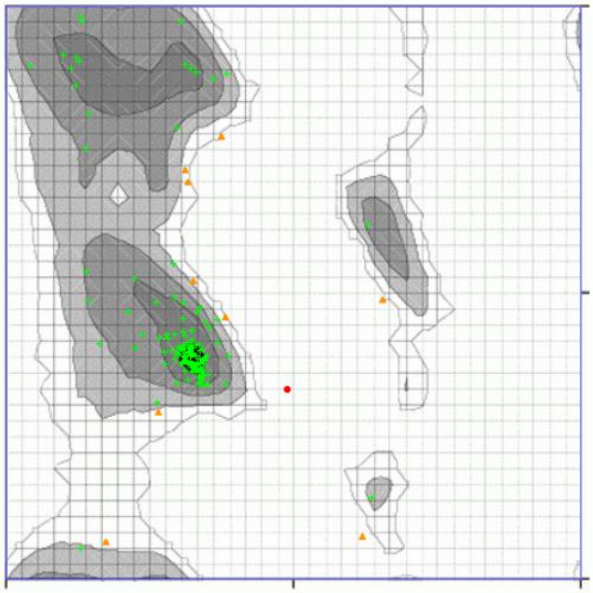


Figure: (1). Shows a Ramachandran plot of PI3K (P110 α) subunit wild-type protein models to validate the structure.

These identified active site residues align with those previously reported in other studies as part of the active site (Shah et al., 2002; von Bubnoff et al., 2005; Zunder et al., 2008). Furthermore, ten potential inhibitor molecules were screened and found to target the active site in the mutant codon 545 of the PI3K (P110 α) subunit protein. This approach provides valuable insights into the structure of the PI3K (P110 α) subunit protein and its potential inhibitors, which can considerably aid the drug discovery and design process. Recent studies have demonstrated the usefulness of computational approaches in drug discovery, such as virtual screening and molecular dynamics simulations (Cheol-Min et al., 2006; Apel et al., 2019; Ton et al., 2020). The use of software such as PASS to identify active sites in proteins has also been shown to be effective in drug discovery.

This analysis utilized molecular docking to identify potential inhibitor molecules for the PI3K (P110 α) subunit protein which is presented in Table (2). The identified inhibitors were found to be in agreement with previous docking studies conducted by Garcia-Echeverria and Sellers

(2008); Saad (2017); Saad and Attialla (2017) and Jin et al., (2021). Additionally, the potential inhibitors met the criteria of Lipinski's 'rule of five', which evaluates drug-likeness based on properties such as solubility, partition coefficient, and molecular weight. These findings suggest that the identified inhibitors have the potential to be effective drugs for PI3K (P110 α) subunit protein and can be considered for further research. Recent studies have demonstrated the importance of drug-likeness evaluation in drug discovery, such as Lipinski's 'rule of five' (Bickerton et al., 2012; Pires et al., 2015; Saad 2017; Saad and Attialla 2017). Moreover, the use of computational methods such as molecular docking has been shown to be effective in the identification of potential inhibitors for various proteins (Muryshev et al., 2003; Cheol-Min et al., 2006; Pinzi and Rastelli 2019; Khan et al., 2022).

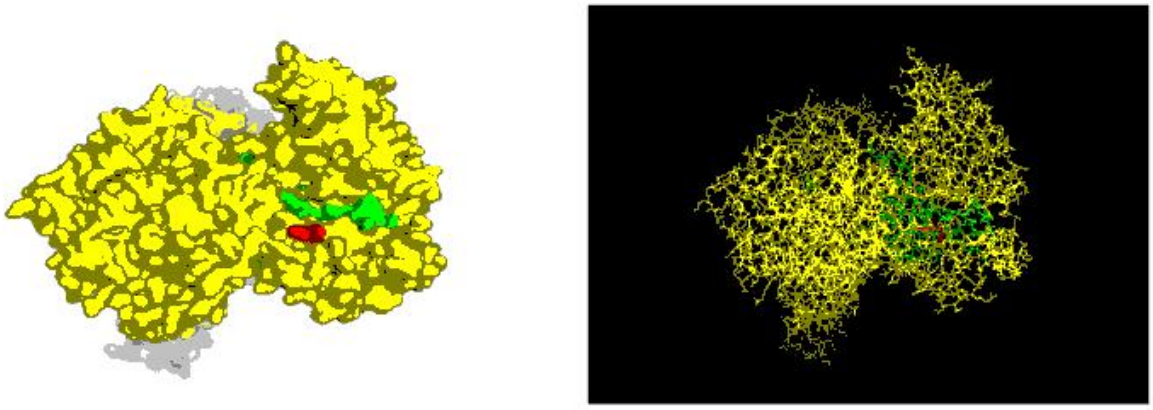


Figure: (3). The active site was identified by PASS green colors show Point mutations at codon 545 red color show active site point of PI3K (P110 α) subunit

Table (2). Docked Energy and Lipinski's Values of Ligand Molecules

Ligand molecules	Molecular formula	Docking energy (kcal/mol)	Xlog P<5	H-Bond donor <5	H-Bond Acceptor <10	Molecular weight (g/mol) <500
Sorafenib	C21H16ClF3N4O3	-12.90	4.200	3	7	464.50
Pilaralisib	C21H16N6O2S2	-11.30	0.4303	0	2	443.00
vemurafenib	C23H18ClF2N3O3S	-10.01	2.6878	1	3	466.00
BEZ235	C30H23N5O	-9.10	-0.6025	1	1	463.00

CONCLUSION

By conducting computational analysis and examining available experimental data, we have identified the most effective ligand molecules for inhibiting the function of the mutant PI3K (P110 α) subunit protein. We specifically focused on ligands that adhere to Lipinski's 'rule of five'. These molecules have demonstrated the ability to halt the growth and proliferation of cancer cells, making them highly promising candidates for future drug development and research endeavors.

Duality of interest: The author has confirmed there is no conflict of interest in this manuscript.

Contribution: The author has carried out all the work related to this manuscript**.**

Funding: There was specific funding for this work.

REFERENCES

An, X., Bai, Q., Bing, Z., Liu, H., Zhang, Q., Liu, H., & Yao, X. (2020). Revealing the Positive Binding Cooperativity Mechanism between the Orthosteric and the Allosteric Antagonists of CCR2 by Metadynamics and Gaussian Accelerated Molecular Dynamics Simulations. *ACS chemical neuroscience*, *11*(4), 628–637. https://doi.org/10.1021/acschemneuro.9b00630

Apel A. Robert K.Y. Cheng R.K.Y. Tautermann C. H. Brauchle M., Huang C. Pautsch A., Hennig M., Nar H.and Schnapp G.(2019). Crystal Structure of CC Chemokine Receptor 2A in Complex with an Orthosteric Antagonist Provides Insights for the Design of Selective Antagonists.Structure, 27(3): 427-438.e5, https://doi.org/10.1016/j.str.2018.10.027.-0812

Bassani, D., and Moro, S. (2023). Past, Present, and Future Perspectives on Computer-Aided Drug Design Methodologies. *Molecules (Basel, Switzerland)*, *28*(9), 3906. https://doi.org/10.3390/molecules28093906

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H.,... Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research*, 28(1), 235-242.

Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S., & Hopkins, A. L. (2012). Quantifying the chemical beauty of drugs. *Nature Chemistry*, 4(2), 90-98.

Cantley, L. C. (2002). The phosphoinositide 3-kinase pathway. *Science*, 296(5573), 1655-1657.

Cheol-Min P. Tetsuro O. Andrew M. Petros A.M. Haichao Zhang H., Nimmer P. M. Henry R. F. and Elmore S. W.(2006).Design, Synthesis, and Computational Studies of Inhibitors of Bcl-XL. *Journal of American Chemical Societ*.128 (50): 16206 –16212. https://doi.org/10.1021/ja0650347

Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: Patterns of nonbonded atomic interactions. *Protein Science*, 2(9), 1511-1519.

Engelman, J. A., Luo, J., & Cantley, L. C. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature Reviews Genetics*, 7(8), 606-619.

Garcia-Echeverria, C., & Sellers, W. R. (2008). Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene*, 27(41), 5511-5526.

Garner, T. P., Lopez, A., Reyna, D. E., Spitz, A. Z., & Gavathiotis, E. (2017). Progress in targeting the BCL-2 family of proteins. *Current opinion in chemical biology*, *39*, 133–142. https://doi.org/10.1016/j.cbpa.2017.06.014

Ikenoue, T., Kanai, F., Hikiba, Y., Obata, T., Tanaka, Y., Imamura, J.,... Matsumoto, K. (2005). Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Research*, 65(11), 4562-4567.

Isakoff, S. J., Engelman, J. A., & Irie, H. Y. (2005). Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Research*, 65(23), 10992-11000.

Janku, F., Lee, J. J., Tsimberidou, A. M., Hong, D. S., Naing, A., & Falchook, G. S. (2011). PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. *PLoS One*, 6(7), e22769.

Jin, Z., Zhao, Y., Sun, Y., Zhang, B., Wang, H., Wu, Y.,... Yang, H. (2021). Structural basis for the inhibition of SARS-CoV-2 main protease by antineoplastic drug carmofur. *Nature Structural and Molecular Biology*, 28, 529-535.

Jorgensen, W. L. (2004). The many roles of computation in drug discovery. *Science*, *303*(5665), 1813-1818.

Kang, S., Bader, A. G., & Vogt, P. K. (2005). Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proceedings of the National Academy of Scienc*es, 102(3), 802-807.

Khan, S., Fakhar, Z., Hussain, A., Ahmad, A., Jairajpuri, D. S., Alajmi, M. F., & Hassan, M. I. (2022). Structure-based identification of potential SARS-CoV-2 main protease inhibitors. *Journal of biomolecular structure & dynamics*, *40*(8), 3595–3608. https://doi.org/10.1080/07391102.2020.1848634

Kim, S., Park, H. S., & Oh, B. H. (2022). Computational design of an apoptogenic protein that binds BCL-xL and MCL-1 simultaneously and potently. *Computational and structural biotechnology journal*, *20*, 3019–3029. https://doi.org/10.1016/j.csbj.2022.06.021

Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: A program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, 26(2), 283-291.

Lee, J. W., Soung, Y. H., Kim, S. Y., Nam, H. K., Park, W. S., Nam, S. W.,... Kim, S. H. (2005). PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene*, 24(8), 1477-1480.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1-3), 3-25.

Lovell, S. C., Davis, I. W., & Arendall, W. B. III. (2003). Structure validation by Cα geometry: φ, ψ and Cβ deviation. Proteins: Structure, Function, and Bioinformatics, 50(3), 437-450.

Moens, U., Kostenko, S., & Sveinbjørnsson, B. (2013). The Role of Mitogen-Activated Protein Kinase-Activated Protein Kinases (MAPKAPKs) in Inflammation. Genes, 4(2), 101–133. https://doi.org/10.3390/genes4020101

Muryshev, A. E., Tarasov, D. N., Butygin, A. V., Butygina, O. Y., Aleksandrov, A. B., & Nikitin, S. M. (2003). A novel scoring function for molecular docking. *Journal of computer-aided molecular design*, *17*(9), 597–605. https://doi.org/10.1023/b:jcam.0000005766.95985.7e

Osweihr W.S M. and. Saad E. I. (2022) Identification of an Inhibitor Molecule for CancerCausing Src Mutant Protein using in silico Analysis. *Libyan Journal of Basic Sciences*. Vol. 19(3) No. 33-45.

Patra, M. C., Maharana, J., Dehury, B., & De, S. (2014). Computational insights into the binding mechanism of antagonists with neuropeptide B/W receptor 1. *Molecular bioSystems*, *10*(8), 2236–2246. https://doi.org/10.1039/c4mb00214h

Pearce, L. R., Komander, D., & Alessi, D. R. (2008). The nuts and bolts of AGC protein kinases. *Nature Reviews Molecular Cell Biology*, 11(1), 9-22.

Pinzi, L., & Rastelli, G. (2019). Molecular Docking: Shifting Paradigms in Drug Discovery. *International journal of molecular sciences*, 20 (18), 4331. https://doi.org/10.3390/ijms20184331

Pires, D. E., Blundell, T. L., & Ascher, D. B. J. J. o. m. c. (2015). pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *58*(9), 4066-4072.

Saad, E. I. (2017). Drug Design for Cancer-Causing PI3K (P110 α) subunit Mutant Protein. *International Journal of Pharmacy & Life Sciences*, 8(11).

Saad IF. and Almabruk AA. (2022). Tracking evolution of collagen subfamily for its utmost possible benefit. *Al-Jabal Academy Journal of Pure and Applied Sciences*. 1(1); 41-49. https://doi.org10.58309/aajpas.v1i1.12 .

Saad, E. I. and Attitalla H. (2017). Molecular Dynamic Simulation and an Inhibitor Prediction of PI3K (P110 α) Subunit Mutant Protein Structured Model as a Potential Drug Target for Cancer. Vol. 1 No. 1:

Sali, A., Potterton, L., Yuan, F., van Vlijmen, H., & Karplus, M. (1995). Evaluation of comparative protein modeling by Modeller. Proteins: Structure, Function, and Genetics, 23(3), 318-326.

Samad, A., Ajmal, A., Mahmood, A., Khurshid, B., Li, P., Jan, S. M., Rehman, A. U., He, P., Abdalla, A. N., Umair, M., Hu, J., & Wadood, A. (2023). Identification of novel inhibitors for SARS-CoV-2 as therapeutic options using machine learning-based virtual screening, molecular docking and MD simulation. *Frontiers in molecular biosciences*, *10*, 1060076. https://doi.org/10.3389/fmolb.2023.1060076

Samuels, Y., and Ericson, K. (2006). Oncogenic PI3K and its role in cancer. *Current Opinion in Oncology*, 18(1), 77-82.

Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S.,... Velculescu, V. E. (2004). High frequency of mutations of the PIK3CA gene in human cancers. *Science*, 304(5670), 554.

Sen, D., Debnath, P., Debnath, B., Bhaumik, S., & Debnath, S. (2022). Identification of potential inhibitors of SARS-CoV-2 main protease and spike receptor from 10 important spices through structure-based virtual screening and molecular dynamic study. *Journal of biomolecular structure & dynamics*, *40*(2), 941–962. https://doi.org/10.1080/07391102.2020.1819883

Shah, V. P., Midha, K. K., Dighe, S., McGilveray, I. J., Skelly, J. P., Yacobi, A.,... Viswanathan, C. T. (2002). Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies. *Journal of Pharmaceutical Sciences*, 91(1), 1-10.

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W., Jr (2013). Computational methods in drug discovery. *Pharmacological reviews*, *66*(1), 334–395. https://doi.org/10.1124/pr.112.007336

Ton, A. T., Gentile, F., Hsing, M., Ban, F., & Cherkasov, A. (2020). Rapid Identification of Potential Inhibitors of SARS-CoV-2 Main Protease by Deep Docking of 1.3 Billion Compounds. *Molecular informatics*, *39*(8), e2000028. https://doi.org/10.1002/minf.202000028.

Uniyal, A., Mahapatra, M. K., Tiwari, V., Sandhir, R., & Kumar, R. (2022). Targeting SARS-CoV-2 main protease: structure based virtual screening, in silico ADMET studies and molecular dynamics simulation for identification of potential inhibitors. *Journal of biomolecular structure & dynamics*, *40*(8), 3609–3625. https://doi.org/10.1080/07391102.2020.1848636

von Bubnoff, N., Peschel, C., and Duyster, J. (2005). Resistance of Philadelphia-chromosome positive leukemia towards the kinase inhibitor imatinib (STI571, Glivec): A targeted oncoprotein strikes back. Leukemia, 19(8), 1437-1442.

Yuan, T. L., and Cantley, L. C. (2008). PI3K pathway alterations in cancer: Variations on a theme. *Oncogene*, 27(41), 5497-5510.

Zhao, H., and Vogt, P. K. (2008). Helical domain and kinase domain mutations in p110α of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proceedings of the *National Academy of Sciences*, 105(7), 2652-2657.

Zunder, E. R., Knight, Z. A., and Shokat, K. M. (2008). Discovery of drug-resistant and drug-sensitizing mutations in the oncogenic PI3K isoform p110α. Cancer Cell, 14(2), 180-192.

Al-Mukhtar Journal of Sciences 38 (4): 345-353, 2023
Doi: https://doi.org/10.54172/e76ycq22

Research Article **Open Access**

Characterization and Isolation of Fungi from Domestic Pigeon Droppings in the Governorate of Erbil and its Suburban Area

Neveen N. Rajab[1*] and Nadeem A. Ramadan[2]

***Corresponding author:** neveen.rajab@su.edu.krd, Department of Biology, Faculty of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq.

Second Author: Nadeem.ramadan53@yahoo.com, Hadbaa University College, Mosul, Iraq.

Received:
07 July 2023

Accepted:
23 November 2023

Publish online:
31 December 2023

Abstract

A total of 200 samples of domestic bird droppings were collected from 54 regions in Erbil Governorate and its suburbs, and 23 of the isolates were fungi obtained by using the Niger bird seed medium and Sabouraud dextrose agar medium. The fungal genera were identified through macroscopic, microscopic, and phenotypic properties on agar plates and slides by staining with Lactophenol cotton blue stain. Findings belong to nine genera (*Aspergillus*, *Alternaria*, *Chrysosporium*, *Cunninghamella*, *Helminthosporium*, *Mucor*, *Penicillium*, *Rhizomucor*, and *Rhizopus*). Among the isolates, six species belonging to *Aspergillus* were identified which also had the highest frequency (44.4 %) in total genera, while the lowest frequency was (0.1 %) for *Alternaria* sp., *Chrysosporium* sp., *Cunninghamella* sp. Results reveal that 17 sites' samples of bird droppings produced negative results when tested on Niger bird seed agar, while only two locations produced no results when tested on Sabouraud dextrose agar. Thus, Sabouraud dextrose agar (SDA) is thought to be better than Niger bird seed agar (NBSA) for isolating fungi since it is considered a generally rich media for them.

Keywords: Fungi, Pigeon droppings, *Aspergillus* sp.

INTRODUCTION

Mycotic infections are the most common in all types of birds. However, they are less dominating when compared to bacterial and viral illnesses. Aspergillosis, Candidiasis, Dactylariosis, Cryptococcosis, Favus, Rhodotorulosis, Torulopsis, Mucormycosis, Histoplasmosis, and Cryptococcosis are among the fungal pathogens. (Dhama et.al. 2013). As environmental indicators, pigeons and free-flying wild birds (Doves) (Streptopelia) play a critical role in public health. Several fungal species are commonly detected in bird feces, especially yeasts of the genera Cryptococcus Vuill, Candida Berkh, Trichosporon Behrend, and Rhodotorula, in addition to filamentous fungi belonging to the genera Aspergillus Michelli, and Penicillium spp (Elhariri, Hamza et al. 2015 ; Mendes et al., 2014; Santos et al., 2009; Fraga et al., 2011).

Approximately 50% of all birds are regarded to be reservoirs and carriers of fungi that could be dangerous to both birds and humans (Dynowska et al. 2015). Grisin et al. (2017) concluded that birds and their droppings can carry over 60 diseases, many of which are airborne and can be transmitted to humans merely by being close to them.

This is because dried bird droppings are generally very fertile ground for fungal species growth, due to high concentrations of nitrogenous bases and because as the droppings age, they contain higher concentrations of fungi than when recently eliminated (Silva & Paula,1963; Mendes et al. 2014; Elhariri et al. 2015).

Fungal species connected with bird nests have been studied in both terrestrial and wetland bird nests; nevertheless, data on this topic is still limited. The body temperature of birds (about 39-43^{o}C) and the unique conditions that exist in nests make them an ideal setting for fungus development. Many suitable substrates encourage microbial growth such as plant materials used to build nests, bird feces, animal remains, and keratin-rich substrates (feathers or hair). The nest position also affects the diversity of fungal species inside the pigeon nest, which affects the source of the nutrients (Hubálek et.al. 1976; Kornilłowicz-Kowalska et.al. 2011). Recent research has focused on teratophiliac fungi (fungi that obstruct wetland bird nests due to high amounts of keratin and favorable climatic conditions, such as greater humidity). (Kornilłowicz-Kowalska et.al.2011& 2013).

Our research aims to find out what types of molds inhabit pigeon droppings in Erbil city and its suburbs.

MATERIALS AND METHODS

Sample Collection

Two hundred samples of pigeon droppings were collected from 54 different regions in Erbil city and its suburbs from November 2021 to October 2022. Samples have been collected and transferred in sterilized and sealed containers into the advanced mycology laboratory at Science College/ Salahaddin University.

Isolation of Fungi

A biosafety cabinet was used for homogenizing and processing all samples. 20 grams of Pigeon (*Columbia Livia)* dropping samples were suspended in sterilized phosphate-buffered saline (PBS) at a 1:5 ratio by vortexing for 5 minutes and centrifuged at 500^{X} g for 5 min. 100 µL from the supernatant from each tube was inoculated onto Niger Bird Seed Agar **(**NBSA**)** in a Petri dish and onto Sabouraud dextrose agar (SDA) plates containing 1.0 g creatinine and 40 mL chloramphenicol (50 µg/mL). The Petri dishes were incubated in the dark at 25 °C in humid conditions for 3-10 days (Chae et. al. 2012)

Identification methods

In general, the identification of filamentous fungi was based on classic taxonomy (macro and microscopic characteristics). The surface and the reverse of the colonies were observed, as well as diameter, conidial color, texture, and presence of soluble pigments (Tell, 2005; Dugan, 2006; Balajee, et.al. 2007).

RESULTS

In Erbil city and its surroundings, 200 samples of regional pigeon excretion were gathered from 54 different areas, and 23 of the isolates were fungi obtained by using the Niger bird seed medium. The fungal genera were identified through macroscopic and microscopic properties on agar plates and slides, which belongs to 8 genera (*Aspergillus, Alternaria, Chrysosporium, Helminthosporium, Mucor, Penicillium, Rhizomucor and Rhizopus*). Among the isolates, four species belong to the genus *Aspergillus,* while for *Alternaria, Chryspsprium, Helminthospori-*

um, and *Rhizomucor* only the genus has been identified with one isolate for each, as shown in table-1. *Aspergillus niger* and *Mucor* had the highest prevalence of the isolates (8.7%) in Bnaslawa. Other species (*Alternaria, Chrysosporium,* frequency (4.3 %) in most of the areas, as shown in the Table 1.

Table (1). Mold isolated from pigeon droppings on Niger bird seed agar medium.

No.	Sample source	Mold	No. of colonies	%Frequency
1.	Bahare nwey	0	0	0
2.	Bahare kon	0	0	0
-+3.	Barzan	0	0	0
4.	Berkote nwey	0	0	0
5.	Bnaslawa	*Aspergillus niger*	2	8.7
		A.ochraceous	1	4.3
		Mucor sp.	2	8.7
6.	Chnar	*A.fumigatus*	1	4.3
		A.ochraceous	1	4.3
		Rhizopus sp.	1	4.3
7.	Chwarchra	*A.candidus*	1	4.3
		Penicillium sp.	1	4.3
8.	Daratu	0	0	0
9.	Darwazae shar	0	0	0
10.	Framanbaran	0	0	0
11.	Galawezh	0	0	0
12.	Havalan	*A.niger*	1	4.3
		Mucor sp.	1	4.3
13.	Kany gany	0	0	0
14.	Kasnazan	*A.niger*	1	4.3
		Rhizomucor sp.	1	4.3
15.	Mahala arab	*Helminthosporium sp.*	1	4.3
16.	Makhmur	*Penicillium sp.*	1	4.3
17.	Mala omer	0	0	0
18.	Mamostayan	A.ochraceous	1	4.3
		Penicillium sp.	1	4.3
		Rhizopus sp.	1	4.3
19.	Masif salahaddin	0	0	0
20.	Mufti	0	0	0
21.	Nawroz	0	0	0
22.	Science college	0	0	0
23.	Setaqan	0	0	0
24.	Shawes	0	0	0
25.	Shurtawa	*A.niger*	1	4.3
26.	Tayrawa	*A.niger*	1	4.3
27.	Xanaqa	*Alternaria sp.*	1	4.3
28.	Zanko	*Chrysosporium sp.*	1	4.3
29.	Zanko village	0	0	0
Total		23		

The findings in Table-1 and Table-2 reveal that 17 sites' samples of bird droppings produced negative results when tested on Niger bird seed agar. While only 2 locations produced no results when tested on Sabouraud dextrose agar. Thus, Sabouraud dextrose agar is thought to be better than NBSA for isolating fungi.

It is worth mentioning that no results were obtained in 17 regions from NBSA media. In contrast a total of 76 isolates were gained from Sabouraud dextrose agar, as mentioned in Table-2.

They belong to the genera (*Aspergillus, Cunninghamella sp., Fusarium, Mucor, Penicillium, Rhizomucor,* and *Trichoderma*) among them, the highest frequency was for unidentified *Penicillium* sp. (9.2%) in Nawroz and (5.2%) in Bahare kon respectively. While (3.9%) was the frequency obtained for the genera (*Aspergillus niger, Aspergillus fumigatus, A.ochraeous, A.terrus, A.flavus, Cunninghamella* sp., *Mucor* sp., *Rhizomucor* sp., and *Penicillium* sp.) in most of the regions. It merits mentioning that only two areas gave negative results, and no fungi have been identified, which were Chwarchra and Mamostayan.

In the isolating fungi on NBSA and SDA, eleven genera have been detected. *Aspergillus* had a superior number of (6 species) and the highest frequency (44.4%), followed by *Penicillium* (2.6%) and *Mucor* sp. (1.6%). While the *Fusarium, Rhizomucor Rhizopus,* and *Trichoderma* were recorded on (0.2 %) of the isolates. However, among isolates, *Cunninghamella, Chrysosporium, Alternaria,* and *Helminthosporium* had the lowest prevalence (0.1%), as shown in Table-3 and Fig. 1.

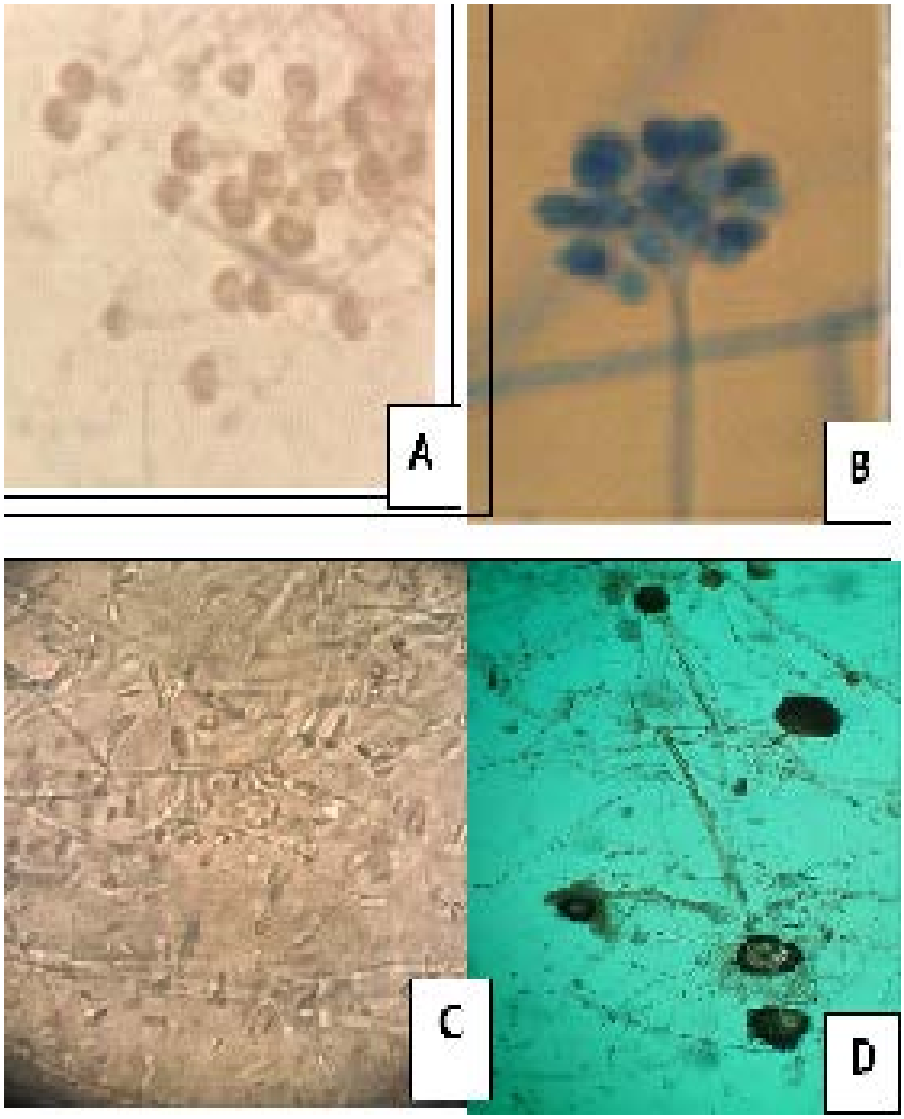


Figure (1). Shows the most composition of fungi isolated from droppings of domestic birds. a- *Aspergillus* b- *Cunninghamella,* c- *Fusarium,* d- *Rhizopus.*

Table (2): Molds isolated from pigeon droppings on Sabouraud Dextrose agar medium.

No.	Sample source	Mold	No. of colonies	% Frequency
1.	Bahare nwey	*Aniger*	1	1.3
		A.fumigatus	1	1.3
		Penicillium sp.	2	2.6
2.	Bahare kon	*A.niger*	1	1.3
		Penicillium sp.	4	5.2
3.	Barzan	*A.niger*	1	1.3
		Mucor sp.	1	1.3
		Penicillium sp.	1	1.3
4.	Berkote nwey	*A.niger*	3	3.9
5.	Bnaslawa	*A.niger*	2	2.6
		A.ochraceous	1	1.3
		Mucor sp.	3	3.9
		Penicillium sp.	1	1.3
6.	Chnar	*A.flavus*	1	1.3
7.	Chwar chra	*0*	0	0
8.	Daratu	*A.niger*	1	1.3
9.	Darwazae shar	*Penicillium sp.*	1	1.3
10.	Framanbaran	*Mucor sp.*	3	3.9
		Trichoderma sp.	2	2.6
11.	Galawezh	*A.niger*	2	2.6
		A.terrus	1	1.3
		Cannicamella sp.	1	1.3
		Fusarium sp.	2	2.6
		Rhizopus sp.	1	1.3
12.	Havalan	*A.flavus*	1	1.3
		Mucor sp.	1	1.3
13.	Kany gany	*A.niger*	1	1.3
14.	Kasnazan	*Rhizomucor sp.*	1	1.3
15.	Mahala arab	*Mucor sp.*	1	1.3
		Penicillium sp.	1	1.3
16.	Makhmur	*Penicillium sp.*	1	1.3
17.	Mala omer	*A.niger*	1	1.3
		Mucor sp.	1	1.3
		Penicillium sp.	3	3.9
18.	Mamostayan	*0*	0	0
19.	Masif salahaddin	*Aniger*	1	1.3
		Penicillium sp.	1	1.3
20.	Mufti	*A.niger*	1	1.3
		Mucor sp.	1	1.3
21.	Nawroz	*A.niger*	2	2.6
		Penicillium sp.	7	9.2
22.	Science college	*A.niger*	1	1.3
23.	Setaqan	*A.niger*	1	1.3
24.	Shawes	*A.niger*	3	3.9
		Mucor sp.	2	2.6
		Penicillium sp.	1	1.3
25.	Shurtawa	*Aniger*	1	1.3
		A.flavus	1	1.3
26.	Tayrawa	*A.niger*	1	1.3
27.	Xanaqa	*A.niger*	1	1.3
28.	Zanko	*A.niger*	1	1.3
29.	Zanko village	*A.ochraceous*	1	1.3
Total		76		

Table:(3). Total mold colonies isolated from the pigeon droppings in Erbil and its suburbs.

Fungi	Media		Total	% Frequency	Total	% Frequency
	NBSA	SDA				
	Total colony number					
Aspergillus candidus	1	0	1	1.0		
A.flavus	0	3	3	3.0		
A.fumigatus	1	1	2	2.0	44	44.4
A.niger	6	26	32	32.3		
A.ochraceous	3	2	5	5.0		
A.terrus	0	1	1	1.0		
Alternaria sp.	1	0	1	1.0	1	0.1
Cunninghamella, sp.	0	1	1	1.0	1	0.1
Chyrysosporium sp.	1	0	1	1.0	1	0.1
Fusarium sp.	0	2	2	2.0	2	0.2
Helminthosporium sp.	1	0	1	1.0	1	0.1
Mucor sp.	3	13	16	16.0	16	1.6
Pencillium sp.	3	23	26	26.0	26	2.6
Rhizomucor sp.	1	1	2	2.0	2	0.2
Rhizopus sp.	2	0	2	2.0	2	0.2
Trichderma sp.	0	2	2	2.0	2	0.2
Total	23	76	99	-	-	-

DISCUSSION

Table:(3). Total mold colonies isolated from the pigeons' dropping in Erbil and its suburbs.

This study isolated and characterized several fungi groups known to pose significant opportunistic risks in their presence within pigeon feces and contamination of the human environment and should, therefore, raise public health concerns, especially for older adults and the immunosuppressed. The growth of bacteria and fungi is highly grown in a nitrogen-rich environment that has been contaminated with birds' droppings (John et.al. 2001). The chemical characteristics and composition of pigeon feces (pH, uric acid, and nitrogen) provide a good substrate for fungal spore propagation. Pathogenic fungus abundance has been connected to weather (humidity, temperature, and radiation), vegetation, and bacteria associated with guano (Lee et.al.2017).

Humans have been known to suffer considerable morbidity and mortality from *Aspergillus* species. They are linked to several clinical manifestations, including disseminated infections, respiratory infections, subcutaneous infections, rhino-cerebral infections, skin and nail infections, ear infections, and keratitis (Rodrigues et.al. 2014).

In the current study, the genus *Aspergillus* was the most prevalent among the isolates, with a frequency of (44%) followed by *Penicillium* sp. with a frequency of (26%) These findings are in accordance with (Maryam, *et.al*.2013), who proved that pigeon droppings are associated with different pathogenic fungal species, including *Penicillium* spp. (n=30), *Apergillus* spp. (n=25), *Mucor* spp. (n=18), *Rhizopus* spp. (n=14), *Paecilomyces* spp. (n=11), *Fusarium* spp. (n=4), and *Cladosporium* spp. (n=2). Similarly, (Hashemi et. al. 2014) reported the presence of these opportunistic fungi such as *Aspergillus, Alternaria, Rhizopus, Mucor,* and yeast-like fungi in various domestic birds presented to veterinary clinics in Tehran. However, our findings partially agree with Abbas et al. (2017), who indicated that *Penicillium* (19%) achieved the highest frequency in the droppings of pigeons. It was followed by *Mucor* (9%), *Rhizopus* (7%), *A. niger* (6%), *A. fumigatus* (5%), *A. flavus* (4%), *Cladosporium* (3 %), and *Alternaria* (2%).

Abulreesh et al. 2015, reported that Mucorales were represented by 8 species related to 6 genera, from which R. stolonifer, *S. racemosum,* and *Mucor hiemalis* were the most common and appeared in 16.1%, 11.6%, and 10.7% of the examined samples, respectively. According to the diversity of genera, the genus Aspergillus ranked second to Mucorales isolated from pigeon fecal samples and was represented by five species, namely, *A. flavus*, *Aspergillus niger* (*A. niger*), *Aspergillus parasiticus, Aspergillus tamarii,* and *Aspergillus terreus*. So our findings are disagreeing with it. Some of the unfavorable outcomes in our study could be attributed to hatchlings defecating in the nest, which raises the salt and alkalinity of the nest lining, promoting alkali-tolerant fungus species. (Korniłłowicz-Kowalska et. al. 2018) The adoption of culture-based methods for isolating fungi may have also resulted in a restricted spectrum of fungal species. Traditional methods are also more widespread and less expensive than high-throughput sequencing procedures. However, this approach has several disadvantages, including the impossibility of detecting unculturable elements (Pasanen,2001; Rastogi 2011). Also, the effectiveness of culture-based analysis of fungal species is affected by factors such as incubation temperature and culture medium type. (Meletiadis et. al. 2001; Marshall & Poulson-Cook, 1998).

On the other hand, the isolation of both yeasts and molds (i.e., fungi) from clinical and environmental samples can be complicated by various factors. Fungal growth in clinical or environmental samples may often be inhibited due to increased growth rates of bacteria as well as bacterial production of deleterious metabolites. These factors can fail to detect fungi in mixed cultures (Hockey et al., 1982). Additionally, fungi frequently have various and particular dietary requirements, which might limit the range and intensity of an organism's growth in the absence of a certain nutrient (Gao & Liu, 2010).

It should be noted that the addition of antibiotics such as chloramphenicol to growth media may also have a negative impact on the growth of certain fungal species (Touimi-Benjelloun et al., 1976). Thus, it can be concluded that there is a potential role of pigeons, as well as other birds, in the propagation of zoonotic yeasts in the environment affecting humans and other animals, which needs to be further investigated.

CONCLUSION

We concluded that pigeon droppings may be a source of many health problems since they contain different types of molds, which may cause clinical manifestations in humans and other animals.

ACKNOWLEDGEMENT

I would like to thank and acknowledge the Department of Biology-College of Science-Salahaddin University/Erbil for enabling this work.

ETHICS

Animal Research Ethics Committee (AREC): Reference No.: 4S/482 on 20/9/2021.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Neveen N. Rajab has collected the samples, performed the data analysis, and written up the paper. Nadeem A. Ramadan supervised the work, reviewed the paper,

and participated in writing the paper.

Funding: No specific funding was received for this work. This work was self-funded.

REFERENCES

Abbas, M.S., Yassein, S.N. and Khalaf, J.M., 2017. Isolation and identification of some important mycological isolates from dropping of birds in Baghdad. *J Entomol Zool Stud*, *5*(53):671-673.

Abulreesh, H.H., Organji, S.R., Elbanna, K., Osman, G.E.H., Almalki, M.H.K. and Abdel-Mallek, A.Y., 2015. First report of environmental isolation of Cryptococcus neoformans and other fungi from pigeon droppings in Makkah, Saudi Arabia, and in vitro susceptibility testing. *Asian Pacific Journal of Tropical Disease*, *5*(8):622-626.

Balajee, S.A., Houbraken,J.,Verweij,P.E,Hong,S.B,Yaghuchi,T.,Varga,J.,Samson, R.A. 2007. *Aspergillus* species identification in the clinical setting. *Stud Mycol.* **59**:39-46.

Dhama, K, Chakraborty, S, Verma, A.K, Tiwari, R, Barathidasan, R, Singh, S.D. 2013. Fungal / Mycotic Diseases of Poultry-diagnosis, Treatment and Control: A Review. *Pakistan Journal of Biological Sciences* 16(23):1626-1640.

Dugan, F.2006. The Identification of Fungi: An Illustrated Introduction. With key, Glossary, and Literature. The American Phytopathological Society.

Dynowska, M., Biedunkiewicz, A., Kisicka, I., Ejdys ,E.,Kubiak ,D., Sucharzewska ,E. 2015. Epidemiological importance of yeasts isolated from the beak and cloaca of healthy Charadriiformes. *Bulletin of Veterinary Institution Poultry* 59:65-69.

Elhariri, M., Hanza, D., Elhelw, R. and Refai, M 2015. Lovebirds and Cockatiels Risk Reservoir of *Cryptococcus neoformans*, a Potential Hazard to Human Health. *J. Vet. Sci .Med .Diagn*. 4:4.

Fraga, M.E., Medeiros, M.E. and Neves, D.M., 2011.Study Aspergilli during the quarantine period parrot CenterScreening of Wild Animals (CETAS) IBAMA, Seropédica RJ.Revista Brasileira de Medicina Veterinária, 33:68-72.

Grisin, T., C., Bories, P. M., Loiseau, Bouchemal, K. 2017. Cyclodextrin-mediated self-associating chitosan micro-platelets act as a drug booster against *Candida glabrata* mucosal infection in immunocompetent mice. *Int. J. Pharm*. **19**:381–389.

Gao, L. and Liu, X., 2010. Nutritional requirements of mycelial growth and sporulation of several biocontrol fungi in submerged and on solid culture. *Microbiology*, *79*:612-619.

Hashemi, S.J., Jabbari, A.G., Bayat, M. and Rafei, S.M. 2014. Prevalence of *Cryptococcus neoformans* in domestic birds referred to veterinary clinics in Tehran.*Eur.J.Exp.Biol.* **4(1)**: 482-486.

Hockey, L.J., Fujita, N.K., Gibson, T.R., Rotrosen, D., Montgomerie, J.Z. and Edwards Jr, J.E., 1982. Detection of fungemia obscured by concomitant bacteremia: *in vitro* and *in vivo* studies. J. Clinical Microbiology,16: 1080-1085.

Hubálek, Z.1976.Influence of pH on the occurrence of fungi in bird's nests. Z. Allg.*Mikrobiol* **16**, 65–72.

John, F.T. Spencer and Alicia, L.Ragout de Spencer.2001.Food Microbiology Protocols.*Methods in Biotechnology* **14**. 495.

Korniłłowicz-Kowalska, T.; Kitowski, I. 2013. *Aspergillus fumigatus* and other thermophilic fungi in nests of wetland birds. *Mycopathologia* **175**: 43–56.

Korniłłowicz-Kowalska, T.; Kitowski, I.; Iglik, H. 2011. Geophilic dermatophytes and other keratinophilic fungi in the nests of wetland birds. *Acta Mycol*. **46**: 83–107.

Korniłłowicz-Kowalska, T.; Kitowski, I.; Bohacz, J.; Kwiatkowska, E. Fungal Frequency and Diversity in the Nests of Wetland Birds from Poland: relationships between Birds, Nest Properties and inhabiting Fungi. Avian Biol. Res. 2018, 11, 245–262.

Lee, WD; Fong, JJ; Eimes, JA and Lim, YW 2017. Diversity and abundance of human-pathogenic fungi associated with pigeon faeces in urban environments. *Mol.Ecol*.**26**: 4574-4585.

Chae, H., et al. (2012). "Rapid direct identification ofCryptococcus neoformans from pigeon droppings by nested PCR usingCNLAC1 gene." Poultry Science 91(8): 1983-1989.

Elhariri, M., et al. (2015). "Lovebirds and cockatiels risk reservoir of Cryptococcus neoformans, a potential hazard to human health." J Vet Sci Med Diagn 4 4: 2.

Pasanen, A.L. A review: Fungal exposure assessment in indoor environments. Indoor Air 2001,11,87–98.

Rastogi, G.; Sani, R.K. Molecular techniques to assess microbial community structure, function, and dynamics in the environment.In Microbes and Microbial Technology; Ahmad, I., Farah, A., John, P., Eds.; Springer Science & Business Media, Agricultural and Environmental Applications: Berlin/Heidelberg, Germany, 2011; pp. 29–57.

Rodrigues, A.M., de Hoog, G.S., de Cássia Pires, D., Brihante, R.S.N., da Costa Sidrim, J.J., Gadelha, M.F.,Colombo, A.L. and de Camargo, Z.P. 2014. Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. BMC Infect. Dis. 14(1): 219.

Santos, L.L., Ferreira, F.M., Lopes, S.F., Condas, L.A., Muro, M.D. and Lugarini, C., 2009. Search for Cryptococcus neoformans and Candida spp. in feces of captive parrots and passerines. Arquivos de Ciências Veterinárias eZoologia da UNIPAR,12:5-9.

Silva, M.E. and Paula, L.A., 1963. Isolation of Cryptococcus neoformans from excrement and nests of pigeons (Columba livia) in Salvador, Bahia (Brazil). Revista do Instituto de Medicina Tropical de São Paulo 5:9-11.

Tell, L.A., 2005. Aspergillosis in mammals and birds: impact in veterinary medicine. Medical Mycology, vol. 43, no.s1,suppl. 1, pp.71-73.http://dx. doi.org /10.1080/13693780400020 089

Touimi-Benjelloun, A., Bonaly, R. and Reisinger, O., 1976. Study of the Rhodotorula rubra cell wall. VII Influence of chloramphenicol on its morphology and phosphatase activity (author's transl). Chemico-biological Interactions, 12(3-4):403-414.

Al-Mukhtar Journal of Sciences 38 (4): 354-359, 2023
Doi: https://doi.org/10.54172/njjw7j72

Research Article **Open Access**

The Level of 25-Hydroxy Vitamin D3 in Gingival Crevicular fluid and Serum Pre-Post Scaling and Root Planning in Chronic Periodontitis Patients in Western Libya: Comparative Study

Aeshah A. Altayf

***Corresponding author:**
Aishabasher981@gmail.com
Faculty of Nursing, Sabratha University, Surman, Libya.

Received:
14 August 2023

Accepted:
16 December 2023

Publish online:
31 December 2023

Abstract

Despite the critical role of vitamin D in the body, it can be affected by several factors that may cause deficiency and imbalances in vitamin D levels. The study aimed to evaluate 25-Hydroxy vitamin D3 in chronic periodontitis patients. The total study participants were 40 patients. They were divided into (20) as a study group with moderate to severe chronic periodontitis, and (20) chronic gingivitis patients were chosen as a control group. Gingival crevicular fluid and serum samples were collected at baseline and six weeks after therapy for the study group and at baseline for the control group. Systemic and local levels of 25-hydroxy vitamin D3 were measured using an enzyme-linked immunosorbent assay (ELISA) technique. The study group of diseased patients received initial periodontal therapy (SRP). The study showed that respective local 25-Hydroxy vitamin D3 levels significantly increased from baseline up to six weeks after (SRP) (3.41ng/ml versus 4.57ng/ml), *P*=0.022. The respective systemic 25-Hydroxy vitamin D3 levels significantly increased after six weeks from SRP (39.88ng/ml versus 41.48 ng/ml), *P*=0.001.

Keywords: Chronic periodontitis, 25-Hydroxy Vitamin D3, Libya.

INTRODUCTION

Current knowledge about the pathogenesis of periodontal disease suggests that its central cause is the loss of a healthy balance between microbial virulence agents and host inflammatory response(Amano, 2010; Genco, 1992; Lee et al., 2000). The immune system, while protecting the host against microbial dental plaque, also participates in attacking the host. Inflammation and tissue destruction are early and mediated processes in response to bacterial infection (Hauschka et al., 1989; Kornman et al., 1997 ; Madianos et al., 2005). Periodontal diseases may differ in their etiological factors and pattern of progression. This variability can be attributed to differences in the presence of factors that might modify the host response to microbial pathogens. Chronic periodontitis (CP) and aggressive periodontitis, forms of inflammatory periodontal disease, differ from each other in terms of the magnitude, sequel, and control of the response (Armitage, 2004) 5. The destruction of soft and hard tissues seen in periodontitis is caused by a large number of cytokines as well as due to the presence of other effects or molecules released by resident and migrating cells (Genco, 1992; Koide et al., 2010) .

Calcium-binding protein of bone accounts for 10–20% of the non-collagenous protein in bone matrix. It has three residues of a calcium-binding amino acid, gamma-carboxyglutamic acid (Gla), that allow specific conformational changes enabling its binding to hydroxyapatite and later accumulation in bone matrix (Hauschka, 1986; Lee et al., 2000).

Vitamin D plays a critical role in mediating calcium absorption and regulating musculoskeletal health (Giovannucci et al., 2008). It has also been demonstrated to function in the regulation of cardiovascular health, immune responses, wound healing, and cancer prevention (Kilkkinen et al., 2009). Vitamin D is a fat-soluble vitamin obtained from three sources. Endogenous synthesis of vitamin D occurs in the skin and is induced via ultraviolet radiation. It may also be obtained exogenously through dietary sources that include oily salt-water fish (mackerel, salmon, sardines, and tuna), cod liver oil, and egg yolk. Many countries, including the United States of America, fortify dairy products with vitamin D due to its scarcity in natural foods(Gorham et al., 2007; Kilkkinen et al., 2009). The aim of this study was to evaluate the level of 25-Hydroxy vitamin D3 in gingival crevicular fluid, serum pre-post scaling and root planning in chronic periodontitis patients.

MATERIALS AND METHODS

The present study was an analytical case control study, carried out on 40 patients whose ages ranged from 35-60 years (both males and females) and were selected from Surman Hospital dental department, dental clinic, Western Libya from Feb 2022 to May 2022. Only 20 patients were diagnosed as having chronic periodontitis after obtaining a proper case history and thorough clinical examination. 20 have been suffering from chronic gingivitis.

Participants:

The selected patients were (40), free from any systemic disease, and no medication had been received for three months prior to the study. Furthermore, none of them had previous periodontal treatment including scaling, root planning, and periodontal surgery in the last six months. On the other hand, smokers and pregnant females were excluded from the study**.** Verbal informed consent was taken from each participant in the study.

The clinical parameters were measured before and after treatment (at baseline, after 6 weeks), bleeding on probing index, plaque index, gingival index, clinical attachment level, and probing pocket depth.

Statistical analysis:

Data were presented as mean and standard deviation (SD) values. Data were presented as frequencies (n) and percentages (%). The chi-square (*x2*) test was used to compare between the two groups. The significance level was set at $P \leq 0.05$ by using the Statistical Package of Social Science SPSS, Version 20.

Ethical consideration:

The study was conducted at the University of Sabratha according to standards of ethics for scientific research. All participants provided verbal consent prior to participation.

RESULTS

Table 1 shows the demographic characteristics and baseline data of the patients enrolled in the study. The mean value and standard deviation of the age of individuals was 40.8 ± 7.7 years in

the study group. Their age ranged from 35-56 years, and 35.3 ± 3.4 were in the control group, their age ranged from 30-43 years.

Table (1). Shows Mean demographic characteristic data (age).

Parameters	Control Group (M±SD)	Study Group (M±SD)
Age (years)	30 – 43	35 - 56
Mean	35.3±3.4	40.8±7.7
Numbers	20	20

Table 2 shows the variation of mean values and standard deviation of GI, PI, BOP, PD, and CAL of individuals who participated in the study. For the study group, the mean value and standard deviation of the gingival index (GI) at baseline was 1.88 ± 0.29, and after SRP, it was 0.72 ± 0.22. Thus, there was a statistically significant decrease in GI values post-operatively ($P < 0.001$). Moreover, the mean value and standard deviation of plaque index (PI) before treatment was 2.02 ± 0.44. After treatment, the mean value was 0.72 ± 0.28. So, there was a highly statistically significant decrease between values of PI before and after treatment in the study group ($P < 0.001$).

It was observed that there was a statistically significant reduction in bleeding on probing (BOP) scores at baseline in the study group with mean values of 0.91 ± 0.08 compared to scores after treatment which was 0.18 ± 0.09 ($P < 0.001$). The mean value and standard deviation of probing pocket depth (PD) at baseline in the study group was 5.27 ± 0.77 mm and after therapy, it was 4.35 ± 0.73 mm. As a result, there was a statistically significant decrease in mean PD post-operatively ($P < 0.022$). Moreover, the mean value and standard deviation of clinical attachment level (CAL) at baseline in the study group was 5.84 ± 0.79 mm. After treatment, the mean value and standard deviation of CAL in the same group was 5.02 ± 0.71 mm. Therefore, in periodontitis patients; there was a statistically significant decrease in mean CAL post-operatively ($P < 0.033$).

Table (2). The mean values (SD) of GI, PI, BOP, PD, and CAL of the study and control group before and after (SRP).

Parameter	Before (Baseline)	After (SRP)	*P*-Value
GI	1.88±0.29	0.72±0.22	0.001
PI	2.02±0.44**	0.72±0.28	0.001
BOP	0.91±0.08*	0.18±0.09	0.00
PD	5.27±0.77	4.35±0.73	0.022
CAL	5.84±0.79	5.02±0.07	0.033

* Highly Significant ($P < 0.001$).
GI = Gingival Index, PI = Plaque Index, BOP = Bleeding on Probing
PD = Pocket depth in millimeter, CAL = Clinical attachment level in millimeter.

Table 3 shows the comparison of GCF and serum levels in 25-Hydroxy vitamin D3. The mean value and standard deviation of 25(OH) vitamin D3 in the study group, GCF at baseline was 3.41 ± 2.25 ng/ml, and after therapy (SRP) was 4.57 ± 2.33 ng/ml. As a result, there was a statistically significant increase in the mean post-operatively at a *p*-value of 0.022. Moreover, in the control group, it showed statistically significantly higher. The mean value and standard deviation of SRP in serum at baseline were 39.88 ± 13.64 and after therapy (SRP), was 41.48 ± 13.58 with *P*-value 0.001.

Table 3: shows the comparison of GCF and serum levels in 25-Hydroxy vitamin D3.

25(OH) VitD3	Study group	Control group	*P* –value
GCF	3.41±2.25	4.57±2.33	0.022
Serum	39.88±13.64	41.48±13.58	0.001

GCF=Gingival Crevicular Fluid.

DISCUSSION

Chronic periodontitis is a chronic condition where bacterial biofilms lead to host responses within periodontal tissues. Previous reports revealed evidence that periodontal disease is linked to low serum 25-hydroxy vitamin D3 concentrations in addition to recognized risk factors like diabetes and smoking. Thus, the present clinical study was designed to assess the levels of 25-Hydroxy vitamin D3 in GCF and serum before and after scaling and root planning (SRP) in chronic periodontitis patients.

The results of our study showed improvement of all clinical periodontal parameters (GI, PI, BOP, PD, and CAL) after SRP in chronic periodontitis patients (study group) and exhibited significant improvements of all values after therapy compared to baseline records. These findings are in agreement with several studies and many consensus reports as SRP is considered the basic therapeutic modality of chronic periodontitis. In the present study, there was a statistically significant increase in the mean GCF 25-Hydroxy vitamin D3 after performing SRP in the study group.

However, there was no statistically significant variation in mean values of serum 25-OH vitamin D3 level after SRP of the same group. These findings come in agreement with other investigators. However, they evaluated 25-OH vitamin D3 in aggressive periodontitis individuals.Previous studies found that low vitamin D levels in GCF have been associated with increased tooth loss, clinical attachment loss, and maternal periodontal disease during pregnancy. These findings are consistent with our results.

Investigators found evidence for the association of periodontal disease with low serum 25(OH) D concentrations. This result comes in disagreement with our findings that showed no difference in serum 25(OH) D3 concentrations between periodontitis patients at baseline and after therapy and also compared to the chronic gingivitis group. The patients selected for participation in the previous study were suffering from aggressive periodontitis. We can only speculate why the association between serum 25(OH) D3 concentrations and clinical attachment loss (CAL) was limited to the older subjects of both sexes. One reason may be the higher prevalence and extent of AL in older subjects than in younger subjects. In this way, older subjects may be more susceptible to the potential benefit of vitamin D3.

In another study, it was found that there was a significant inverse association between serum 25(OH) D3 concentrations and periodontal disease in both men and women aged ≥50 years. This association was independent of race or ethnicity, socioeconomic status, estrogen use among the women, smoking, and gingival bleeding.

It was concluded from the current study that 25-hydroxy vitamin D3 might have an important role in the pathogenesis of periodontal disease and could be used as an adjunctive therapeutic modality for the prevention and treatment of different types of periodontitis. Future intervention therapeutic studies with vitamin D supplementation are also recommended in both chronic peri-

odontitis and aggressive periodontitis patients to completely evaluate the role that vitamin D might play in the treatment of periodontal disease.

CONCLUSION

Scaling and root planning (SRP) is the mainstay of treatment for periodontal diseases and was effective in improving clinical parameters in patients with chronic periodontitis. 25-hydroxy vitamin D3 might have an important role in the pathogenesis of periodontal disease and could be used as an adjunctive therapeutic modality for the prevention and treatment of different types of periodontitis.

Duality of interest: The author has confirmed there is no conflict of interest in this manuscript.

Contribution: The author has carried out all the work related to this manuscript**.**

Funding: No specific funding was received for this work

REFERENCES

Amano, A. (2010). Host–parasite interactions in periodontitis: microbial pathogenicity and innate immunity. *Periodontology 2000, 54*(1), 9-14.

Armitage, G. C. (2004). Analysis of gingival crevice fluid and risk of progression of periodontitis. *Periodontology 2000, 34*(1), 109-119.

Genco, R. J. (1992). Host responses in periodontal diseases: current concepts. *Journal of periodontology, 63*, 338-355.

Gorham, E. D., Garland, C. F., Garland, F. C., Grant, W. B., Mohr, S. B., Lipkin, M., Newmark, H. L., Giovannucci, E., Wei, M., & Holick, M. F. (2007). Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *American journal of preventive medicine, 32*(3), 210-216.

Giovannucci, E., Liu, Y., Hollis, B. W., & Rimm, E. B. (2008). 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Archives of internal medicine, 168(11), 1174-1180.

Hauschka, P. V. (1986). Osteocalcin: the vitamin K-dependent Ca2+-binding protein of bone matrix. *Pathophysiology of Haemostasis and Thrombosis, 16*(3-4), 258-272.

Hauschka, P. V., Lian, J. B., Cole, D., & Gundberg, C. M. (1989). Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological reviews, 69*(3), 990-1047.

Kilkkinen, A., Knekt, P., Aro, A., Rissanen, H., Marniemi, J., Heliövaara, M., Impivaara, O., & Reunanen, A. (2009). Vitamin D status and the risk of cardiovascular disease death. *American journal of epidemiology, 170*(8), 1032-1039.

Koide, M., Kinugawa, S., Takahashi, N., & Udagawa, N. (2010). Osteoclastic bone resorption induced by innate immune responses. *Periodontology 2000, 54*(1), 235-246.

Kornman, K. S., Page, R. C., & Tonetti, M. S. (1997). The host response to the microbial challenge in periodontitis: assembling the players. *Periodontology 2000*, *14*(1), 33-53.

Madianos, P., Bobetsis, Y., & Kinane, D. (2005). Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. Journal of clinical periodontology, 32, 57-71.

Lee, A. J., Hodges, S., & Eastell, R. (2000). Measurement of osteocalcin. *Annals of clinical biochemistry*, *37*(4), 432-446.

Al-Mukhtar Journal of Sciences 38 (4): 360-366, 2023
Doi: https://doi.org/10.54172/d4mcqc03

Research Article **Open Access**

Compliance of Hand Hygiene among Health Care Professionals and Allied Health Workers in the Prevention of Bacterial Contamination

Raul I. Gambalan[1*], Janette L. Calo[2], Jerlinda P. Roa[3] and Salih A. S. Alsalimi[4]

***Corresponding author:** raul.gambalan@omu.edu.ly Faculty of Nursing, Omar Al-Mukhtar University, Libya

Second Author: janette.calo@omu.edu.ly Faculty of Nursing, Omar Al-Mukhtar University, Libya

Third Author: jerlindar@omu.edu.ly Department Faculty of Nursing, Omar Al-Mukhtar University, Libya

Fourth Author: salehelsalme@gmail.com Neurosurgery Department, Hawari General Hospital, Benghazi City, Libya

Received:
30 September 2023

Accepted:
25 December 2023

Publish online:
31 December 2023

Abstract

This study examined the hand hygiene compliance in the operating theatre set-up and its effect on how to deter pathogenic microorganisms and prevent transmitting them. Poor hand hygiene practices are unacceptable and break the law of our infection control practices, thus transmitting microorganisms to our clientele and fellow health care practitioners. Respondents' profiles like gender, age, occupation, years of employment, level of respondents' behaviour in performing proper hand hygiene, feedback on proper hand hygiene, and fulfilment of health care supplies and facilities were assembled. Survey questionnaires were distributed, and SPSS was utilized for the data analysis. Fifty-five (55) participants joined the current research study. Each participant answered a three (3) page questionnaire formulated by the researchers and confidentiality of their identity was strictly observed. The majority of respondents of this research study are nurses, mostly female, with 4 to 6 years of experience as operating theatre staff. Feedback on proper hand hygiene was significant, and respondents were "Fulfilled" by limited supplies and facilities. This study was conducted during the COVID-19 pandemic to evaluate the adherence of our operating theatre staff to hand hygiene practices along with the supplemental of additional information and knowledge of disease transmission prevention.

Keywords: Allied Health Workers, Compliance, Hand Hygiene, Health Care Professionals, Prevention of Bacterial Contamination.

INTRODUCTION

Hand Hygiene or handwashing is the act of cleansing both hands with the use of clean running water and anti-septic soap (Helba, 2014; Goldman, 2008). Proper hand hygiene reduces the spread of potentially deadly micro-organisms to patients and the risk of healthcare provider colonization of infection caused by germs acquired from the patient (Kredie & Kalkman, 2011; Helba, 2014; Control & Prevention, 2021; Organization, 2009, 2017).

Surgical Hand scrubbing is being observed inside our operating department by scrubbing together all surfaces and crevices of the hands using a surgical scrub soaked with an anti-septic solution and water. It is the most basic and effective measure that prevents and controls the transmission of infectious agents (Control & Prevention, 2021). In the event of the absence of soap and water, the 15-seconds vigorous scrubbing should be observed using alcohol-based

hand sanitizers to prevent the transfer of micro-organisms for both patients and fellow healthcare providers (Control & Prevention, 2021; Organization, 2017).

This study provides additional information for healthcare professionals and allied healthcare workers working inside the operating theater. The additional information regarding our study about proper hand hygiene falls under the infection control program that is part of the cleanliness cited by Florence Nightingale that a person's health was the direct result of environmental influences (DeLaune & Ladner, 2011; Smeltzer, 2010).

This study was conducted to examine compliance with hand hygiene in the operating theatre set-up to deter pathogenic microorganisms and prevent transmitting them. Poor hand hygiene practices are unacceptable and break the law of our infection control practices thus, transmitting microorganisms to our clientele and fellow healthcare providers(DeLaune & Ladner, 2011; Helba, 2014).

MATERIALS AND METHODS

Design:

This is a questionnaire-based research study. Approvals were obtained from the hospital administrator and operating theater supervisor. A self-administered questionnaire was utilized, which consisted of four (4) parts, including respondents' demographic data, behavior, feedback, and fulfillment on health care supplies and facilities.

Research questionnaires were translated from English to Arabic to cater to operating theater staff who do not have an English background. The respondents' behavior will be measured using 10 questions with a rate of 1 to 3, wherein 1 is the lowest and 3 is the highest, in addition to feedback on proper hand hygiene, which consists of six (6) statements, and fulfillment on health care supplies and facilities, which also consists of six (6) statements.

To have more understanding of the data of this study, SPSS (Statistical Package for the Social Sciences) was utilized by the researchers for data analysis.

Study Participants and Locale:

The study investigates compliance with hand hygiene practices among healthcare professionals and allied health workers in the prevention of bacterial contamination. The researchers gathered 55 participants, which included surgeons, staff nurses, anesthesiologists, anesthesia technicians, help nurses, and porters for elective and trauma/emergency surgeries. Operating theaters in all government healthcare facilities of Benghazi City were part of the questionnaire's distribution.

RESULTS AND DISCUSSION

Table 1 showed that the majority of the respondents belong to the female group. This means, there are more female than male healthcare workers employed in the operating department. The table shows that according to age, the majority of the respondents belong to the 30-39 years of age bracket with 58.2 percent. These were followed by 18-29, 40-49- and 50-59-year old age bracket with 18 (32.7 percent), 4 (7.3 percent) and 1 (1.8 percent) respondents, respectively.

This finding indicates that healthcare workers employed in operating theaters in Benghazi City, Libya, belong to the early adulthood group. According to Havighurst (DeLaune & Ladner, 2011), this age group is establishing a career or occupation. This could be attributed to the fact

that the younger generation of healthcare workers are very eager to find stable employment for the betterment of their future and chosen career.

Table (1). Respondents' Profile in Terms of Gender, Age, Occupation, and Years of Employment

Demographics	Parameters	(f)	%
Gender	Male	27	49.1
	Female	28	50.9
Age	18-29	18	32.7
	30-39	32	58.2
	40-49	4	7.3
	50-59	1	1.8
	60 and above	0	0
Occupation	Porter	4	7.3
	Help Nurse	9	16.4
	Nurse	17	30.9
	Anesthesia Technician	7	12.7
	Anesthesiologist	7	12.7
	Surgeon	11	20.0
Years of Employment	1-3	11	20.0
	4-6	20	36.4
	7-9	9	16.4
	10-12	7	12.7
	13-15	3	5.5
	16-18	2	3.6
	19-21	2	3.6
	25 and above	1	1.8

In terms of employment, the table indicates that 17 or 30.9 percent of the respondents were nurses. Next in line were help nurses with 9 or 16.4 percent, followed by surgeons with 11(20.0 percent), and the last, who landed on the same spot were the anesthesia technicians and anesthesiologists both with 7, or 12.7 percent score. This means that the vast majority of the respondents are nurses. Florence Nightingale ((DeLaune & Ladner, 2011) stated that nurses are the core or heart of healthcare.

After scrutinizing the information sheet of the operating theater personnel working in operating as to their length of service, it disclosed that 20 or 36.4 percent of the majority have less than 7 years of clinical experience in operating theaters, while the longest who served were 2 or 19-21 percent respondents with 6-10 years of experience in the healthcare industry which are longer as compared to the rest of the respondents.

Further, it could be reflected from the previous table that most (11 or 20 percent) of them have rendered less than 4 years of service followed by those who rendered 7-9 years (9 or 16.4 percent), 10-12 years (7 or 12.7 percent), and those 16 to 18 and 19 to 21 years of experience who garnered 3.6 percent. No one among the respondents renders more than 21 years of service.

Table 2 shows the respondents' behavior in performing hand hygiene. Ranked the highest is number 4 "Emergency cases including trauma are a higher priority than hand hygiene" having a

mean of 2.5818. This means that operating theater personnel have difficulties in performing hand hygiene prior to handling cases like this. Being an operating nurse who handled both elective and emergency operations always believed that performing hand hygiene is necessary in order to prevent additional infection to the patient. Next is number 2 “I have concrete knowledge about hand hygiene practices”, having a mean of 2.5455. Hand hygiene is one of the basic nursing skills that a student should learn before going further on to the invasive skills. Number 1 came as number 3 “I'm following proper hand hygiene practices all the time”, scoring a mean of 2.5091. The last was number 8 with a mean score of 2.0364 " Junior/New staff have not been properly instructed in hand hygiene protocols". For senior staff nurses, it is our duty to provide orientation to the newly hired staff, from protocols down to the small details of every surgery.

Table (2). Respondents Behavior in Performing Hand Hygiene

Statement	Mean	Interpretation
1. I'm following proper hand hygiene practices all the times	2.5091	Highly significant
2. I have concrete knowledge about hand hygiene practices	2.5455	Highly significant
3. Sometimes there are more important things to be done than hand hygiene	2.3273	Significant
4.Emergency cases including trauma are a higher priority than hand hygiene	2.5818	Highly significant
5.Donning gloves to decrease the need for hand hygiene	2.3091	Significant
6. Frustrated when others are unable to perform hand hygiene	2.1273	Significant
7. I'm reluctant to request others to be part of hand hygiene practices	1.9818	Not significant
8. Junior/New staff have not been properly instructed in hand hygiene protocols	2.0364	Significant
9. I feel guilty if I skip hand hygiene	2.3273	Significant
10. Following hand hygiene protocols is easy	2.4182	Significant
Average Mean	2.3163	Significant

Table (3). Respondents’ Feedback on Proper Hand Hygiene

Statement	Mean	Interpretation
1. Sometimes I forget about hand hygiene	2.0364	Significant
2. Hand hygiene is an important part of my job	2.5455	Highly significant
3. The frequency of hand hygiene makes it difficult to follow as necessary	2.1273	Significant
4. Infection Control Committee have a positive influence on the staff hand hygiene	2.1818	Significant
5. Bulletin boards of Infection Control Committee reminds all the staff about hand hygiene	2.2000	Significant
6. Lectures related to hand hygiene is difficult to attend due to work pressure	2.1455	Significant
Average Mean	2.2060	Significant

The table above shows that 37 out of the 55 respondents, comprising 2.5455 of the total mean score, belong to the second statement that “Hand hygiene is an important part of my job" which supports the study that hand hygiene is the single most effective preventive measure against acquiring hospital infections (Kredie & Kalkman, 2011; Control & Prevention, 2021). The rest have significant feedback on proper hand hygiene.

Table 4 above shows respondents are fulfilled with the availability of enough soap, antiseptic for hand washing, alcohol rub, and paper/clothes for drying hands (mean score, 2.0000). However, the availability of these products on a regular basis is still low in this study (mean score, 1.9273) due to limited supplies from the main store. " Updated infection control bulletin boards" (mean score, 1.8000) landed at the bottom part due to the absence of posting educational materials for proper hand hygiene, which will provide a great reminder to operating theater personnel that hand hygiene is important. Findings on the “continuing education program related to hand hygiene in the hospital” received a significant(fulfilled) result from the respondents (mean score, 1.9273), which means that it is important to carry out training programs related to hand hygiene regularly for healthcare workers as it has been associated with increased compliance with hand hygiene practices and reduction of infection.

Table (4). Respondents Fulfillment on health care supplies and facilities

Statement	Mean	Interpretation
1.Availability of enough supplies/equipment’s in performing hand hygiene	1.9273	Unfulfilled
2. Updated infection control bulletin boards	1.8000	Unfulfilled
3. Availability of enough soap, antiseptic for hand washing, alcohol rub, and paper/clothes for drying hands	2.0000	Fulfilled
4. Availability of clean and sterile gloves in OT	2.4545	Highly fulfilled
5. Number of functional sinks with running water	2.5455	Highly fulfilled
6.Continuing Education program related to hand hygiene in the hospital	1.9273	Fulfilled
Average Mean	2.1091	Fulfilled

CONCLUSION

Outcomes and conclusions were drawn from this research study. The null hypothesis was rejected on respondents’ behavior on proper hand hygiene, with an average mean of 2.31, and feedback on proper hand hygiene practices, with an average mean of 2.20, that there is a significant relationship between the respondents’ behavior and feedback on proper hand hygiene. Lastly, respondents are fulfilled on health care supplies and facilities despite limitations.

In light of the findings and conclusions of the study, the researchers provide suggestions and recommend additional studies, in addition to increasing the number of respondents for future research studies. The hospital administration should provide additional posters inside the scrub sinks that further elaborate and illustrates the relevance of proper hand hygiene. The administration should also include proper hand hygiene as part of the regular training and seminars for healthcare staff. Infection control bulletin boards must be up-to-date by posting relevant educational information

about proper hand hygiene. And, despite the imported products for surgical hand scrubbing, the supplies are not enough. The hospital administration should provide more hand hygiene supplies like soap, alcohol, and paper clothes, equally divided to all operating theaters.

ACKNOWLEDGMENT

The researchers would like to extend their heartfelt gratitude, first and foremost, to God Almighty Father for the grace and glory. We are acknowledging our family and friends, for their unconditional support, our supportive Dean, Prof.Sabah H. Lamlom and the respondents who extended their cooperation and shared their time in filling up the questionnaire, to the management and staff of various hospitals, specifically operating theaters in Benghazi City, Libya, and to the publisher of this research study in promoting and developing vast researches. Thank you, and God Bless Us All.

Duality of interest: We, the authors of this research study declare that there is no duality of interest associated with this manuscript.

Author contributions:
Author 1: Developed Introduction, Statement of the Problem, Null Hypothesis, Theoretical and Conceptual Framework, Related Literature, Methodology and over all facilitator.
Author 2 and 3: Formulation of Significance of the Study, Scope and Delimitation, Definition of terms, Analysis and Interpretation of Data.
Author 4: Banking of Related Literature, Finalization of Summary, Conclusion and Recommendations, Translation of questionnaires from English to Arabic, and follow-up with statistician.

Funding: We, the authors of this research study states the funding we utilized came from our own expense for the work reported in their manuscript including transportation and questionnaires reproduction.

REFERENCES

Control, C. f. D., & Prevention. (2021). Guideline for hand hygiene in health-care settings. *Recommendation of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force*.

DeLaune, S. C., & Ladner, P. K. (2011). *Fundamentals of Nursing: Standards & Practice*. Cengage Learning.

Goldman, M. (2008). Patient Safety. Pocket Guide to the Operating Room. F. A. Davis Company.

Organization, W. H. (2009). *Guide to hygiene and sanitation in aviation*. World Health Organization.

Organization, W. H. (2017). Evidence of hand hygiene as the building block for infection prevention and control: an extract from the systematic literature reviews undertaken as the background for the WHO guidelines on core components of infection prevention and control programmes at the national and acute health care facility level.

Krediet, A.C., & Kalkman, C.J et.al., (2011). Hand-hygiene practices in the operating theatre: an observational study. British Journal of Anaesthesia

Phillips, N. (2013). *Principles of Aseptic and Sterile Technique.* Berry & Kohn's Operating Room Technique. Mosby, Elsevier Inc. .

Smeltzer, S. C. (2010). *Hand Hygiene Methods. Brunner & Suddarth's textbook of medical-surgical nursing* (Vol. 1). Lippincott Williams & Wilkins.

Helba, S.(2014) Preventing Perioperative Disease Transmission. Surgical Technology for the Surgical Technologist: A Positive Care Approach. DELMAR Cengage Learning.

Al-Mukhtar Journal of Sciences 38 (4): 367-374, 2023
Doi: https://doi.org/10.54172/bmmbk673

Research Article **Open Access**

Medical Errors in Terms of Cause, Departments in which they Occur, and Solutions from the Point of View of Doctors in Benghazi Medical Center

Lamya S. El Adouli *[1] and Gehan F. Elboseifi [2]

***Corresponding author:** lamya.eladoli@uob.edu.ly
Department of Health Administration, Faculty of Public health, University Benghazi, Libya

Second Author: lamya.eladoli@uob.edu.ly
Department of Occupational Health, Faculty of Public health, University Benghazi, Libya

Received:
28 September 2023

Accepted:
20 December 2023

Publish online:
31 December 2023

Abstract

Medical errors primarily negatively affect patients, medical service providers, as well as health organizations in terms of quality. The prime purpose of this study was to develop an understanding of medical errors in terms of causes, departments with the highest incidence of them, the most to blame for their occurring, and strategies to prevent medical errors from occurrence from the perspective of doctors in Benghazi Medical Centre. A cross-sectional design was chosen to conduct this study, and data was collected from doctors working at Benghazi Medical Center. 281 samples were selected at random. The Epi Info tool calculated the sample size with a 95% confidence interval, and the response rate was 100%. The instrument of data collection was a self-administration questionnaire. (SPSS version 22) was used to analyze the collected data. The results show that the most common errors occurred in the emergency room (66.9%). In addition, it has been found that one of the most critical factors that contribute to the occurrence of medical errors was the lack of necessary resources for some tasks (566%). 45.6% of doctors blamed the administration and (57.7%) suggested that seminars and workshops were the best solution to solve the medical errors issue. Results show that a lack of required resources was generally the main reason for medical errors. In addition, the emergency room is a typical location for medical errors to take place. Through workshops and seminars, doctors determined the best way to stop medical errors. Additionally, the study participants attributed the occurrence of medical errors to the administration in their respective departments.

Keywords: Medical Errors, Doctors, Benghazi Medical Center, Cross Section Study.

INTRODUCTION

Patient safety can generally be improved by recognizing the unfavorable events that transpired, drawing lessons from them, and implementing preventative actions(Rodziewicz et al., 2022). Improving patient safety requires the healthcare system to be able to provide timely, efficient, patient-centered, safe, effective, and equitable care (MacGillivray, 2020). Globally, medical errors are the primary cause of death and a significant public health issue. The patient is the first casualty of medical errors. Prior research indicates that medical errors exacerbate patients' initial problems, which they require medical attention for (Liederbach et al., 2001). Notable consequences of incidences of medical errors, such as post-traumatic stress disorder, prolonged

hospital stays, and extreme circumstances, can result in persistent quality of life changes or even death (Ottosen et al., 2018; Tevlin et al., 2013). The second victim is the doctors. Surveys such as that conducted by Waterman et al. with a large sample size of 3,171 physicians have shown that doctors reported feeling more anxious (61%), losing confidence (44%), having trouble sleeping (42%), being less satisfied with their jobs (42%), and having their reputations harmed (13%) as a result of medical errors(Waterman et al., 2007).

The most common types of medical errors were those related to hospital infection rates, diagnostic errors, injuries from needles or cutting devices, and problems from the use of drugs that cause side effects (Alduais et al., 2014). Significant variations were found in the etiology of medical errors in earlier research. Age extremes, novel procedures, inexperienced physicians and nurses, and complex or emergency care are the most likely causes of medical errors (Weingart et al., 2000). Several things are known to make the problem worse, including poor communication, insufficient documentation, poor handwriting, spelling errors, low nurse-to-patient ratios, and medications with similar names (Hannawa, 2018). However, other studies showed that the acts or inactions of the patient may also have a substantial impact on medical mistakes(Hannawa et al., 2017; Seisser, 2003). The classification and identification of errors must be made obvious in order to reduce medical errors. However, it is a challenging task that may be made simpler by creating a strong classification system. Every year, dangerous medical practices affect millions of patients globally, resulting in 2.6 million fatalities just in low- and middle-income nations (Rodziewicz et al., 2022). The study aimed to shine new light on medical errors in Benghazi Medical Center BMC, Benghazi City.

MATERIALS AND METHODS

Study type:
The study design was a cross-sectional study.

The subjects of this study:
doctors who were working at Benghazi Medical Center during the study period. There were 281 random samples chosen. The number of samples was computed by the Epi Info program, with a 95 percent confidence interval, and the response rate was 100%. Doctors were chosen as subjects for the study because they are presumed to be the second victims of medical errors (Plews-Ogan et al., 2016).

Study setting:
The Benghazi Medical Center, a public hospital in Benghazi, was chosen at random from among the city's other public hospitals as the site of the study. Data used in this study were obtained from a self-administered questionnaire. The questionnaire was adopted from a previous study and utilized to investigate the issue of medical errors (Ahmed et al., 2019). The questionnaire was composed of three sections: Section one: gathering demographic information about the participants (age, gender, qualifications, profession, departments they worked in (emergency, general, cardiology, radiology, (ear, nose, and throat (ENT)), blood, oncology, the central care unit and operations), and years of experience. Section two: was about the participants' knowledge about medical errors and the questions of whether they had seen or been part of the medical errors. The third section, the last of which were suggestions to avoid and reduce these problems.

Ethical considerations:
Names or other personal identifiers were not recorded anywhere in the researcher's data. The

data collection timeframe was approximately two weeks. Data management and analysis were performed using (SPSS22)

Statistical tests:
Frequencies and percentages were the statistical tests used in this study.

RESULTS

According to demographic data, the majority of respondents were female, with a percentage of 60.34%, while the percentage of males was 39.9%, as shown in Table 1. In addition, more than half of the respondents ranged in age between 30 and 39 years (52.7%), followed by those who were in the age groups of 25–29 years (23.1%), 40–49 years old (13.9%), 59–50 years old (5.3%), and less than 25 years old (5.0%), respectively. As shown in Table (1), the results show that for the years of experience variable, the percentage of the highest years of experience was more than 10, and its percentage was 33.5%, followed by experience from 5 to 10 was (19.6%), then two years the percentage was (17.8%), from 3 to 5 years was (15.3%), and a year or less was (13.9%). As shown in Table 1, the results show that for the years of experience variable, the percentage of the highest years of experience was from 5 to 10 years, and its percentage was 33.5%, followed by experience of more than 10 years at 19.6%, then two years at 17.8%, from 3 to 5 years at 15.3%, and a year or less at 13.9%. In terms of educational qualification, 39.5% held a bachelor's degree, followed by doctorates (27.5%), masters (16.4%), diplomas (13.2%), and other qualifications (3.9%).

Table (1): Demographics and characteristics of participants included in the study (n = 281)

Characteristic	Variables	Number. (%)
Gender	Male	112 (39.9)
	Female	169 (60.1)
(Age group)	25<	(5.0) 14
	25-29	(23.1) 65
	30-39	(52.7) 148
	40-49	(13.9) 39
	50-59	(5.3) 15
Level of education	Bachelor's degree	(39.5) 111
	Masters	(16.4) 46
	Doctorate	(27.0) 76
	Diploma	(13.2) 37
	Other	(3.9) 11
(Years of experience)	One year or less	(13.9) 39
	Two years	(17.8) 50
	Years 3-5	(15.3) 43
	Years 5-10	55 (19.6)
	More than 10 years	(33.5) 94

Approximately more than half of the participants indicated that the most common place for medical errors to occur was in the emergency room. There were responses to this question (30.6) where it was the operating room. The intensive care unit (29.2%) came in third place. Just a small number of participants answered (the wards), who totaled 16 doctors (Table 2).

Table (2): The most common places where medical errors occur.

Valid	Frequency (yes) answered	Percentage %
Emergency room	188	66.9
Operation room	86	30.6
Intensive care unit	29.2	29.2
The wards	45	16.0

Table 3 presents the most common causes behind the medical errors in BMC. Just over half of the participants answered the lack of necessary resources for some tasks (56.6%), followed by the failure of the communication system. Few participants (6.0) indicated that (Failure to comply with set policies) was the most common cause of medical error. The experience and satisfaction of participants are shown in Table 4. Table 5 shows that participants blamed more administrators in their departments, and 128 of those who participated indicated that.

Table 6 demonstrates that the best solutions, from the point of view of doctors, were seminars and workshops. A little over half indicated that this is the best solution (57.7). Only a small number of respondents indicated that the best solutions were the reporting and analysis of problems (76, 73), respectively.

Table (3). The most common cause of medical errors

Valid	Frequency (yes) answers	Percentage %
The lack of necessary resources for some tasks	159	56.6
Failure of the communication system	68	24.2
Human error	64	22.8
Patients with poor collaboration	24	8.5

Table (4). The experience and satisfaction of participates.

Valid	Frequency (yes) answers	Percentage %
Does a doctor's inexperience lead to more medical mistakes?	247	87.9
Are you satisfied with your position at the hospital?	167	59.4

Table (5). The one who is most to blame for mistakes in your department.

Valid	Frequency (yes) answers	Percentage %
Administration	128	45.6
Fellow staff	44	15.7
Patients	34	12.1
The System running the facility	101	35.9
Other departments	33	11.7

Table (6). The best solution to the problem of medical errors.

Valid	Frequency (yes) answers	Percentage %
Educating healthcare professionals about medical responsibility through seminars and workshops	162	57.7
Encourage health sector workers and auditors to report medical mistakes	76	27.0
Addressing medical errors by conducting an investigation to determine the root of the issue and creating a mechanism to prevent this error from happening again.	73	26.0
Creating a climate at work where employees in the health industry can work fewer hours, rethink the shift structure, and consider fewer patients being admitted to the hospital.	50	17.8

DISCUSSION

The majority of the sample had more than ten years of experience. This detail suggested that the experiences of the individuals were diverse. The opinions expressed in this survey were representative of the entire sample, as evidenced by the 100% sample response rate. The majority of sample respondents—more than half—agreed that emergency rooms were the primary setting where medical errors were frequently observed. Previous research conducted in Kuwait and Saudi Arabia (Ahmed et al., 2019; AlJarallah & AlRowaiss, 2013) placed the operating room higher than the emergency room, which contradicted the results of our study. Usually, there are reasons for the occurrence of errors in the emergency department, the most important of which was the high workload in Benghazi Medical Center, as the health system has been suffering from poor application of the referral system for years and an unstable system of triage. All emergency cases are received in just two hospitals in Benghazi, including the Benghazi Medical Center. Also, working in the emergency room requires high degrees of cooperation and effective communication. The participants in the study indicated that the second reason for the occurrence of errors is poor communication. Studies indicate that one of the causes of errors in the emergency room was ineffective communication (Eisenberg et al., 2005; Zimmer et al., 2021).

The study's participants also indicated that the causes of medical errors were due to a lack of necessary supplies, which is an obvious explanation for why this would have occurred in the emergency department. The results of several other authors are consistent with this intriguing link between medical errors and a scarcity of resources in the emergency room. High decision density and resource constraints are associated with a high occurrence of preventable medical errors in the emergency department (Asadi et al., 2018; Rowland & Adefuye, 2022).

The majority of participants revealed the most common cause of medical errors to be a lack of resources for some tasks and a shortage of equipment. The World Health Organization underlines that in order to prevent, diagnose, treat, and cure diseases safely and effectively, medical devices are a crucial part of health systems (W.H.O, 2022) Libya’s health system has a 50% availability rate for basic equipment, according to the Service Availability and Readiness Assessment (SARA) (Cakmak et al., 2017). Without the best medical equipment, it is impossible for doctors to practice their profession. Medical practitioners require the best equipment available today in order to perform effectively and efficiently while carrying out their jobs. The current study supports previous research on the point that the lack of essential equipment causes medical errors (Maphumulo & Bhengu, 2019; Moyimane et al., 2017). In addition, according to the findings of this study, the second major cause of medical errors was poor communication. In

many studies, miscommunication was the first reason for medical errors (Alsaleh et al., 2021).

The result of this study indicates that inexperience increases medical errors among doctors. The result is in line with earlier literature (Abd Elwahab & Doherty, 2014) that found junior physicians were particularly at risk for mistakes and their detrimental effects. Results from this study showed that slightly less than half of the doctors participating in the study felt job dissatisfaction. It seems possible that lack of satisfaction is one of the reasons behind medical errors. According to a study done in Taiwan, patient safety practices are directly and substantially correlated with healthcare workers' job satisfaction (Al-Surimi et al., 2022).

Doctors put the primary blame on management in their departments for medical errors. This result is compatible with a study conducted in Egypt and another in Australia. This result is completely different from the study of Kuwait (Ahmed et al., 2019), and this may be due to the size of the hospital and the high workload for the Benghazi Medical Hospital, as the hospital is among the hospitals with 1200 beds. On the contrary, Kuwait Hospital, considered a tertiary hospital, is somewhat smaller. There may be a connection between hospital size and management inaccuracies but there is no clear link. Larger hospitals usually have more complicated organizational structures, greater number of patients, and more healthcare workers, which raises the risk of management errors. These errors can be caused by issues like poor communication, coordination problems, and challenges with applying standardized protocols and processes throughout a big organization.

Based on the results, the opinions of the doctors participating in the study regarding the role of the institutions in reducing medical errors were that they should conduct workshops. When an adverse event happens, all members of the healthcare team must ensure efficient interprofessional communication, identify and report a medical error as soon as it is discovered, and offer prompt assistance to their colleagues. All these activities require effective training, especially for new graduates, to avoid making mistakes, as indicated by the results of this study.

CONCLUSION

Therefore, in general, it seems that the most common cause of medical errors at Benghazi Medical Center was a lack of necessary resources. In addition, the department where the most medical errors occurred was the emergency room. The doctors find that the best solution to prevent medical errors is by conducting workshops and seminars. Furthermore, the participants in the study blamed the administration in their departments for the occurrence of medical errors.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Contribution is equal between authors.

Funding: This work is not funded.

REFERENCES

Abd Elwahab, S., & Doherty, E. (2014). What about doctors? The impact of medical errors. *the surgeon, 12*(6), 297-300.

Ahmed, Z., Saada, M., Jones, A. M., & Al-Hamid, A. M. (2019). Medical errors: Healthcare professionals' perspective at a tertiary hospital in Kuwait. *PloS one, 14*(5), e0217023.

Al-Surimi, K., Almuhayshir, A., Ghailan, K. Y., & Shaheen, N. A. (2022). Impact of Patient Safety Culture on Job Satisfaction and Intention to Leave Among Healthcare Workers: Evidence from Middle East Context. *Risk Management and Healthcare Policy*, 2435-2451.

Alduais, A. M. S., Mogali, S., Al Shabrain, B., Al Enazi, A., & Al-awad, F. (2014). Barriers and strategies of reporting medical errors in public hospitals in Riyadh city: A survey-study. *IOSR Journal of Nursing and Health Science*, *3*(5), 72-85.

AlJarallah, J. S., & AlRowaiss, N. (2013). The pattern of medical errors and litigation against doctors in Saudi Arabia. *Journal of family & community medicine*, *20*(2), 98.

Alsaleh, F. M., Alsaeed, S., Alsairafi, Z. K., Almandil, N. B., Naser, A. Y., & Bayoud, T. (2021). Medication errors in secondary care hospitals in Kuwait: The Perspectives of healthcare professionals. *Frontiers in Medicine*, *8*, 784315.

Asadi, P., Modirian, E., & Dadashpour, N. (2018). Medical errors in emergency department; a letter to editor. *Emergency*, *6*(1).

Cakmak, C., Demir, H., & Kidak, L. B. (2017). A research on examination of medical errors through court judgments. *J Turgut Ozal Med Cent*, *24*(4), 443-449.

Eisenberg, E. M., Murphy, A. G., Sutcliffe, K., Wears, R., Schenkel, S., Perry, S., & Vanderhoef, M. (2005). Communication in emergency medicine: implications for patient safety. *Communication monographs*, *72*(4), 390-413.

Hannawa, A., Wu, A., & Juhasz, R. (2017). *New horizons in patient safety: Understanding communication: Case studies for physicians*. Walter de Gruyter GmbH & Co KG.

Hannawa, A. F. (2018). "SACCIA Safe Communication": Five core competencies for safe and high-quality care. *Journal of Patient Safety and Risk Management*, *23*(3), 99-107.

Liederbach, J., Cullen, F. T., Sundt, J. L., & Geis, G. (2001). The criminalization of physician violence: Social control in transformation? *Justice Quarterly*, *18*(1), 141-170.

MacGillivray, T. E. (2020). Advancing the Culture of Patient Safety and Quality Improvement. *Methodist DeBakey cardiovascular journal*, *16*(3), 192.

Maphumulo, W. T., & Bhengu, B. R. (2019). Challenges of quality improvement in the healthcare of South Africa post-apartheid: A critical review. *Curationis*, *42*(1), 1-9.

Moyimane, M. B., Matlala, S. F., & Kekana, M. P. (2017). Experiences of nurses on the critical shortage of medical equipment at a rural district hospital in South Africa: a qualitative study. *Pan African Medical Journal*, *28*(1), 157-157.

Ottosen, M. J., Sedlock, E. W., Aigbe, A. O., Bell, S. K., Gallagher, T. H., & Thomas, E. J. (2018). Long-Term Impacts Faced by Patients and Families After Harmful Healthcare Events. *Journal of Patient Safety*, *17*(8), e1145-e1151.

Plews-Ogan, M., May, N., Owens, J., Ardelt, M., Shapiro, J., & Bell, S. K. (2016). Wisdom in medicine: what helps physicians after a medical error? *Academic Medicine, 91*(2), 233-241.

[Record #114 is using a reference type undefined in this output style.]

Rowland, M., & Adefuye, A. (2022). Human errors and factors that influence patient safety in the pre-hospital emergency care setting: Perspectives of South African emergency care practitioners. *Health SA Gesondheid, 27*(1).

Seisser, M. A. (2003). Patient Safety Initiatives Explored at Third Annual Medical Errors and Patient Safety Conference. *The Journal for Healthcare Quality (JHQ), 25*(2), 47-48.

Tevlin, R., Doherty, E., & Traynor, O. (2013). Improving disclosure and management of medical error–an opportunity to transform the surgeons of tomorrow. *the surgeon, 11*(6), 338-343.

W.H.O, W. H. O. (2022). Global atlas of medical devices 2022.

Waterman, A. D., Garbutt, J., Hazel, E., Dunagan, W. C., Levinson, W., Fraser, V. J., & Gallagher, T. H. (2007). The emotional impact of medical errors on practicing physicians in the United States and Canada. *The Joint Commission Journal on Quality and Patient Safety, 33*(8), 467-476.

Weingart, N. S., Wilson, R. M., Gibberd, R. W., & Harrison, B. (2000). Epidemiology of medical error. *Bmj, 320*(7237), 774-777.

Zimmer, M., Czarniecki, D. M., & Sahm, S. (2021). Communication of preclinical emergency teams in critical situations: A nationwide study. *PloS one, 16*(5), e0250932.

Al-Mukhtar Journal of Sciences 38 (4): 375-383, 2023
Doi: https://doi.org/10.54172/23ayac75

Research Article **Open Access**

Does Assisted Reproductive Technology have an Effect on Human Secondary Sex Ratio?

Agzail S. Elhddad, [1] and Faiza M. Younis[2*]

***Corresponding author:** agzail.elhddad@gmail.com
Department. of Obstetrics and Gynecology, Faculty of Medicine, Omer Al- Mukhtar University, Libya

Second Author: faizamyounis2006@gmail.com
Department. of Obstetrics and Gynecology, Faculty of Medicine, Omer Al- Mukhtar University, Libya

Received:
10 November 2023

Accepted:
26 December 2023

Publish online:
31 December 2023

Abstract

Assisted reproduction technology (ART) is up-trending. Therefore, the number of babies born by ART is increasing. ART may influence the secondary sex ratio (SSR) compared to natural conception. to evaluate the effect of ART on SSR. a pilot retrospective medical records review study was conducted on 201 live-birth babies born to couples who attended Albayda Fertility Centre. 97 babies were born by ART procedures [intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI)], and the remaining were born by non-ART (medical treatment or post-hysteroscopy). The overall SSR was 46%, and the SSR for non-ART babies was (54.8%) higher than that for the ART offspring (47.4%). However, the difference was non-significant. More girls were born as a result of ICSI (SSR = 43.7%). In contrast, more males were born following IUI (SSR = 47.7%). But the difference was insignificant. Regarding the stage of embryo transfer (ET) in babies born by ICSI, a higher, but non-significant difference (Fisher's Exact test = 0.9) was found in the blastocyst stage than in the cleavage stage. ART might cause a bias in sex ratio at birth, and this change in SSR was found to be affected by the ART procedure applied. The mechanism of these effects is still controversial, and larger multi-centric studies are still warranted.

Keywords: ART, Natural Conception, SSR, IUI, ICSI, Cleavage Stage Embryo Transfer, Blastocyst Embryo Transfer.

INTRODUCTION

Assisted reproductive technology (ART) to treat infertility has developed rapidly since the first in-vitro-fertilization (IVF) baby was born in 1978 (Dean et al., 2010). The number of babies born from ART is increasing rapidly. Worldwide, approximately five million babies have been born as a result of ART (Sandin et al., 2013).

Primary sex ratio (PSR) is the ratio after conception, defined according to the number of ova fertilized by X or Y-bearing spermatozoa (Wang et al., 2010). The ratio at birth is known as the secondary sex ratio (SSR); it is the ratio of male births to total births (Chen et al., 2017; Dean et al., 2010). Others defined SRR as the ratio of male live births to 100 female live births (Bu et al., 2014). The primary sex ratio was found to range between 107 and 170 males / 100 female live births (Bonduelle et al., 2002), whereas the crude secondary sex ratio was estimated as 51.3% (Dean et al., 2010; Wang et al., 2010). This drop in the natural conception SSR is at-

tributed to several environmental and biological factors, such as maternal age, metabolic diseases in women, stressors (war, earthquake), toxins (smoking, pollutants, and pesticides), and race (Rueness et al., 2012). There are three assisted reproductive techniques (ART): intrauterine insemination (IUI), intracytoplasmic sperm injection (ICSI), and in vitro fertilization (IVF). These ART methods not only differ technically but also may have different effects on SSR (Dean et al., 2010).

These ART methods may affect SSR compared to natural conception as the gametes were exposed to external influences during ART, which might vary from the effect of internal factors during natural pregnancy (Chen et al., 2017). Whether the difference in SSR among ART babies results from natural causes (biological or environmental reasons) or as a result of ART per se is still controversial (Dean et al., 2010).

Thatcher et al. found that the SSR of live-born offspring following in-vitro fertilization/embryo transfer (IVF /ET) was significantly higher than normal (64.1%) (Thatcher et al., 1989), whereas in 2000 Ghazzawi and his colleagues reported that a higher percentage of female babies (61.7%) were born following the transfer of embryos fertilized by intracytoplasmic sperm injection (ICSI) (Ghazzawi et al., 2000). A higher SSR in babies born after IUI and a lower SSR after ICSI was reported. This reduction in SSR following ICSI could be explained by the use of ICSI, which is dominantly indicated in the management of male factor infertility (Luke et al., 2009). IUI is the first line of infertility treatment before proceeding to more invasive and expensive procedures such as IVF and ICSI (Dean et al., 2010). Different trials have been undertaken for separating Y- and X-bearing spermatozoa based on the motility of the sperms, but controversial results have been reported (Supramaniam et al., 2019). No direct evidence was found that the sperm preparation procedures for separating motile spermatozoa to be used for either IUI or IVF would lead to an imbalance of male and female babies from the norm (Javed et al., 2019; Yan et al., 2006).

(Fernando et al., 2012) reported that SSR in assisted reproduction technology (ART) babies born from ICSI procedures is lower than those conceived with IVF (Fernando et al., 2012). Dean et al. (2010) demonstrated that the day of embryo transfer (the stage of embryonic development during an ART cycle) also had an effect on the SSR, with a higher proportion of male births reported following a blastocyst stage transfer when compared with dividing stage embryo transfer, and this was found to be independent of the fertilization method (IVF or ICSI) (Dean et al., 2010).

The stability of the secondary sex ratio is of great importance in preserving social stability and improving economic development as happening worldwide. In Libya, more babies were born as a result of ART. To our knowledge, no research has assessed SSR in Libya, and therefore, we sought to evaluate the influence of assisted reproductive techniques on SSR to babies delivered within Albayda Fertility Center to confirm or rule out the effect of assisted reproductive techniques on the secondary sex ratio.

MATERIALS AND METHODS

A pilot retrospective medical records review study was conducted on 201 live-birth offspring born after different modalities of treatment to infertile couples who attended Albayda Fertility Centre/Libya from 2020 to 2022. The subjects included singletons and twins (none was monozygotic), and only one mother gave birth to triplets.

This study was conducted to evaluate the potential influences of ART on the secondary sex ratio (live-birth male to total live-birth). The data variables included in the study were: the mother's and father's age at conception, cause of infertility, type and duration of infertility, how the mother got pregnant, and the baby's gender at birth. The gender of the included babies in the current study was not subject to the sex selection process. The semen samples used in IUI were fresh, and the samples were prepared by swim-up technology for both IUI and ICSI. The embryos transferred in ICSI either in cleavage or blastocyst stage were fresh (no frozen embryos).

The included live-birth babies were divided into two groups: the ART group (babies resulting from IUI and ICSI) and the non-ART group (babies of women who got pregnant by lifestyle management, medical treatment, or pregnancy after hysteroscopy). ART group was divided into IUI and ICSI subgroups, and the ICSI subgroup was further divided according to the embryonic stage at embryo transfer (cleavage stage and blastocyst stage).

Statistical analyses

Statistical analyses were performed using SPSS-26. The continuous data were presented as mean and standard deviation, and Student t-test was used to compare the data. Categorical data were represented as numbers and proportions, and the Chi-square statistic test was used to compare the categorical data. The level of significance was considered at a P value < 0.05.

RESULTS

Two hundred and one live-birth babies delivered to Libyan infertile couples were included in the study. 55.8% of the included couples were presented with primary subfertility and 44.3% presented with secondary subfertility (Figure 1). The duration of infertility ranged between one and fourteen years. The mean age of the wives and husbands was 30.4 (5.2) and 37 (6.3) years respectively. Most of the women (98%) had no antenatal or postnatal complications, and only three mothers had their pregnancy complicated with pre-eclampsia.

Most of the newborns (91.5%) were term, and only 17 (8.5%) were preterm (less than completed 37 gestational weeks) The birth weight ranged between one and four kilograms, and the mean birth weight was 2.8 (0.4) Kg. Neonatal complications occur only in 16 cases (7.9 %), neonatal death in twelve newborns, and most of them are preterm, and four have congenital anomalies.

Table: (1). Demographic data of ART& non-ART group

Variable		ART	Non-ART	Test of significance	*P*
Maternal age		29.8 (4.5)	31 (5.7)	*t*= 1.6	0.09
Paternal age		36.4 (5.9)	37.8 (6.6)	*t*= 1.5	0.1
Infertility type	Primary	63	49	X^2 = 6.4	^{FE}0.01*
	Secondary	34	55		
Infertility duration		4 (2.4)	3.7 (2.5)	*t*= 1.1	0.3
Infertilit aetiology	Female	68	68	X^2 = 4.5	^{FE}0.2
	Male	9	10		
	Combined	3	11		
	Unexplained	17	15		
Gestational age	Term	87	97	X^2 = 0.8	^{FE}0.45
	Preterm	10	7		
Birth weight		2.8 (0.5)	2.9 (0.4)	*t*= 1.7	0.2
Neonatal complications		12	4	X^2 = 4.9	^{FE}0.03*
Maternal complications		2	1	X^2 = 1.3	^{FE}0.4

X2: Chi square test FE: Fisher's exact test

*: Statistically significant at p≤ 0.05

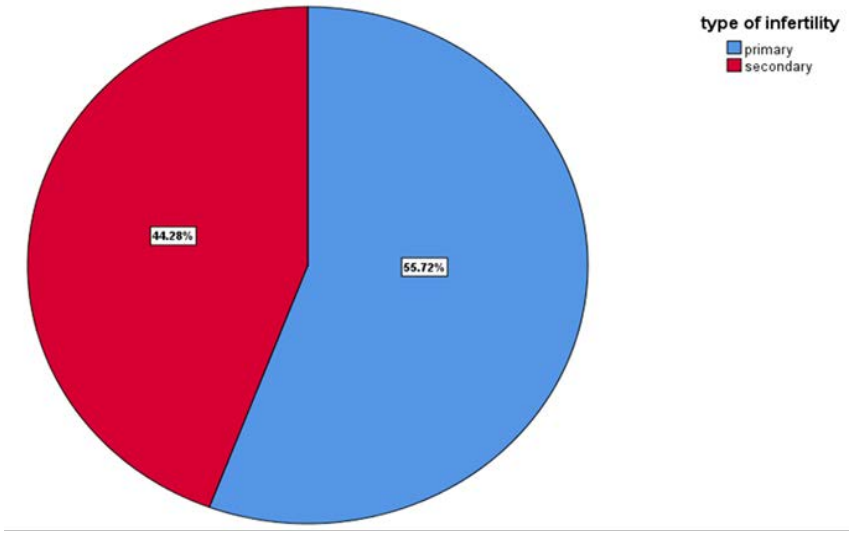


Figure: (1). type of infertility of the included couples

Out of two hundred and one live births during the period of study, 97 newborns were delivered as a result of IUI and ICSI (ART group), and 104 newborns as a result of medication or following hysteroscopy (non-ART group).

Comparing the ART with the non-ART group:

Regarding maternal and paternal age, infertility duration, gestational age, and birth weight, the difference did not reach the significant level between the two studied groups. Primary infertility was significantly more frequent in the ART group than in the non-ART group (p=0.01). Neonatal complications were found to be more significant in the ART newborns (p=0.03) (Table 1). The causes of infertility were due to female problems in most of the cases, followed by unexplained infertility, then male-related causes, and the least was due to combined male and female causes. However, the difference was non-significant between the ART and non-ART groups regarding the causes of infertility (Figure 2).

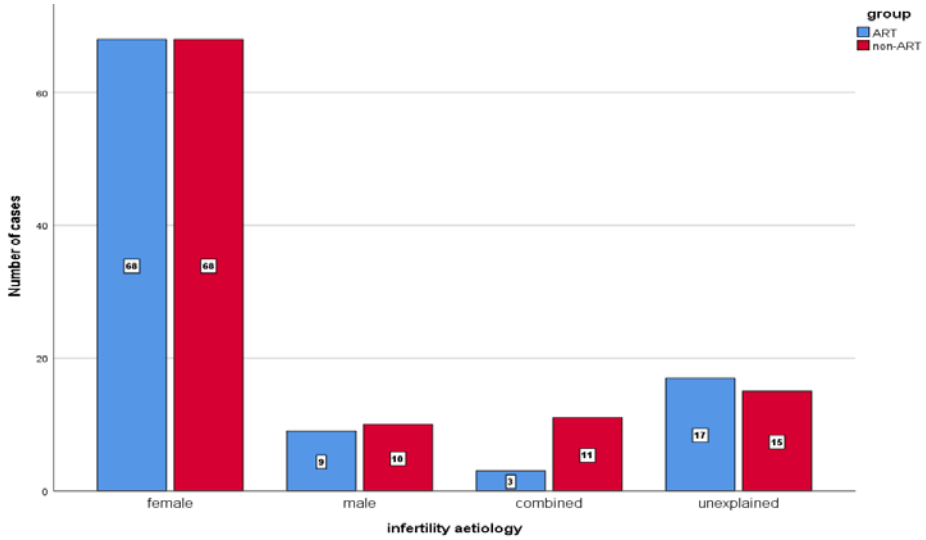


Figure: (2). causes of infertility in ART and non-ART group

The secondary sex ratio (SSR) for all the included newborns was (93/201= 46%). The SSR for ART newborns (46/97=47.4%) was lower than that for the non-ART newborns (57/104=

54.8%), but the difference was not significant (p = 0.325) (Figure 3& Table 2).

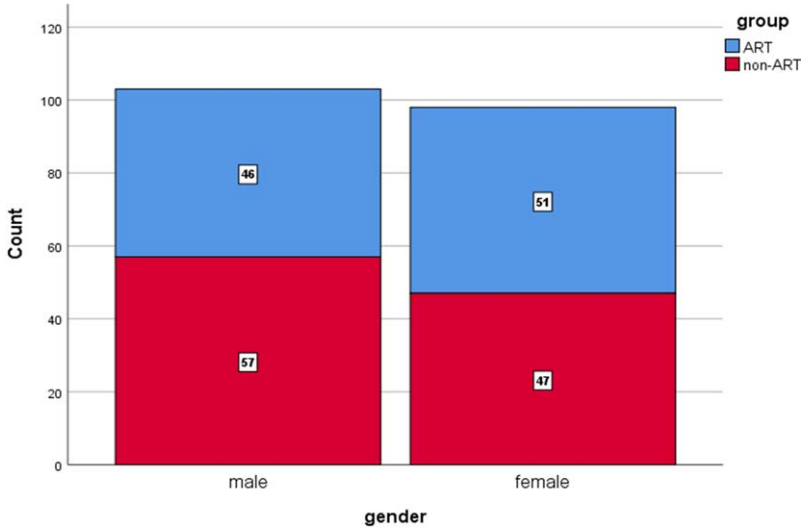


Figure: (3). SSR in the Art and non-ART group

Table: (2). SSR for ART and non-ART newborns

	Male	Female	SSR	Test of significance	*P*
ART	46	51	47.4%	X^2= 1.1	^{FE}0.3
Non-ART	57	47	54.8%		

In the ART group, SSR for IUI 47.7% was higher than SSR for ICSI (43.7%), but the difference was not significant P = 0.83 (Table 3). With regard to the embryonic stage of development of the embryo within the ICSI sub-group, SSR for the blastocyst stage (54%) was higher than that for the cleavage stage of the embryo transfer (45%). However, the difference was non-significant (Fisher's Exact test = 0.9) (Figure 4).

Table: (3). Comparison of SSR between IUI and ICSI

	Male	Female	SSR	Test of significance	*P*
IUI	31	34	47.7%	X^2= 1.3	^{FE}0.83
ICSI	14	18	43.7 %		

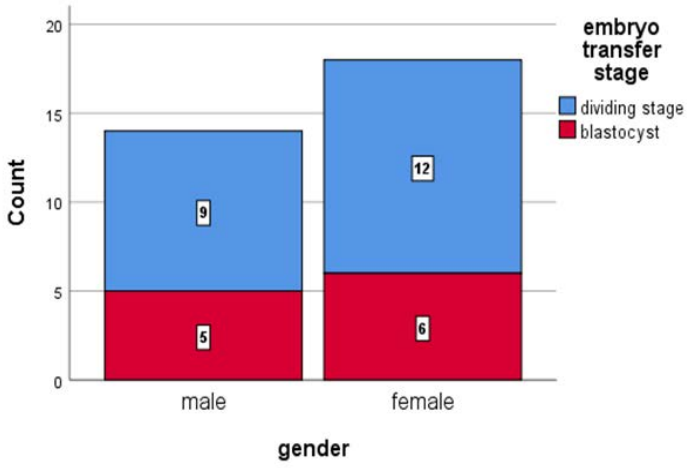


Figure: (4). SSR within ICSI subgroup according to the stage of embryo transfer

DISCUSSION

A pilot retrospective medical records review study was conducted during the period from January 2020 to December 2022 at Albayda Fertility Center on 201 offspring delivered after different modalities of treatment of infertile couples and analyzed the effect of assisted reproductive technique (ART) on the gender of newborns (SSR).

In the current study, SSR for the whole study population (ART and non-ART babies) was 46%, and this result was lower than what was reported (51.22%) in a previous study (James, 2012). This could be explained by the small sample size, geographical differences, or demographic structure of the participants. In this study, there was no conventional IVF, and it was reported that the SSR after standard IVF was actually higher than that for the population sex ratio, and also following ICSI (Bonduelle et al., 2002; Källén et al., 2005; Wang et al., 2020).

A study from the United Kingdom reported the SSR of babies born through ART to be 50.98% (Supramaniam et al., 2019). Chinese multi-centric research from 18 reproductive centers found that the SSR was 51.8% and concluded that ART may affect the SSR (Bu et al., 2014). In the present study, the SSR for the ART group (IUI & ICSI) was 47.4% lower than that for the non-ART group (SSR= 54.8%). In contrast, a previous study (Hann et al., 2018) compared the ART offspring with the babies born after natural pregnancy. They reported a similar SSR between the two sets of babies, suggesting that ART did not affect SSR.

In this study, SSR is higher in pregnancies achieved by IUI (47.4%) compared to that by ICSI (43.7%), but the difference was not significant. This result was in accordance with a previous study (Maalouf et al., 2014).

Increased SSR in IUI and IVF (closer to natural conception) more than that for ICSI, as in these methods (IUI and IVF), the sperms compete for fertilization, while in ICSI, the fertilizing sperms selected by embryologists (Maalouf et al., 2014) and so the natural selection of sperm is bypassed. Also, the gametes in ICSI are exposed to more manipulation than in IUI and IVF (Chen et al., 2017). The reduced SSR after ICSI compared to IUI and natural pregnancy could be explained by the reduced number of Y-bearing spermatozoa in a male partner, as ICSI is greatly indicated in the treatment of male factor infertility that is frequently associated with poor spermatogenic function (Ménézo, 2006; Tarín et al., 2014). All these could explain the higher SSR in IUI than in ICSI group in the current study.

Regarding the stage of embryo transfer; this study revealed that embryo transfer at a blastocyst stage results in SSR (54%) higher than after early stage (dividing) embryo transfer. A similar study (Dean et al., 2010; Majeed et al., 2019; Supramaniam et al., 2019) revealed that a predominance male gender is seen with blastocyst stage embryo transfers with IVF or ICSI (Majeed et al., 2019). In contrast, other studies concluded that blastocyst transfer was not associated with an increased SSR (Al Dibouni et al., 2016; Milki et al., 2003; Żądzińska et al., 2011). Blastocyst stage embryo transfers were thought to lead to a higher proportion of males, secondary to the quick growth potential of male embryos due to their higher ability of pyruvate and glucose uptake compared to female counterparts (Bonduelle et al., 2002; Ray et al., 1995).

ART has been a newly employed technology in Libya in recent years, and therefore, the sample size was relatively small. A larger multi-centric study will be more informative.

CONCLUSION

ART might alter the secondary sex ratio. This change in the sex ratio at birth was found to be affected by the ART procedure used. The mechanism of these effects is still controversial, so larger and multi-centric studies are still warranted.

ACKNOWLEDGEMENT

The authors would like to express great thanks to the participants for their contribution to the study and the reception staff for their help in the data collection.

ETHICS

There are no ethical issues regarding the current research.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Contribution is equal between authors.

Funding: No specific funding was received for this work.

REFERENCES

Al Dibouni, Z., Shah, T., Wheat, S., Griffin, D. K., & Thornhill, A. R. (2016). Incidence, sex ratio and perinatal outcomes of IVF and ICSI monozygotic twin pregnancies following either cleavage or blastocyst stage embryo transfer. *Hum Genet Embryol, 6*, 132.

Bonduelle, M., Liebaers, I., Deketelaere, V., Derde, M.-P., Camus, M., Devroey, P., & Van Steirteghem, A. (2002). Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Human Reproduction, 17*(3), 671-694.

Bu, Z., Chen, Z.-J., Huang, G., Zhang, H., Wu, Q., Ma, Y., Shi, J., Xu, Y., Zhang, S., & Zhang, C. (2014). Live birth sex ratio after in vitro fertilization and embryo transfer in China-an analysis of 121,247 babies from 18 centers. *PloS one, 9*(11), e113522.

Chen, M., Du, J., Zhao, J., Lv, H., Wang, Y., Chen, X., Zhang, J., Hu, L., Jin, G., & Shen, H. (2017). The sex ratio of singleton and twin delivery offspring in assisted reproductive technology in China. *Scientific reports, 7*(1), 7754.

Dean, J., Chapman, M., & Sullivan, E. (2010). The effect on human sex ratio at birth by assisted reproductive technology (ART) procedures–an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002–2006. *BJOG: An International Journal of Obstetrics & Gynaecology, 117*(13), 1628-1634.

Fernando, D., Halliday, J. L., Breheny, S., & Healy, D. L. (2012). Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. *Fertility and sterility, 97*(3), 579-584.

Ghazzawi, I. M., Sarraf, M., & Alhasani, S. (2000). Children born after ICSI: Are we altering sex ratio? *International Journal of Gynecology and Obstetrics*(70), B66.

Hann, M., Roberts, S. A., D'Souza, S. W., Clayton, P., Macklon, N., & Brison, D. R. (2018). The growth of assisted reproductive treatment-conceived children from birth to 5 years: a national cohort study. *BMC medicine, 16*(1), 1-11.

Javed, M., Laqwer, R., Mahouk, B., Kannachath, A., Najashi, S., & Sufyan, H. (2019). Does gradients or swim up procedure produce baby of desired gender. *Obstetrics & Gynecology International Journal, 10*.

Källén, B., Finnström, O., Nygren, K.-G., & Olausson, P. O. (2005). In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. *Fertility and sterility, 84*(3), 611-617.

Luke, B., Brown, M. B., Grainger, D. A., Baker, V. L., Ginsburg, E., Stern, J. E., & Group, S. f. A. R. T. W. (2009). The sex ratio of singleton offspring in assisted-conception pregnancies. *Fertility and sterility, 92*(5), 1579-1585.

Maalouf, W. E., Mincheva, M. N., Campbell, B. K., & Hardy, I. C. (2014). Effects of assisted reproductive technologies on human sex ratio at birth. *Fertility and sterility, 101*(5), 1321-1325.

Majeed, N. A., Zheng, C., Polyakov, A., Pucci, M., Tarmizi, M. H., & Win, M. M. C. (2019). Secondary sex ratio of assisted reproductive technology babies. *Asian Pacific Journal of Reproduction, 8*(2), 88-92.

Ménézo, Y. J. (2006). Paternal and maternal factors in preimplantation embryogenesis: interaction with the biochemical environment. *Reproductive biomedicine online, 12*(5), 616-621.

Milki, A. A., Jun, S. H., Hinckley, M. D., Westphal, L. W., Giudice, L. C., & Behr, B. (2003). Comparison of the sex ratio with blastocyst transfer and cleavage stage transfer. *Journal of assisted reproduction and genetics, 20*, 323-326.

Ray, P., Conaghan, J., Winston, R., & Handyside, A. (1995). Increased number of cells and metabolic activity in male human preimplantation embryos following in vitro fertilization. *Reproduction, 104*(1), 165-171.

Rueness, J., Vatten, L., & Eskild, A. (2012). The human sex ratio: effects of maternal age. *Human Reproduction, 27*(1), 283-287.

Sandin, S., Nygren, K.-G., Iliadou, A., Hultman, C. M., & Reichenberg, A. (2013). Autism and mental retardation among offspring born after in vitro fertilization. *Jama, 310*(1), 75-84.

Supramaniam, P., Mittal, M., Ohuma, E., Lim, L., McVeigh, E., Granne, I., & Becker, C. (2019). Secondary sex ratio in assisted reproduction: an analysis of 1 376 454 treatment cycles performed in the UK. *Human reproduction open, 2019*(4), hoz020.

Tarín, J. J., García-Pérez, M. A., Hermenegildo, C., & Cano, A. (2014). Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles. *Reproductive Biology and Endocrinology, 12*, 1-8.

Thatcher, S., Restrepo, U., Lavy, G., & Decherney, A. (1989). In-vitro fertilisation and sex ratio. *The Lancet, 333*(8645), 1025-1026.

Wang, M., Liu, X., Zhang, H., Li, L., Liu, R., Zhang, H., & Yu, Y. (2020). Associated factors of secondary sex ratio of offspring in assisted reproductive technology: a cross-sectional study in Jilin Province, China. *BMC Pregnancy and Childbirth, 20*, 1-5.

Wang, Y. A., Sullivan, E. A., & Chambers, G. (2010). Assisted reproductive technology in Australia and New Zealand 2008.

Yan, J., Feng, H. L., Chen, Z.-J., Hu, J., Gao, X., & Qin, Y. (2006). Influence of swim-up time on the ratio of X-and Y-bearing spermatozoa. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 129*(2), 150-154.

Żądzińska, E., Rosset, I., Mikulec, A., Domański, C., & Pawłowski, B. (2011). Impact of economic conditions on the secondary sex ratio in a post-communist economy. *Homo, 62*(3), 218-227.

Al-Mukhtar Journal of Sciences 38 (4): 384-394, 2023
Doi: https://doi.org/10.54172/fa3np476

Research Article **Open Access**

Role of Otoacoustic Emissions in Hearing Assessment of Children with Autism

Faria M. Elbabour

***Corresponding author:** E-mail addresses: faria.elbabour@uob.edu.ly Department of Otolaryngology-Head & Neck Name, Faculty of Medicine, University of Benghazi, Libya

Received:
29 November 2023

Accepted:
28 December 2023

Publish online:
31 December 2023

Abstract

Evaluation of hearing is essential in the diagnostic process of children suspected of having autism spectrum disorder (ASD). The study aimed to examine the efficiency and feasibility of utilizing otoacoustic emissions as an objective test to assess hearing in children diagnosed with ASD. A pilot study was conducted over a 3-year period (from September 2019 to November 2022) in the Audiology Department at the Center for Specialty Surgeries, Benghazi, Libya. A total of 61 children with autism aged between 2 and 15 years, referred for hearing evaluation, were enrolled in the study. The clinical procedure consisted of an otoscopic ear examination and Otoacoustic Emission (OAE) recording. OAE testing was attempted on the study sample (n 51) who met the inclusion criteria and completed successfully for 44 participants (86%): 40 cases (91%) from the first attempt, while 4 cases (9%) needed a second or more visit. Four autistic children (9%) had absent otoacoustic emissions in one ear. Testing could not be completed for 7 children (14%) due to behavioral difficulties. The results of this study showed the feasibility of using Distortion Product Otoacoustic Emissions (DPOAEs) as an objective tool in the initial assessment of hearing for children with autism, for whom obtaining reliable behavioral responses can be challenging.

Keywords: Autism, Autism Spectrum Disorder, Children, Hearing, Otoacoustic Emissions.

INTRODUCTION

Autism spectrum disorder (ASD) is defined by the American Psychiatric Association's Diagnostic Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR) as a neurodevelopmental disorder characterized by lifelong challenges in social communication and interaction, in addition to the presence of restricted interests and repetitive behaviors (American Psychiatric Association, 2022).

Globally, the prevalence of autism has noticeably increased over time. It is estimated that about one in 100 children has autism spectrum disorder, with a significant male predominance (Zeidan et al., 2022; WHO, 2022; Maenner et al., 2023). In the United States, according to the 2020 Centers for

Disease Control and Prevention (CDC) Surveillance Report, ASD prevalence was one in 36 per 1000 children aged 8-years (CDC, 2023; Maenner et al., 2023). Based on a systemic review conducted in the Arab Gulf region, the prevalence of autism in the six Gulf Cooperation Council countries ranged from 1.4 to 29 per 10,000 persons (Salhia et al., 2014). In Libya, the prevalence varied between one in 190 and one in 178 per 1000 children in 2011 and 2015, respectively (Zeglam et al., 2012a; Zeglam et al., 2012b; Zeglam et al., 2016).

Hearing conditions in children with autism spectrum disorder are variable. Some children with ASD don't exhibit any hearing difficulties. While other autistic children have some degree of auditory abnormalities, such as hypersensitivity to sounds, difficulty hearing in background noise, unresponsiveness to certain verbal stimuli, hearing loss, and tinnitus (Danesh et al., 2015; Dayem et al., 2018). The co-existence of hearing loss and ASD was reported in the literature (Rosenhall et al., 1999; Roper et al., 2003). Gallaudet Research Institute survey (2009-2010) revealed that up to 40% of hearing-impaired additional have children comorbidity, including visual impairment, learning disabilities, cerebral palsy, and autism (Szymanski et al., 2012). Some research claimed a higher prevalence of ASD among hearing-impaired children than in normal-hearing children, while other studies indicated no difference between the ASD population and the general pediatric population (Beers et al., 2014). It is estimated that one in 59 children with hearing loss is also diagnosed with ASD (Szymanski et al., 2012). Results of the Zeglam et al. (2016) study conducted at Al-Khadra Teaching Hospital in Tripoli revealed a prevalence of 11 per 1000 (1.1%) of sensorineural hearing loss among Libyan children with ASD.

Evaluation of hearing is essential in the diagnostic process of children suspected of having ASD who presented with early communication difficulties and or delayed language development (Tas et al., 2007; Li et al., 2021). Co-occurrence of hearing loss and ASD can significantly delay the diagnosis of either condition (Mandell et al., 2005; Fitzpatrick et al., 2014; Danesh et al., 2015). Moreover, it is probable that children with hearing loss are being misdiagnosed with ASD (Fitzpatrick et al., 2014). Furthermore, previous research identified several common risk factors between hearing loss and ASD, for example, prematurity, low birth weight, hypoxia, and viral infections (Limperopoulos et al., 2008; Danesh et al., 2015; Allen et al., 2020).

Reliability of hearing thresholds obtained by behavioral audiometry for children with ASD can be poor due to the atypical behavior some ASD children may display during conventional audiological testing, such as limited ability to communicate, uncooperativeness, inability to follow test instructions, dislike wearing headphones, or anxiety to test booth or examiner (Schafer, 2021). Therefore, objective hearing measurement tools, such as otoacoustic emissions (OAEs) and auditory brainstem response (ABR), may be considered valuable in the assessment of the auditory system function of children with autism (Tas et al., 2007; Beers et al., 2014; Bennetto et al., 2017).

Otoacoustic emissions are commonly used in the hearing assessment of difficult-to-test patients who cannot provide reliable behavioral responses (Demopoulos 2016). OAEs are also used to screen hearing in neonates and test for functional hearing loss. Measurement of OAEs in children with developmental disabilities has numerous advantages, namely easy to record, non-invasive, doesn't require behavioral cooperation or response, and provides information about cochlear status in a short time.

The presence of OAEs indicates normally functioning outer hair cells which correspond with essentially normal hearing sensitivity. It also denotes the proper function of the external and middle ear conductive mechanism of hearing. However, OAE testing has some disadvantages; it is very sensi-

tive to noise, greatly affects the middle ear status, can't be used to estimate the degree of hearing loss, and doesn't evaluate neural auditory pathways (Tas et al., 2007; Cunningham, 2011; Kaf et al., 2013; Al-Meqbel, 2016).

Unlike OAEs, estimating hearing thresholds by Auditory Brainstem Response (ABR) may not be possible without sedation for autistic children at an average age of diagnosis, over 3 years old (CDC, 2019; van't Hof et al., 2021). Additionally, running ABR is time-consuming in comparison to recording OAEs. Although ABR is considered the accepted method for predicting hearing thresholds in suspected ASD children (Elmawgoud et al., 2017; Kamita et al., 2020), the previously mentioned drawbacks render ABR unnecessary for assessing the hearing of ASD children without any parental concern of hearing problems especially in developing countries like Libya where shortage of hearing healthcare professionals and cost and availability of medical equipments and supplies are major barriers and constraints (Zeglam et al., 2016).

The present study had two main aims. First, to examine the efficiency and feasibility of utilizing Distortion Product Otoacoustic Emissions (DPOAEs) as an objective initial test to evaluate hearing in children diagnosed with ASD and to help identify those who need comprehensive audiological assessment. It was expected that unsedated OAE tests couldn't be successfully completed in the autism pediatric population. Second, to compare the emission responses of children with autism spectrum disorder and normally developing peers. It was hypothesized that levels of OAEs of children with ASD would be significantly higher than of normally developing children.

MATERIALS AND METHODS

This study was conducted at the Audiology Department at The Center for Specialty Surgeries, Benghazi, Libya, which is one of the two main hearing referral centers in eastern and southern Libya. Verbal informed consent was taken from the parents of all participants.

PARTICIPANTS

Autism Group: Initially, a total of 61 children diagnosed with autism aged between 2 and 15 years enrolled in this study, 46 boys and 15 girls (Ratio 3:1). Among them, 10 children with autism were excluded.

Control Group: 23 age-matched normally developing children, 8 males and 15 females (Ratio 8:15) randomly recruited from patient companions/hospital visitors with non-ear, nose, and throat-related problems.

SAMPLING PROCEDURES

Audiological evaluations consisted of asking parents about any concerns about hearing problems, doing otoscopic examinations, and measuring DPOAEs. Clinical data for all participants who met the study inclusion criteria was examined retrospectively:

Inclusion Criteria

- Referral by a psychologist or a special needs and autism center.
- No parental concern of hearing impairment.
- Normal otoscopic examination (no external auditory canal or middle ear pathology)
- DPOAE test results recorded for frequencies: 1500, 2000, 3000, 4000, & 6000 Hz for both ears

Exclusion Criteria

- Uncertain diagnosis of autism or not documented by a specialist.
- Abnormal otoscopic examination (occluded external auditory canal, congested tympanic membrane, or presence of pressure equalizing tubes).
- DPOAE test results were recorded only for one ear.

Diagnostic DPOAE measurements were performed in a quiet room using Interacoustics Eclipse (A/S DK-5610 Assens, Denmark) DPOAE probe for each ear while participants were awake and still. The DPOAEs are elicited by two pure tone stimuli [L1=65 and L2=55 dB sound pressure level (SPL)] at primary frequencies of f1 & f2 at a fixed ratio of f2/f1 = 1.2. The DPOAE response was measured at the distortion product generated at (2f1-f2) for targeted five frequency regions: 1500, 2000, 3000, 4000, & 6000 Hz. The testing time was 60 seconds. The detection of the DPOAEs was based on the amplitude of -5.0 dB or more and being at least 6 dB above the average level of the noise floor (Signal-to-Noise Ratio (SNR) = ≥6 dB).

Statistical Analysis

The recorded distortion product otoacoustic emission responses (Distortion Product (DP) level and SNR) of children with autism were investigated and analyzed using Apple Mac Numbers® basic descriptive statistics and compared with those of non-autistic control subjects. The variables from the two groups were compared for the right and left ears separately and both ears together using a two-sample t-test. The level of significance was set at $p<.05$.

Table: (1). Demographic Characteristics of Study Subjects

Variable	Autism Group (n=51)	Control Group (n=23)
Age range, years	2 - 15	2 -12
Age mean, years±SD	6.3±3.39	6.5±2.52
Age median, years	6	6
Gender, n (%)		
Male	40(78%)	8(35%)
Female	11(22%)	15(65%)
Address, n (%)		
Benghazi	44(86%)	18(78%)
Outside Benghazi	7(14%)	5(22%)

SD: Standard Deviation

RESULTS

Table 1 summarizes the participants' demographic characteristics. DPOAE testing was successfully completed for 44 ASD participants (86%), 40 cases (91%) from the first attempt, while the other 4 cases (9%) had to be retested. DPOAE testing couldn't be completed for 7 (14%) ASD children due to uncooperativeness and high noise levels. DPOAEs were present in both ears in 40 (91%) ASD participants, whereas it was present in just one ear in the remaining 4 (9%). Since all children in the control group cooperated, DPOAEs were measured from the first trial and were absent in only one out of 46 ears. Figure 1 displays the average time in seconds taken for DPOAE measurements (probe insertion not included) in each ear for both the autism and the control group. Running the -test revealed no significant difference in time spent to complete the DPOAE test between the two

groups (p= >.05).

There was no statistically significant difference in the DP levels in the right ear and left ear between the autism and control groups (Table 2). For both ears together, the highest DPOAE amplitude was recorded at 1500 Hz, with similar results in both groups.

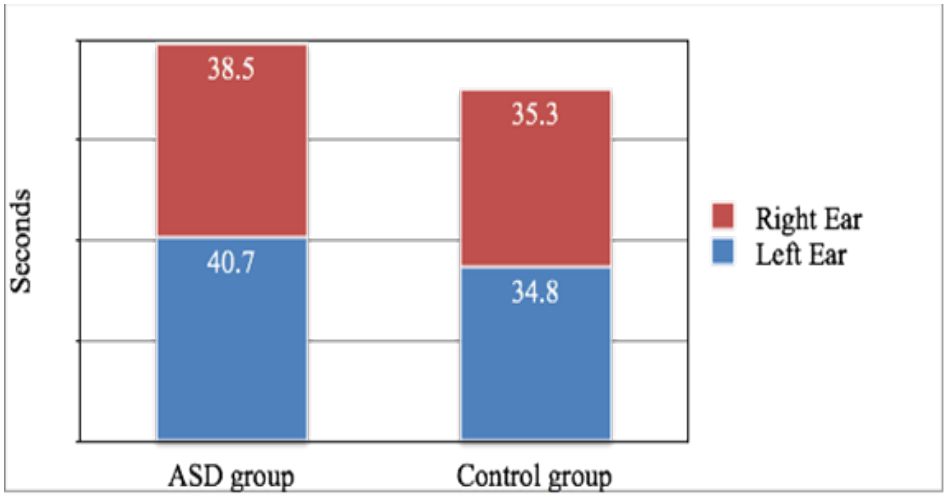


Figure: (1). Average DPOAE test duration in seconds for each ear for both groups

Table: (2). Mean values and standard deviations of DPOAE levels in dB SPL in each ear for both study groups

DPOAE Level (dB SPL)	Right Ear					Left Ear				
Frequency (Hz)	Autism Group		Control Group			Autism Group		Control Group		
	M	SD	M	SD	p-value	M	SD	M	SD	p-value
1500	15.1	7.91	13.64	7.28	.453	13.58	8.16	14.90	5.49	.436
2000	11.42	7.01	12.36	7.34	.613	9.92	9.43	12.15	6.53	.262
3000	7.79	7.05	8.25	6.28	.787	6.38	6.81	8.32	6.69	.269
4000	9.77	6.48	10.53	5.98	.636	8.38	7.82	10.77	5.50	.151
6000	4.19	8.74	4.28	10.60	.974	4.17	8.55	1.65	10.11	.313

DPOAE: Distortion Product Otoacoustic Emission; dB SPL: decibel sound pressure level; Hz: Hertz; M: Mean; SD: Standard Deviation

In regard to signal-to-noise ratio (SNR), the analysis showed significantly higher SNR at 1.5 kHz, 2 kHz, 3 kHz & 4 kHz of the left ears (Figure 2) in the control group than that of the autism group, with the greatest difference observed at 3 kHz (p=<.001). Although there was no significant difference in the right ear SNRs between the two groups, as illustrated in Figure 3, the biggest between-group SNR difference for the right ear was also recorded at 3 kHz. When data from both ears were analyzed together, both groups had the largest combined SNR average at 4 kHz, 19.3 dB in the autism group and 22.3 dB in the control group (table 3). Studying the sex effect on DPOAE responses between 37 males and 7 females in the autism group revealed no significant difference p=>.05.

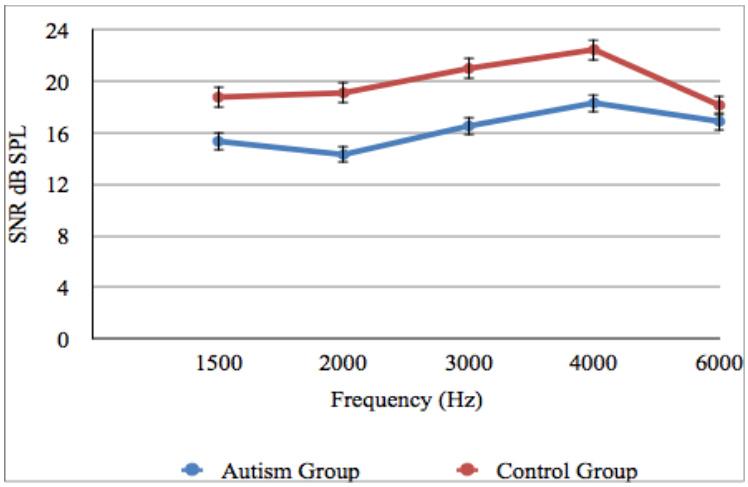


Figure: (2). Comparison of DPOAE SNR at each frequency in left ear between the two groups

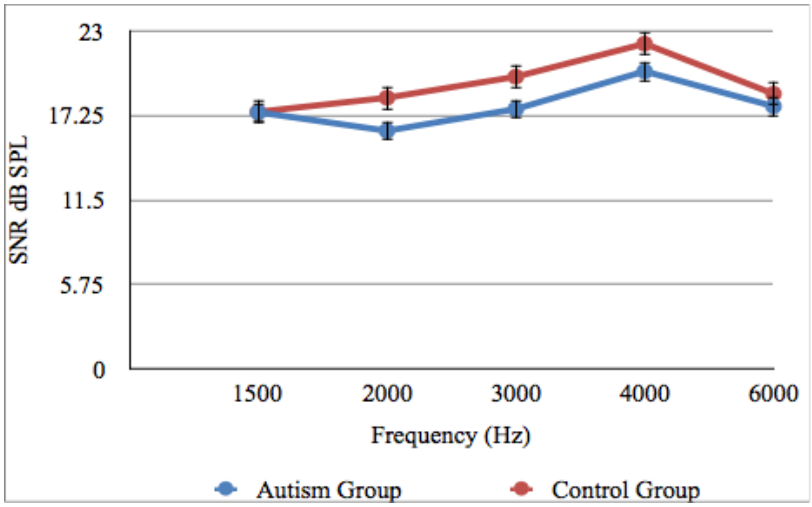


Figure: (3). Comparison of DPOAE SNR at each frequency in right ear between the two group

Table: (3). Mean values of both ears DPOAE SNR at each tested frequency in children with and without autism

Frequency (Hz)	Both ears DPOAE SNR (dB SPL)				p-value
	Autism Group		Control Group		
	M	SD	M	SD	
1500	16.43	5.60	18.17	4.57	.177
2000	15.30	4.88	18.76	4.35	.004**
3000	17.15	4.07	20.48	4.19	.003**
4000	19.33	4.86	22.33	4.23	.011*
6000	17.41	4.92	4.92	5.62	.430

DPOAE: Distortion Product Otoacoustic Emission; SNR: Signal-to-Noise Ratio; dB SPL: decibel sound pressure level; Hz: Hertz; M: Mean; SD: Standard Deviation; *P<.05; **P<.01

DISCUSSION

The lack of cooperation of ASD children reported during audiological testing could be attributed to the sensory sensitivity issues that children with autism are characterized by (Mansour et al., 2021). The abnormal tactile hypersensitivity response to the otoscopic ear examination or the insertion of ear tips of tympanometry or OAE probes into the external ear canal could be the reason behind the high rate of incomplete testing (Andersson et al., 2013; Danesh et al., 2015). In the present study, some strategies for testing hearing in children with autism, as suggested by Brueggeman (2012), were followed, letting the child touch the ear speculum and ear tip, pretending to test a sibling or parent by demonstrating insertion of the ear tips on, or asking parents to practice wearing earphones at home for the few participants who didn't cooperate at the initial appointment. Interestingly, otoscopy was carried out in all autism cases enrolled in the present study. Unlike Kaf, a 2012 study reported a higher percentage of uncompleted otoscopies, whereas DPOAE measurements were obtained without sedation in the majority (86%) of the included autism study sample. Obtaining OAEs under sedation was recommended, especially in severe ASD children, to control motor issues that may interfere with OAE testing (Tas et al., 2017). However, Gungor et al., 2016 observed a significant reduction in DPOAE levels with sedative agents.

As seen in the result section, almost all (92%) study participants had present otoacoustic emissions in both ears, which is suggestive of normal outer hair cell function. A few children (<10% of the autism group and <5% of the non-autism group) had absent emissions in one ear, which is indicative that further evaluation is required to establish diagnosis. Conductive hearing loss due to middle ear disorders is a frequent problem in children with autism, as concluded by Rafal (2016) and Al-Meqbel (2013). Middle ear diseases can impair the transmission of otoacoustic emissions and cause reduced emissions amplitude or absent response (Balatsouras et al., 2012). Tympanometry should be included as an objective tool in the screening of hearing in uncooperative children, particularly autistic children (Rafal et al., 2013; Al-Meqbel, 2016).

Another finding is the higher overall DPOAE SNRs in the control group compared with those in the autism group. A similar finding was confirmed by Danesh & Kaf (2013). While other studies found no difference between the DPOAE SNRs of children with autism and typically developing children (Gravel et al., 2006; Tas et al., 2017). As discussed in the Bennetto et al. (2017) study, the significantly reduced combined DPOAE SNR levels at mid-frequencies without any differences in noise floor may correlate with the speech perception and discrimination challenges persons with autism suffer from. Studying the ear effect on DPOAE SNR responses revealed left ear advantage in the control group. But no significant auditory asymmetry was noted in the autism group, which doesn't agree with what has been mentioned in the literature (Khalfa et al., 2001; Kaf et al., 2013). The inconsistent results could be due to the lack of contralateral suppression of OAEs, as argued in Danesh et al. (2012) study.

This study has several limitations. First, no detailed medical history in the participants' records. Like identifying risk factors for hearing loss, handedness, and severity of autism to compare results accordingly. Second, the inability to get tympanograms for all participants was a restriction, as middle ear status can influence otoacoustic emission response. Third, not measuring DPOAEs below 1500 Hz and above 6000 Hz. Finally, the relatively small sample size for the control group may make it difficult to generalize the outcomes and make them inconclusive.

CONCLUSION

Although no clear evidence exists of a higher risk of hearing loss in children with autism spectrum

disorder, assessment of hearing using objective measurements such as OAE is recommended in children with confirmed and suspected autism. Because the national hearing screening program is not yet routinely implemented on all children in Libya, hence autism spectrum disorder may be identified before hearing loss in those affected by both conditions. Therefore, early identification and timely intervention of hearing impairment in ASD is very important and can reduce the burden of dual disability and improve communication and overall quality of life.

ACKNOWLEDGEMENT

The author wishes to thank the families and children who participated in this research and the staff of the Audiology Unit at the Specialty Surgical Center, Benghazi, Libya, for their support. A portion of this work was presented at the first Scientific International Conference on Autism Spectrum Disorder, Dec 26-29, 2022, Benghazi, Libya.

Duality of interest: The author has no conflict of interest to declare.

Funding: No funding was received for this work.

REFERENCES

American Psychiatric Association. (2022). *Diagnostic and statistical manual of mental disorders* (5th ed., text rev.). https://doi.org/10.1176/appi.books.9780890425787

Al-Meqbel, A. (2016). A Pilot Study of the Relationship between Autism Spectrum Disorder and Hearing Loss. *Journal of Communication Disorders, Deaf Studies & Hearing Aids, 04(01).* https://doi.org/10.4172/2375-4427.1000152

Allen, L., Leon-Attia, O., Shaham, M., Shefer, S., & Gabis, L. V. (2020). Autism risk linked to prematurity is more accentuated in girls. *Plos one*, *15*(8), e0236994.

Andersson, E., Arlinger, S., Magnusson, L., & Hamrin, E. (2013). Audiometric screening of a population with intellectual disability. *International Journal of Audiology*, *52*(1), 50-56. http://dx.doi.org/10.3109/14992027.2012.700773

Balatsouras, D. G., Koukoutsis, G., Ganelis, P., Korres, G. S., Aspris, A., & Kaberos, A. (2012). Transiently evoked otoacoustic emissions in children with otitis media with effusion. *International journal of otolaryngology*, *2012*. https://doi.org/10.1155/2012/269203

Beers, A. N., McBoyle, M., Kakande, E., Santos, R. C. D., & Kozak, F. K. (2014). Autism and peripheral hearing loss: a systematic review. *International Journal of Pediatric Otorhinolaryngology*, *78*(1), 96-101. https://doi.org/10.1016/j.ijporl.2013.10.063

Bennetto, L., Keith, J. M., Allen, P. D., & Luebke, A. E. (2017). Children with autism spectrum disorder have reduced otoacoustic emissions at the 1 kHz mid-frequency region. *Autism Research*, *10*(2), 337-345. https://doi.org/10.1002/aur.1663

Brueggeman, P. M. (2012). 10 Tips For Testing Hearing in Children With Autism. *The ASHA Leader*, *17*(1), 5-7. https://doi.org/10.1044/leader.FTR3.17012012.5

Centers for Disease Control and Prevention. (2023). *Data & statistics on autism spectrum disorder*. Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/ncbddd/autism/data.html

Centers for Disease Control and Prevention. (2019). *Spotlight on: Delay between first concern to accessing services.* Retrieved from https://www.cdc.gov/ncbddd/autism/addm-community-report/delay-to-accessing-services.html

Cunningham, R. F. (2011). Otoacoustic emissions: Beyond newborn hearing screening. *Audiology Online*, 5.

Danesh, A. A., & Kaf, W. A. (2012). DPOAEs and contralateral acoustic stimulation and their link to sound hypersensitivity in children with autism. *International journal of audiology*, *51*(4), 345-352.

Danesh, A. A., Kaf, W. A., Abdelhakiem, M. K., Danesh, D., & Scott, J. (2015). Auditory Manifestations and Intervention in Children with Autism Spectrum Disorders. *Austin J Autism & Relat Disabil*, *1*, 1005.

Dayem Ali, A. A., Youssef Darwish, S., Ahmed Mahmoud, A. M., & Mohammed Hassanain, M. M. (2018). Audiological Profile in Children with Autism Spectrum Disorders. *Egyptian Journal of Hospital Medicine*, *72*(2). https://dx.doi.org/10.21608/ejhm.2018.9078

Demopoulos, C., & Lewine, J. D. (2016). Audiometric profiles in autism spectrum disorders: Does subclinical hearing loss impact communication?. *Autism Research*, *9*(1), 107-120. https://doi.org/10.1002/aur.1495

Elmawgoud, S. M., Emam, A. M., & Ali, H. Y. (2017). The rate of consistency between auditory brainstem response and Gilliam Autism Rating Scale among autistic children. *Clin Otolaryngol*, *8*(03), 00242. DOI: 10.15406/joentr.2017.08.00242

Fitzpatrick, E. M., Lambert, L., Whittingham, J., & Leblanc, E. (2014). Examination of characteristics and management of children with hearing loss and autism spectrum disorders. *International journal of audiology*, *53*(9), 577-586. https://doi.org/10.3109/14992027.2014.903338

Gravel, J. S., Dunn, M., Lee, W. W., & Ellis, M. A. (2006). Peripheral audition of children on the autistic spectrum. *Ear and Hearing*, *27*(3), 299-312. DOI: 10.1097/01.aud.0000215979.65645.22

Gungor, G., Sutas Bozkurt, P., Yener, H. M., Yilmaz, Y. Z., Sarı, E., Atas, A., ... & Hayir, D. (2016). Comparison of anesthetic agents on otoacoustic emissions in children: propofol vs ketamine. *Pediatric Anesthesia*, *26*(7), 752-758. DOI: 10.1111/pan.12936

Kaf, W. A., & Danesh, A. A. (2013). Distortion-product otoacoustic emissions and contralateral suppression findings in children with Asperger's Syndrome. *International journal of pediatric otorhinolaryngology*, *77*(6), 947-954. https://doi-org.ezproxylocal.library.nova.edu/10.1016/j.ijporl.2013.03.014

Kamita, M. K., Silva, L. A. F., Magliaro, F. C. L., Kawai, R. Y. C., Fernandes, F. D. M., & Matas, C. G. (2020). Brainstem auditory evoked potentials in children with autism spectrum disorder. *Jornal de pediatria*, *96*, 386-392. https://doi.org/10.1016/j.jped.2018.12.010

Khalfa, S., Bruneau, N., Roge, B., Georgieff, N., Veuillet, E., Adrien, J. L., ... & Collet, L. (2001). Peripheral auditory asymmetry in infantile autism. *European Journal of Neuroscience*, *13*(3), 628-632.DOI: 10.1046/j.1460-9568.2001.01423.x

Li, S., Cheng, C., Lu, L., Ma, X., Zhang, X., Li, A., ... & Gao, X. (2021). Hearing Loss in Neurological Disorders. *Frontiers in cell and developmental biology*, *9*. https://doi-org.ezproxylocal.library.nova.edu/10.3389/fcell.2021.716300

Limperopoulos, C., Bassan, H., Sullivan, N. R., Soul, J. S., Robertson Jr, R. L., Moore, M., ... & du Plessis, A. J. (2008). Positive screening for autism in ex-preterm infants: prevalence and risk factors. *Pediatrics*, *121*(4), 758-765. DOI: 10.1542/peds.2007-2158

Maenner, M. J., Warren, Z., Williams, A. R., Amoakohene, E., Bakian, A. V., Bilder, D. A., ... & Shaw, K. A. (2023). Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020. *MMWR Surveillance Summaries*, *72*(2), 1-14. http://dx.doi.org/10.15585/mmwr.ss7202a1

Mandell, D. S., Novak, M. M., & Zubritsky, C. D. (2005). Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics*, *116*(6), 1480-1486. https://doi.org/10.1542/peds.2005-0185

Mansour, Y., Burchell, A., & Kulesza, R. J. (2021). Central Auditory and Vestibular Dysfunction Are Key Features of Autism Spectrum Disorder. *Frontiers in Integrative Neuroscience*, *15*. https://doi.org/10.3389/fnint.2021.743561

Rafal, Z. (2013). Conductive hearing loss in children with autism. *European Journal of Pediatrics*, *172*(8), 1007-1010. https://dx.doi.org/10.1007%2Fs00431-013-1980-0

Roper, L., Arnold, P., & Monteiro, B. (2003). Co-occurrence of autism and deafness: diagnostic considerations. *Autism*, *7*(3), 245-253. https://doi.org/10.1177/1362361303007003002

Rosenhall, U., Nordin, V., Sandström, M., Ahlsen, G., & Gillberg, C. (1999). Autism and hearing loss. *Journal of autism and developmental disorders*, *29*(5), 349-357. https://doi.org/10.1023/A:1023022709710

Salhia, H. O., Al-Nasser, L. A., Taher, L. S., Al-Khathaami, A. M., & El-Metwally, A. A. (2014). Systemic review of the epidemiology of autism in Arab Gulf countries. *Neurosciences Journal*, *19*(4), 291-296. PMID: 25274588 PMCID: PMC4727667

Schafer, E. (2021). 20Q: Auditory issues in children with autism spectrum disorder - audiologists wanted. AudiologyOnline, Article 27860. Available at www.audiologyonline.com

Szymanski, Christen A., Patrick J. Brice, Kay H. Lam, and Sue A. Hotto. "Deaf children with autism spectrum disorders." *Journal of autism and developmental disorders* 42 (2012): 2027-2037. https://doi.org/10.1007/s10803-012-1452-9

Tas, A., Yagiz, R., Tas, M., Esme, M., Uzun, C., & Karasalihoglu, A. R. (2007). Evaluation of hearing in children with autism by using TEOAE and ABR. *Autism*, *11*(1), 73-79. https://doi.org/10.1177/1362361307070908

Taş, M., Yılmaz, Ş., Bulut, E., Polat, Z., & Taş, A. (2017). Otoacoustic Emissions in Young Children with Autism. *Journal of International Advanced Otology*, *13*(3). https://doi.org/10.5152/iao.2017.3105

van't Hof, Maarten, Chanel Tisseur, Ina van Berckelear-Onnes, Annemyn van Nieuwenhuyzen, Amy M. Daniels, Mathijs Deen, Hans W. Hoek, and Wietske A. Ester. "Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019." *Autism* 25, no. 4 (2021): 862-873. https://doi.org/10.1177/1362361320971107

World Health Organization. (2022). *Autism*. World Health Organization. Retrieved from https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders

Zeglam, A. M., & Maouna, A. J. (2012). Prevalence of autistic spectrum disorders in Tripoli, Libya: the need for more research and planned services. *Eastern Mediterranean Health Journal*, *18*(2). DOI: 10.26719/2012.18.2.184

Zeglam, A. M., & Al-Bloushi, H. (2012). Autism today in Libya: Is it a tip of an iceberg?(A comparative study). *Middle East J Fam Med*, *10*(8), 34-8.

Zeglam, A. M., & Al-Ksaik, S. M. (2016). Hearing assessment in autistic spectrum disorder; is it unnecessary and pointless procedure in developing countries?. https://doi.org/10.26719/2020.26.2.176

Zeidan, J., Fombonne, E., Scorah, J., Ibrahim, A., Durkin, M. S., Saxena, S., ... & Elsabbagh, M. (2022). Global prevalence of autism: a systematic review update. *Autism Research*, *15*(5), 778-790.https://doi.org/10.1002/aur.2696.