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Sexual Dimorphism and Morphological Variation in the Populations of *Akis costitubera Marseul* (Coleoptera: Tenebrionidae) By Using a Geometric Morphometric Approach

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Abstract: The present study was conducted to evaluate the differences related to area of males and females of *Akis costitubera* Marseul by using morphometric geometric technique. The study was accomplished by using photographic records of each individual in ventral views by using a CELESTRON X-150 digital camera. A matrix of photographs for each view was constructed using the TpsUtil program. In each view, we used 26 homologous landmarks which were digitized in the TPSdig2 program. 30 males and 30 females individuals were collected, photographed, and 26 landmarks from ventral views were digitized. We used a multivariate analysis of morphological variation. The results revealed significant differences between male and female ($P<0.005$), and the surface area of females was larger than that for males. The study concludes that sexual dimorphism occurred in the population of *A. costitubera* Marseul where these differences raise the question of whether sexual dimorphism may be modulated by natural selection.

Keywords: *Akis costitubera*, sexual dimorphism, geometric morphology

INTRODUCTION

For centuries, comparison of organisms by anatomical characteristics has been a core element in comparative biology, taxonomic classification, and understanding of biological diversity have been based mainly on morphological descriptions (Adams, Rohlf, & Slice, 2004). In the early twentieth century, where morphological analysis had a similar revolution of quantification, comparative biology entered a transition from the description field and quantitative science (Bookstein, 1996). The study of morphology has had an important emphasis by developing statistical shape analysis. This made the combination of multivariate statistical methods possible and established new ways to visualize the structure (Adams & Funk, 1997 ; Dryden & Mardia,1998).

The main use of Morphometric analysis is for studying shape variations and covariance with other variables. In the last 10 years, the number of studies using geometrical morphometric methods has increased. These new methods have been used and proved to be relevant in a large spectrum of morphometric fields, including species systematic**,** phylogeny, and ontogeny (Auffray, Alibert, Renaud, Orth, & Bonhomme, 1996; David & Laurin, 1996; Fink & Zelditch, 1995; Klingenberg & McIntyre, 1998; Loy, 1993; Naylor, 1996; Zelditch, Bookstein, & Lundrigan, 1993; Zelditch, Fink, & Swiderski, 1995).

The differentiability degrees of pressure in insects and their outcome alteration of habitat suggested that the phenotypic disturbance degrees reflect the ability of an individual to overcome the effects of pressure (Hugo A

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Benítez, Briones, & Jerez, 2010; Hugo Alejandro Benítez, Vidal, Briones, & Jerez, 2010).

As a result of some difficulties taxonomical and biodiversity analysis have been based on morphological descriptions. The geometry captured data of the morphological structure is particularly an interesting method to analyze developed data (Alibert, Moureau, Dommergues, & David, 2001).

In the present study, we apply a geometrical morphometric technique to assess morphological differentiation among males and females of *A. costitubera* Marseul.

MATERIALS AND METHODS

The study was conducted by collecting beetle specimens from the Mass area that is located west of Al-Bayda city in Libya at (32°45' N, 21°37' E). In this study we analyzed morphological differences in 30 males and 30 females during the summer of 2015-2016.

The study site is an area of 10 hectares located very close to Massa region (5 km west), surrounded by *Cupressus sempervirens* L*, Ceratonia silique* L*., Pistacia lentiscus* L*., Phillyrea latifolia* L*., Arbutus bavarii, Olea europaea Rhamnus lycioides L. Erica multiflora L., Globularia alypum*linn*.,Cistusparvi florus*lam., and *Calicotome villosa* trees.

The specimens were collected by using a Pitfall traps that made of glass, about 30 glass jars that measured of 10×6 cm and were buried in the soil in both sites, equipped by an attractant item such as a banana or banana extract, and buried in 12 cm depth. The samples were transferred to the Entomology lab. at the Department of Zoology, OMU, and were killed by Ethyl acetate 99%. Then, the insects were stuffed and the samples were identified, and the target species were chosen for the study conduction.

The study was performed by using photographic records of each individual in ventral views with using a CELESTRON X-150 digital camera. A matrix of photographs, with each photo constructed by using the TpsUtil program (F. Rohlf, 2015). In each photo, 26 homologous landmarks were used (Figure,1), and digitized by using the TPSdig2 program (F. J. Rohlf, 2001). Then we calculate the surface area for all samples, and All statistical tests were performed with the Minitab 16 version program by using t-test to compare the differences between the two genders.

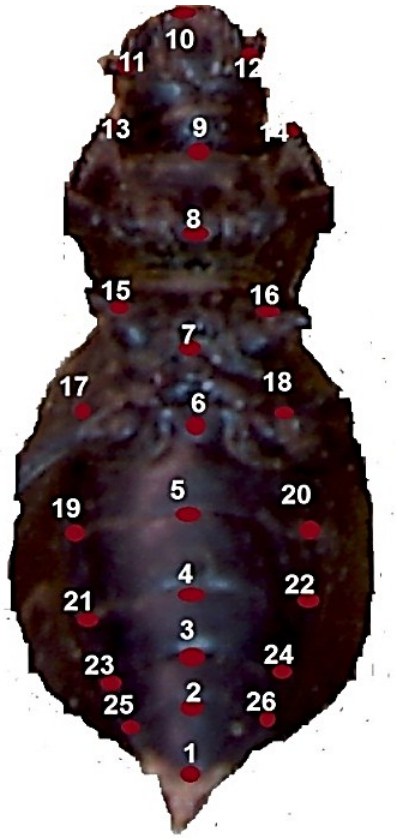

Figure (1). All landmarks description on ventral view of beetle's body without limbs and antenna

RESULTS AND DISCUSSION

By using T-test to compare body surface area in males and females, we noticed a high signification ($P<0.05$) in the population of *A. costitubera* Muresul (Table 1), and (Figure 2).

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Table (1). Comparisons using the mean statistical analysis for body surface area (mm²) among Massa region for males and females of *Akis costitubera* Marseul

Sex	N	±SE	DF	F	P
Females	30	1208.3±41	53	2.99	0.004
Males	30	999 ±56			

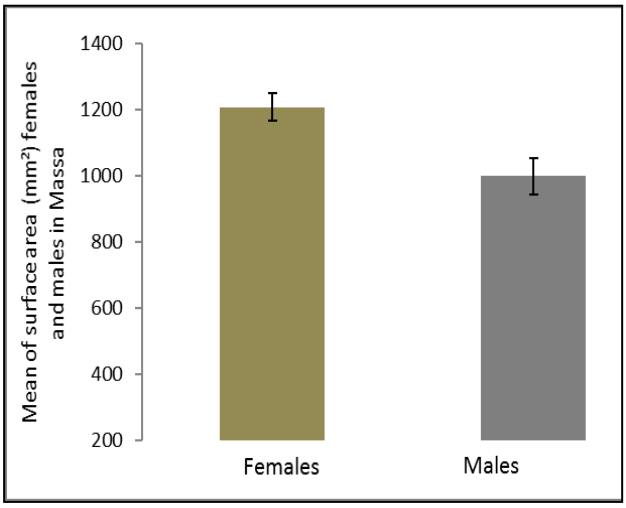

Figure (2). Surface area (mm²) representation of all samples in Massa region for males and females of *A. costitubera* Marseul

This study is the first attempt to statistically examine morphological variation in *Tenebrionidae* species in Libya. As morphology is determined by both genotype and phenotype, it can provide insights into the phylogeny and ecology of beetles, and the selective pressures driving their evolution (Losos & Miles, 1994). A morphological study can also aid the development of a reliable and accurate identification for this species, which is fundamental for studying *Tenebrionids* biology, and ultimately for a better conserving of the species.

The adoption of new techniques to determine variation in the shape of both animals and plants is currently a widely discussed issue. (Lawing & Polly, 2010), geometric morphometric technique can unify methodologies to quantify and visualize shape in all the ways that are possible.

The current study results suggest that there was a significant difference in the body surface area between both adult males and females of *A. costitubera* population in Massa region ($P<0.05$).

As the result suggested that the surface area in females (1208.3±41) is larger than that for males (999 ±56), so the sexual dimorphism is very clear between the individuals of this species in this region. Although these variations are not visible to humans by ocular inspection, they may be sufficient to produce a sexual selection in insects. The variation in the abdomen surface size was greater in females; this is an essential morphological character which allows a female to produce more eggs, and therefore have a greater fecundity and fitness (Andersson, 1994; Hugo A Benítez et al., 2010; Hugo Alejandro Benítez et al., 2010; Cepeda-Pizarro, Vásquez, Veas, & Colon, 1996). Studies of body shape in *Ceroglossus chilensis* have demonstrated that the abdomen surface-size variation between males and females is directly associated with the sex ratio in this species (Hugo A Benítez et al., 2010). Morphological sex dimorphism is much reduced and only visible under a microscope. However in terms of geometric morphometric, the differences are visible in two body regions; the abdomen of females, these variations have been reported to have an adaptive value due to the presence of eggs and changes in the pronotum of the thorax in males, which has been attributed to intrasexual competition in this species (Hugo A Benítez et al., 2010; Hugo Alejandro Benítez et al., 2010).

CONCLUSION

We concluded that studying sex dimorphism differences by using TPSdig techniques could help in taxonomic classification and understanding of biological diversity.

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التمايز الجنسي والاختلاف المورفولوجي لعشائر النوع *Akis costitubera* Marseul (Coleoptera: Tenebrionidae) باستخدام تقنية القياس الهندسي المورفولوجي

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المستخلص: هذه الدراسة بحثت الاختلافات الخاصة بالمساحة السطحية للخنافس من عائلة Tenebrionidae للنوع. *Akis costitubera* M باستخدام تقنية القياس المورفولوجي الهندسي، وقد أجري البحث بالتصوير الرقمي البطني للخنافس وتم تحويل الصور إلى مصفوفات رقمية باستخدام برنامج TpsUtil وشملت كل صورة 26–معلماً متجانس لبطن الحشرة ثم نقلت للبرنامج TPSdig2. تم تصوير 60 عينة بمعدل (30 ذكر–30 أنثي) جمعت في الفترة ما بين شهر يونيو 2015 لغاية شهر أكتوبر 2015، من منطقة الدراسة. بينت النتائج أن هناك فرقا معنوياً كبيراً في مساحة الجسم السطحية بين الذكور والإناث بين أفراد هذا النوع ($P<0.05$) وكانت المساحة السطحية للجسم عند الإناث أكبر من المساحة السطحية للجسم عند الذكور وتستخلص الدراسة وجود اختلافات في المساحة السطحية بين النماذج الجنسية للنوع الواحد، وهذه الاختلافات قد تقودنا للمزيد من الأسئلة التي تتحدث عن علاقة الشكل الظاهري بالاختيار الطبيعي.

الكلمات المفتاحية: *Akis constitubera*، التمايز الجنسي، القياس الهندسي المورفولوجي.

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GIS-Based Groundwater Information System (GWIS) of Al Waseetah area: Case study

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Abstract: Since groundwater is the main water resource in Libya, groundwater information system (GWIS) is required as a decision support system (DSS) where it promises great potential for effective management and assessment of groundwater resources, and enhances the sustainability and efficient use of groundwater resources. This paper discusses the requirements and the design approach for the preparation of GWIS using geographic information system (GIS) techniques, where Al Waseetah area in Al Jabal Al Akhdar region was selected as a case study because of the availability of the hydrogeological data. The system structure and characteristics were defined and designed to store and manipulate the groundwater data, where 155 groundwater wells data were used to test and implement the GWIS system, in addition to remote sensing data as well as previous geological and hydrogeological studies. A series of maps and the hydrogeological map were produced and used to evaluate and interpret the groundwater system in Al Waseetah area.

Keywords: GIS; Water Resources; Groundwater.

INTRODUCTION

Water is an essential commodity to mankind, and the largest available source of fresh water lies underground. As the world's population increases, the demand for fresh water has necessitate the call and need for the development of underground water supplies. Globalization and the inevitable processes of progress in the form of modernization and urbanization have magnified the problem of the search for freshwater supplies. Efforts, systems, and technologies have increased to solve these problems; methods for investigating the occurrence, patterns, and movement of groundwater have improved, better means for extracting ground water have been developed, principles of conservation have been established, and researches of several types have contributed to a better understanding of the subject (Todd, 1963). Management and decision making in water sector essentially are based on Water Information System (WIS), where it represents the key for water resource assessment and sustainable management. These involve different levels of stakeholders concerned with water data in different purposes of assessment and management.

At present, holistic management of groundwater resources suffers from a large number of noted groups involved with respect to their divergent interests on the one hand, and the lack of essential information and the complexity of the geological system on the other. Various hydro-geological, climatic, water-economical, chemical and biological interrelations have to be taken into account. Thus, the traditional approaches in information retrieval and management, for example, single reports in hardcopy form, isolated data maintenance and hardly automation which are characterized by high

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standards and time expenditure, are not sufficient (Uwe Rueppel and Thomas Gutzke, 2004). Also, the groundwater has a spatial extent, therefore, Geographical Information Science and System (GIS) can be a powerful tool for developing solutions for groundwater resource problems. GIS involves the essential developmental system and techniques for assessing water quality, determining water availability, preventing flooding, understanding the natural environment, and managing water resources on a local or regional scale (Asadi et al., 2007). Moreover, Foglini (2004) maintains that GIS is:

- GIS is able to efficiently manage groundwater issues such as groundwater data management (for example stratigraphy, drilling information, aquifers and aquitards geometry, hydrogeological parameters such as transmissivity, specific storage, and horizontal and vertical hydraulic conductivity).
- Tools (or interfaces to tools) to support experts in their data interpretation.
- Groundwater modeling facilities to address underground conceptualization, modeling data input (that is a definition of boundary conditions, internal sources/sinks) and simulation tasks.
- Management policy analysis, including assessment of optimal strategies in water supply, aquifers protection and remediation measures.

There are several Ground Water Information System (GWIS) platforms around the world that are used to collect, input, process, implement, and display data in interactive reports with maps and associated statistics. For example, the GWIS Figure (1) is the local authority of Berlin (Senatsverwaltung von Berlin) uses the GeODin platform developed by FUGRO CONSULT GMBH for regional database which comprises 130,000 boreholes with log profiles, 8000 wells with several thousand chemical groundwater analysis and over 6 million water levels, which is maintained by GeODin running an Oracle database (GeODin, 2018).

Another example is the Australian National Groundwater Information System (NGIS), which is a spatial database for GIS specialists that contains a range of groundwater information submitted by respective Commonwealth States and Territories. The system contains more than 850, 000 borehole locations with associated lithology logs, bore construction logs, and hydrostratigraphy logs. In addition, 2D and 3D aquifer geometries are also available for some areas. Jurisdictional groundwater management area boundaries are the most recent addition to the system (Australia Bureau of Meteorology, 2018). Moreover, the NGIS data can be viewed, visualized, and retrieved through the interactive tool Australian Groundwater Explorer as in figure (2). The majority of the Libyan land is categorized as arid to semi-arid. About 90% of the land is a desert characterized by low rainfall rates, diurnal temperature variations, poor soils, and seasonal winds. Moreover, groundwater accounts for 97% of the total water abstracted for different uses, and the country total water abstraction is about 4.98 billion cubic meters per year (GWA, 2006).

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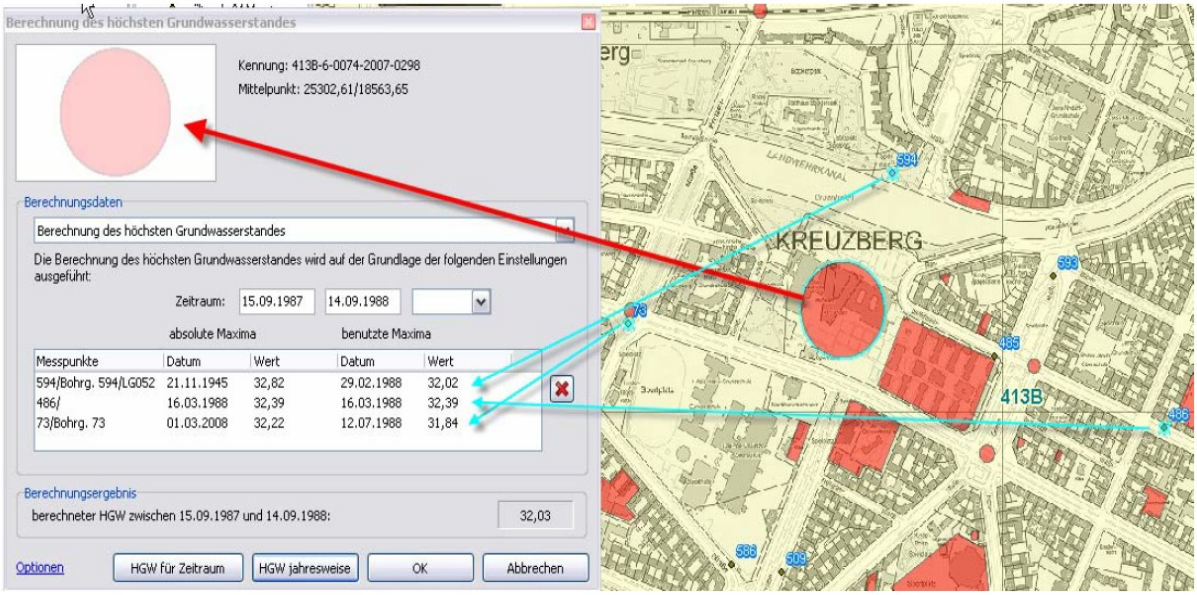

Figure (1). GeODin platform developed by FUGRO CONSULT GMBH (GeODin, 2018)

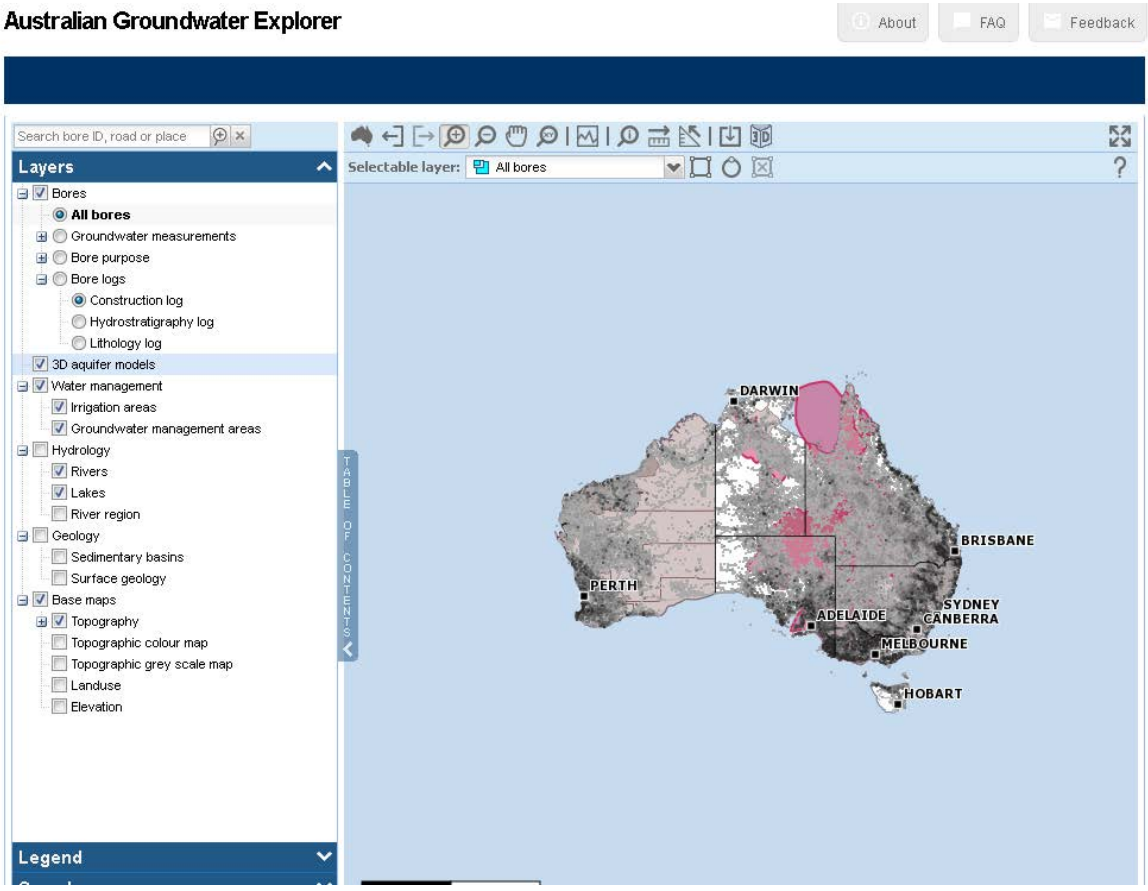

Figure (2). Australian Groundwater Explorer (Australia Bureau of Meteorology, 2018)

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There are many major issues in groundwater management facing the Libyan water sector. One of these issues is the lack of groundwater information system at the country and local levels which need to be addressed based on the introduction of innovative solutions that serve this sector and thus, contribute to information management process of groundwater resources. Moreover, it is important to note that before embarking on the creation of the GWIS to firstly identify the data sources, the water institutions in Libya faces obstacles and challenges, which can be summarized in the following points (Hamad, 2012):

- The overlap of water institutions responsibilities.
- The lack of coordination between the water institutions.
- Organizational instability.
- Limited structuring among the functional levels.
- Inadequate institutional capacity at regional and local levels.
- Limited experience in integrated management.
- less participation of the stakeholders.
- Lack of monitoring of groundwater resources.
- Lack and poor quality of data.

These obstacles and challenges have made it difficult than expected for interested scientists and analysts. Therefore, the goal of this research is to prepare a simple design architect system for GWIS, and the structure of data required for the testing and implementation using real data of groundwater from a small hydrogeological unit.

MATERIALS AND METHODS

In this research, some of the hydrogeological methods were applied in the integration with the science of Geographic Information System (GIS), and that plays the main role in understanding the spatial characteristics of the groundwater system. The design steps of GWIS are summarized as illustrated in figure (3). Where the identification of the data sources is the first step in a system analysis, as the water resources of Libya managed in a traditional way, groundwater data are often dispersed among different water institutions, water users, and other stakeholders, therefore, there is a crucial need for establishing a plan of communication between stakeholders for data collection. In the second step, once the data become available, they will be classified according to their availability either in hard copies or electronically (for example: Excel, GIS, AutoCAD, dbase.... etc.). In the third step, the data will be classified into spatial and non-spatial data. Selection of the spatial database and GIS platforms will be in the fourth step of the system analysis to finish the design and testing of the GWIS.

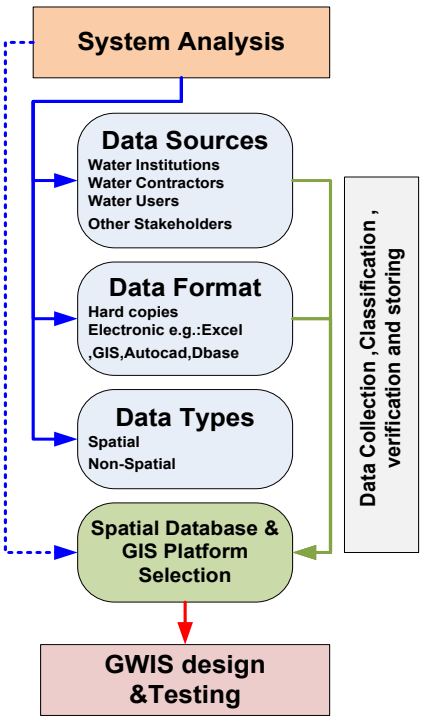

Figure (3). GWIS design steps

The final design of the GWIS is illustrated in figure (4), which consists of two main components. The first is the data component that is divided into spatial data and non-spatial data, and the second component is the GIS interface. Moreover, the processes box in the middle of the figure, which represents the common operations. Therefore, the deliverable will be in different forms (data reports, statistics, maps, and

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charts.etc.) to be available for the beneficiaries including the government, water institutions, water users, and other stakeholders for different purposes of groundwater assessment, planning, management, and decision-making.

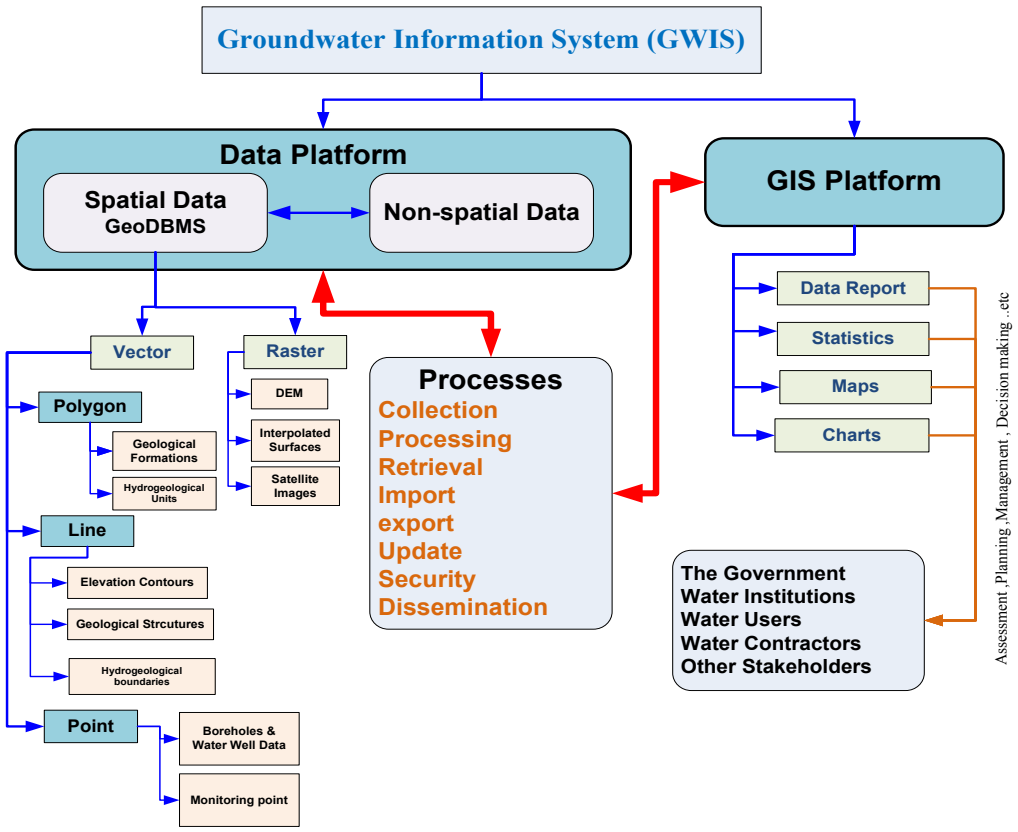

Figure (4). The design architect of GWIS

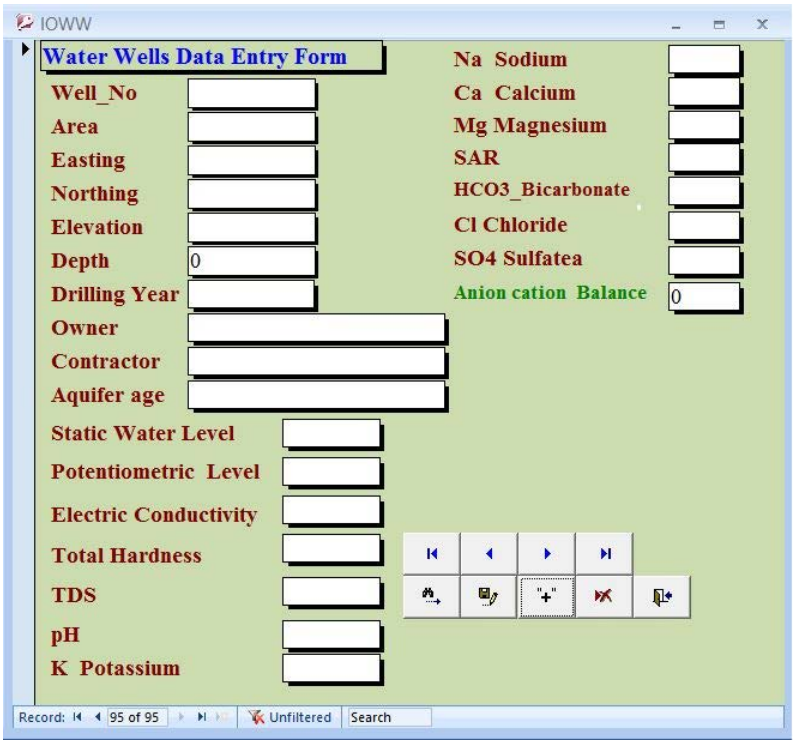

Figure: (5). Input form for water wells data

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The spatial data in the overall data-set component is divided into two categories. These include a vector data containing geological formations, hydrological units, faults, hydrological divides, and water wells data, which are represented by points linked to data entry form using Microsoft access database as in figure (5). The second, is the raster data comprising satellite imagery, digital elevation models, and previous geological and hydrogeological maps; these will be used for delineations and extraction of the geological structures and the hydrological boundaries. In addition to the raster data from the spatial interpolation, which is the process of using points with known values to estimate values at other unknown points (QGIS, 2018). In this research, the raster data are produced by Quantum GIS (QGIS), a very effective open source software using spatial interpolation techniques for interpolating water depth level and the total dissolved solids raster. Vector and raster data will be stored in PostGIS spatial database. The non-spatial data will be stored in folders and DBMS. Also, the following maps will be produced and used for the interpretation of groundwater system:

- Water well location map
- Elevation map
- Depth to water level map
- Total dissolved solids map
- Hydrogeological map

The selected area for implementing the GWIS is known as Al Waseetah area, which is located in Northeast Libya within the Al Jabal Al Akhdar region as presented in figure (6) with an aerial extent of 410 square kilometers. The selection of the area was done according to; first, the availability of the data and secondly, the area represents a subunit of the main hydrogeological unit Al Bayda -Al Bayydah. Moreover, the data shown in table (1) will be used to implement the GWIS.

Table (1). The Collected, Extracted, and Implemented Data**.**

Data	Type	Source
Water wells	vector	Data from 155 water wells from Libyan General water Authority and The General company of water and wastewater in Al Marj and Al-Baydah municipalities, the data contains: • Depth to Water levels • Chemical Analysis results and calculated parameters
Digital Elevation Model (DEM)	Raster	Space Shuttle Radar Topography Mission (SRTM), Spatial resolution of 30 meters
Satellite Image	Raster	Landsat 8, Spatial resolution of 30 meters
Geological Structures	vector	Extracted from satellite image according to geological map of Libya; 1:250 000 sheet, Al Bayda sheet NI34-15 and the geological map of Al Baydah - Al Bayadah area by Hydrogeo Consulting Engineers
Geology	vector	Geological map of Libya; 1:250 000 sheet, Al Bayda sheet NI34-1515 and the geological map of Al Baydah - Al Bayadah area by Hydrogeo Consulting Engineers
Hydrogeological units	vector	Compiled according to previous hydrogeological maps and depth to water level data of 155 water well.
Water Divide	vector	According to previous hydrogeological maps and depth to water level data of 155 water well.
Boundary	vector	Generated from Digital Elevation Model and satellite Image of land sat 8
Coast	vector	Generated from Digital Elevation Model and satellite Image of land sat 8
Escarpment	vector	Generated from Digital Elevation Model and satellite Image of land sat 8
Roads	vector	Open street map
Depth to Water level	Raster	Interpolated using spatial interpolation techniques for the vector point data of 155 water well
Total dissolved solids	Raster	Interpolated using spatial interpolation techniques for the vector point data of 155 water well

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RESULT AND DISCUSSIONS

The data presented in table (1) were used for implementing the GWIS, where data of 155 water wells sourced from the local offices of General Water Authority and General Company of Water and wastewater in Al Marj and Al Bayda municipalities. These data consist of geographic location and the depth to water levels, and chemical analysis results consists of major cations and anions. In addition to the previous geological and hydrogeological work, there were remote sensing data; Digital Elevation Model (DEM) from Space Shuttle Radar Topography Mission (SRTM), and satellite imagery from Landsat 8.

Geological data such as, geological formations, and geological structures, were delineated and extracted using the geological map of Libya Al Bayda-sheet compiled by the Libyan Industrial Research Center in 1974, geological map of Al Baydah - Al Bayadah Area was provided by Hydrogeo Consulting Engineers in 1996, and the interpretations of the satellite imagery and the DEM as in figure (7).

For the data to be interpolated since there are many spatial interpolation methods available with respect to their application in GIS, in this research the spatial interpolation for some of the groundwater data was carried out using Quantum GIS software Interpolation Plug-in, where the depth to water level and total dissolved solids maps were interpolated using Inverse Distance Weighted (IDW) method as in figures (8) and (9).

Hydrogeological boundaries and water divides were delineated according to the hydrogeological map by (Hydrogeo Consulting Engineers 1996), the piezometric map for groundwater of Al Jabal Al Akhdar by (Arghain and Hamad, 2006), and the result of interpolation for depth to water level data.

Spatial analysis of the DEM was carried out to extract the topographic characteristics of the study area, which is illustrated as in table (2).

The hydrogeological map was produced as illustrated in figure (10), where it represents a key tool for the evaluation of a groundwater system in a certain area, and could be interpreted as in the following:

- The surface and subsurface geology of the study area is consisting mainly of the tertiary formations composed of carbonate and quaternary rocks represented by alluvial and coastal sediments characterized by karst and fractures, which represents the main constitutes of the aquifer's material in the area.
- The hydrogeological divides and boundaries are represented by the Mediterranean Sea in the north, the first escarpment in the south, Wadi Al Kuf in the west, and Wadi Al Uwaynah in the east.
- Depth to water level contours ranges from 54.7 to 229 meter, where the flow system characterized by a gradual descending, also the flow direction is toward the north and northeast directions.
- The chemical quality of groundwater, which is expressed by the total dissolved solids (TDS), ranges from 362 to 2788 ppm prevailing high values in the north of the study area and indicating the effect of seawater intrusion which is caused by excessive groundwater pumping due to intensive agricultural activities, as well as urban activities in the coastal region due to the absence of a proper management of the groundwater resources. Moreover, the values of total dissolved solids are decreasing southward toward the second escarpment of Al Jabal al Akhdar, where the aquifer recharge comes from.

Table (2). Topographic characteristics of the study area

Minimum elevation (meter)	10
Maximum elevation (meter)	478
Average elevation (meter)	195
Maximum slope (degree)	84
Average slope (degree)	5
Average aspect	NW (331°)

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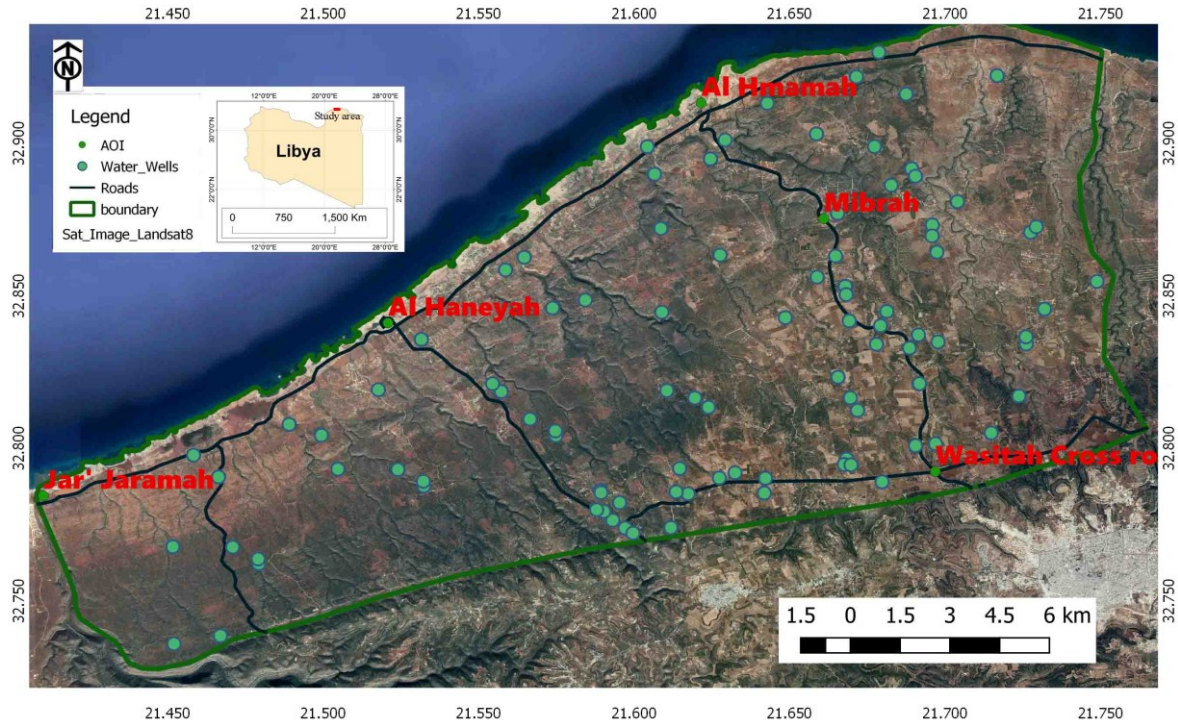

Figure (6). Location map

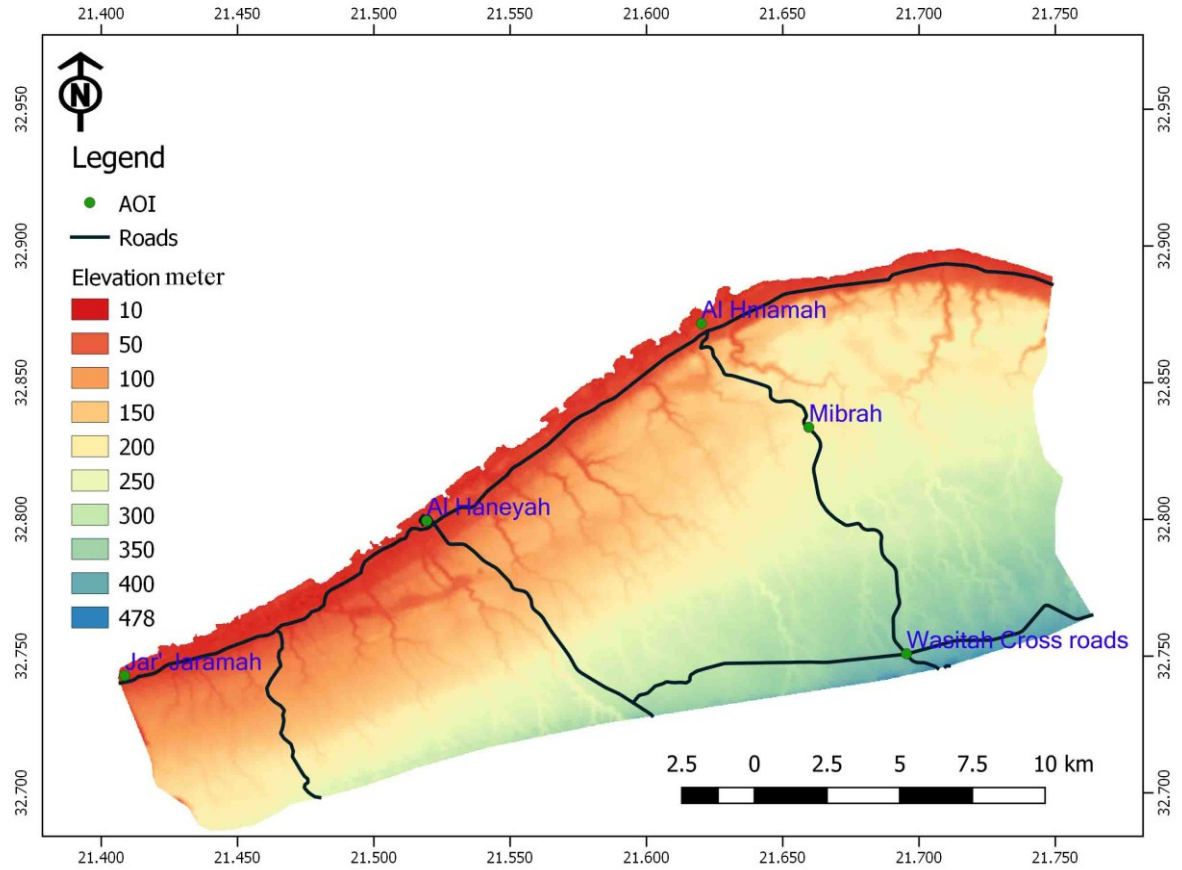

Figure (7). Digital elevation map

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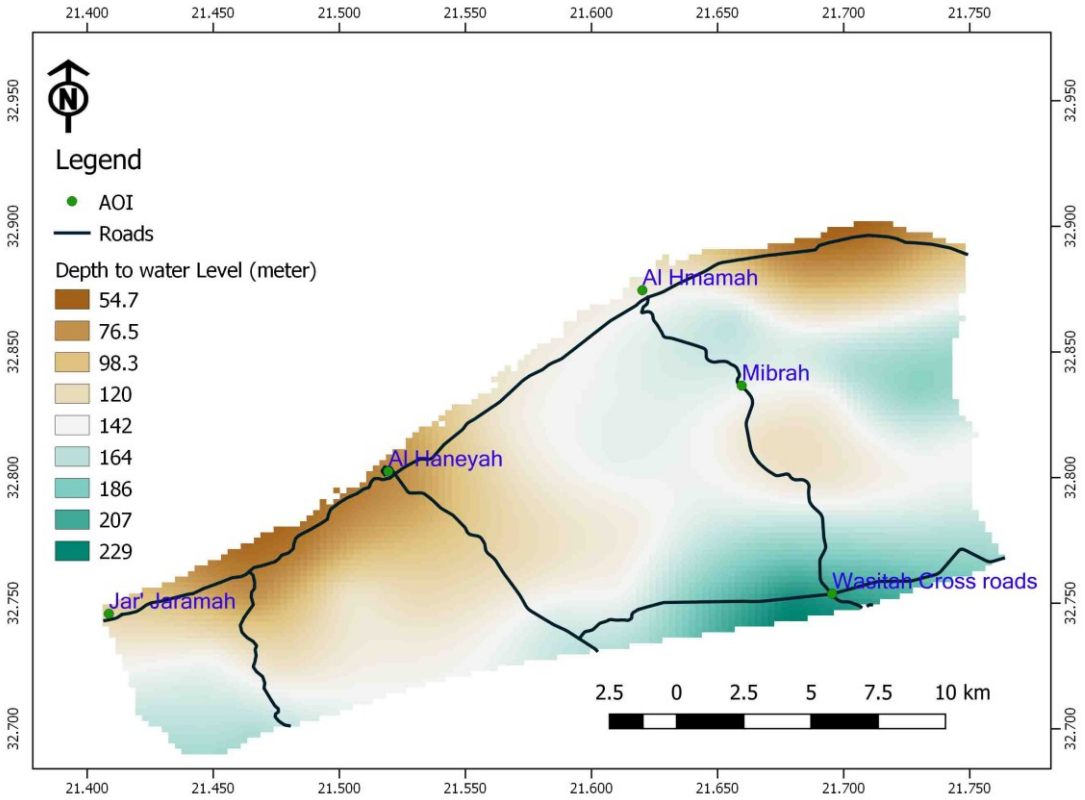

Figure (8). Depth to water level map

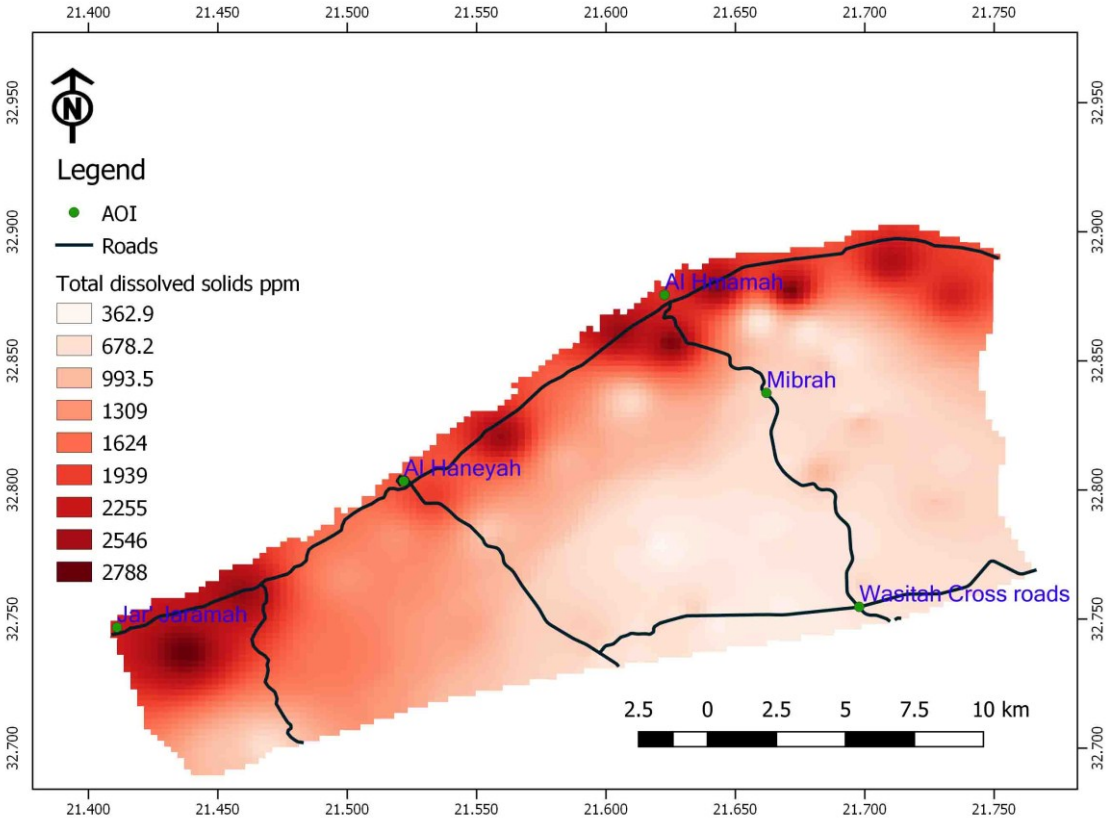

Figure (9). Total dissolved solids map

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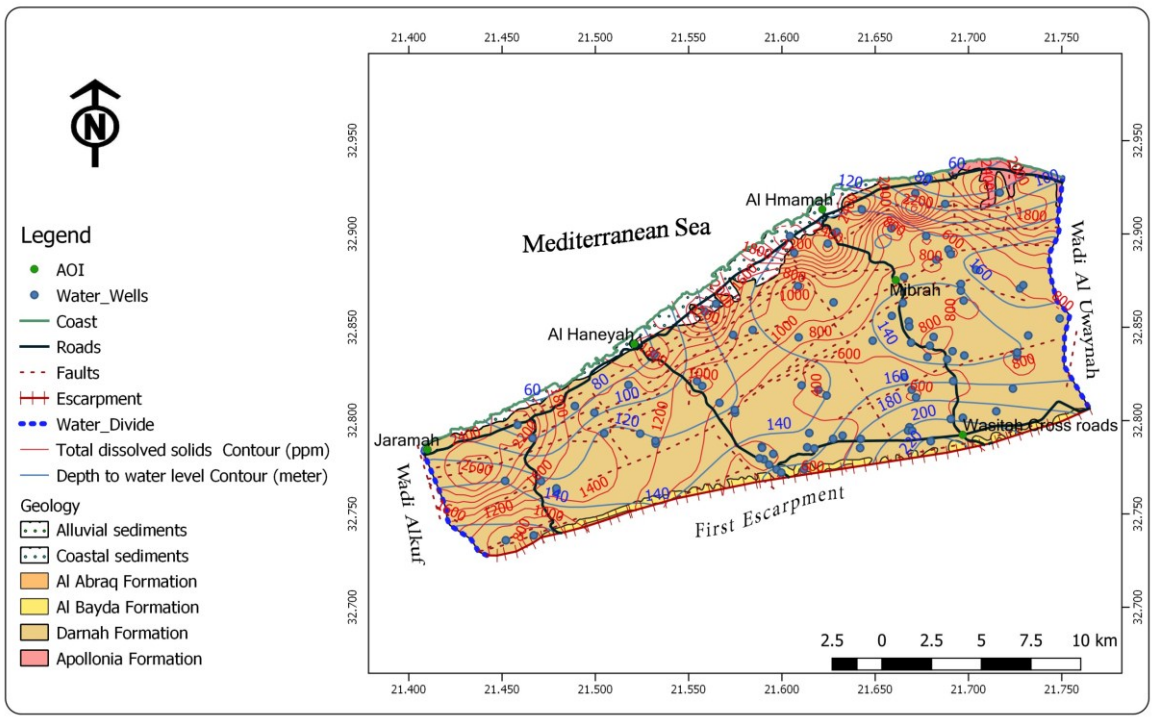

Figure (10). Hydrogeological map

CONCLUSION

Information systems and modern technology have a significant impact in the field of groundwater resources. Many software developers have solutions and services in this field, especially geographic information systems which provides an enabling environment to integrate all the data of groundwater resources that enable storage and processing of the spatial data. These are of concern to stakeholders and decision-makers in the field of groundwater resources. However, the following should be taken into consideration:

- The best GWIS is the system that involves all the stakeholders concerned with groundwater resources to ensure comprehensive and integrative data system and processes.
- Groundwater resource authorities may call and argue that they lack the financial resources and the ability to obtain software tools that would be used for the establishment of GWIS, especially the commercial software. Therefore, open source software such as Quantum GIS has a very good capabilities that could be initiated or even adopted for GWIS.
- The technology must be accompanied by proper data, processing, and implementation methods.
- The focus should shift from two-dimension to three-dimensional capabilities in the future to give more insights into the groundwater system and its hydraulic characteristics.
- Development of simple interface or plug-in linked to open source GIS software will be useful for extracting reports and statistics for users with limited GIS experience.

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نظام معلومات المياه الجوفية (GWIS) القائم على نظام المعلومات الجغرافية منطقة الوسيطة كحالة دراسية

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المستخلص : بما أن المياه الجوفية هي المورد المائي الرئيس في ليبيا ، لذلك فإن نظام معلومات المياه الجوفية (GWIS) مطلوب كنظام لدعم القرار (DSS) ، حيث يعتبر ذا إمكانية كبيرة للإدارة والتقييم الفعال لموارد المياه الجوفية ويعزز الاستدامة والاستخدام الفعال لموارد المياه الجوفية، حيث يناقش هذا البحث المتطلبات ونهج التصميم لإعداد GWIS باستخدام تقنيات نظام المعلومات الجغرافية (GIS) ، وقد تم اختيار منطقة الوسيطة بمنطقة الجبل الأخضر كحالة دراسية نظرا لتوافر البيانات الهيدروجيولوجية، وتم تحديد وتصميم بنية النظام وخصائصه لتخزين بيانات المياه الجوفية ومعالجتها ، كما تم استخدام بيانات 155 بئر مياه جوفية لاختبار وتطبيق نظام GWIS ، بالإضافة إلى استخدام بيانات الاستشعار عن بعد وكذلك الدراسات الجيولوجية والهيدروجيولوجية السابقة ، ومنها أنتجت سلسلة من الخرائط والخريطة الهيدروجيولوجية التي تم استخدامها لتقييم وتفسير نظام المياه الجوفية في منطقة الوسيطة.

الكلمات المفتاحية: نظم معلومات جغرافية، مياه جوفية، نظم معلومات المياه الجوفية، ليبيا.

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The incidence Of Paget's disease of the breast In Benghazi- Libya (During a period of 19 years)

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Abstract: Dermatoses of the nipple and areola are rare. The Paget's disease is a common dermatosis, which is presented in the form of a well-demarcated erythematous area, sometimes erosive, oozing or hyperkeratotic. Because the condition may be confused with benign diseases of the nipple, treatment is frequently delayed. The study aimed to highlight the incidence and make a clinical assessment of patients with Paget's disease of the breast in the eastern part of Libya. The Medical records and histopathological reports of all patients attended the breast clinic in 7th October Hospital in the period from June 1990 till December 2008 were collected and reviewed retrospectively. Demography, clinical features, and biopsy results were noted. The results: The total number of patients who had breast cancer was 897, from which Paget's disease was diagnosed in 19 patients (2.1%). Mean age at presentation was 57.3 years, and all of them were Women. Eczema of the nipple-areola complex and palpable mass occurred in (63.1% and 42.1%) respectively. The metastatic axillary lymphadenopathy at time of presentation were found in 36.8%. We conclude that Paget's disease is serious but commonly misdiagnosed. A thorough history and physical examination are important for every patient who is presented with skin and/or nipple changes of the breast, and physicians should maintain a high index of suspicion for Paget's disease of the breast.

Key words: Paget's disease, eczema, breast carcinoma.

INTRODUCTION

Since the original association between skin changes of the nipple and the subsequent development of breast carcinoma was reported by Sir James Paget in 1874; the condition later termed Paget's disease of the nipple has been a well-documented clinical entity (Paget, 1874). The description of clinical changes of the nipple and areola by Sir Paget includes having the appearance of a florid intensely red raw surface (very finely granular as if the whole thickness of the epidermis was removed). Paget's disease of the breast occurs in approximately 1-3% of all primary breast carcinoma series (Caliskan et al., 2008; Chaudary, Millis, Lane, & Miller, 1986; Dixon, Galea, Ellis, Elston, & Blamey, 1991; Nance FC, 1970) and often be presented clinically as eczematous changes of the nipple, associated with bleeding, itching, and ulceration (Nance FC, 1970; Paone & Baker, 1981) It is extremely uncommon in young women, and the presenting age ranges from 24 to 84 years with a mean age at diagnosis of 55 years(Siponen, Hukkinen, Heikkilä, Joensuu, & Leidenius, 2010). Although approximately 50% of patients present also with an associated palpable mass elsewhere in the breast, 40–50% of patients are diagnosed with Paget's disease without any other clinically breast lesion detected (Maier et al., 1969; Nance FC, 1970; Paone & Baker, 1981). In recent studies, in which mammographic findings have been correlated with clinical findings, the majority of women were presented with nipple changes.

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Histopathologically, a Paget's cell is large rounded cell with few intracellular bridges. It contains a large nucleus and light stain cytoplasm. A Paget's cell is regarded as a mammary gland cell that invades the surrounding tissues (Lever, 1983). Women presented with eczematous changes of the nipple are frequently diagnosed as dermatitis or eczema and treated with topical regimens with the true diagnosis remains elusive for long periods of time (Chaudary et al., 1986; Dixon et al., 1991). To avoid misdiagnosis of Paget's disease of the breast, clinicians must maintain a high level of clinical suspicion. We have documented more than 19 cases of Paget's disease. Our **aim** is to highlight the incidence and clinical assessment of patients with Paget's disease of the breast in the eastern part of Libya.

MATERIALS AND METHODS

All medical records and histopathological reports for patients who attended the breast clinic at 7th October Hospital in the period from June 1990 till December 2008 were collected and retrospectively analyzed. Demography, clinical features, and biopsy results were noted. Libya is a North African country classified under the Eastern Mediterranean Regional Office (EMRO). The overall population is of 6 million with 1.7 million (28%) situated in the eastern part. Benghazi is the second largest city in Libya, located at the coast of the Mediterranean Sea in the northeastern part of Libya with about one million inhabitants.

Statistical analysis

Descriptive statistics were used to describe and illustrate the data.

RESULTS

The total number of patients who had breast cancer was **897**, from which Paget's disease was diagnosed in **19** patients (2.1%). All patients were females; the mean age of patients was 57.3yrs within a range from 30 to 80 years (Figure 2). The clinical features of the patients at presentation are shown in (Figure1). Nipple ulceration and eczematous changes were found in 12 cases (63.1%), discharge and bleeding in 6 (31.5%) cases, and itching and indurations in 3 (15.7%) cases. Lump of the breast was found in 8 (42.1%) cases and pain in 2 (10.5%) cases, Palpable enlarged axillary lymph nodes were presented in 7(36.8%) cases. All patients were treated with total mastectomy and ipsilateral axillary dissection.

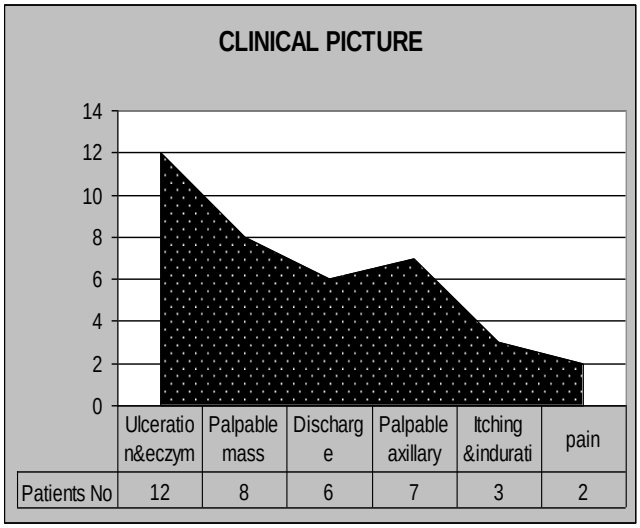

Figure: (1). Clinical picture of patients presented with Paget's disease of breast

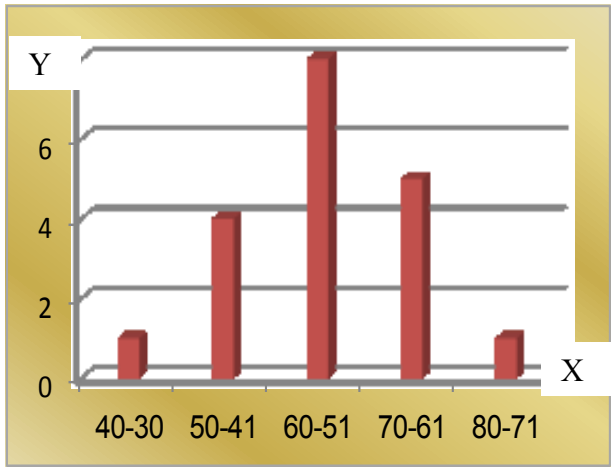

Figure: (2). (X): Ages of patients presented with Paget's disease of breast. (Y): Numbers of patients.

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Figure: (3). Nipple Ulceration and eczema

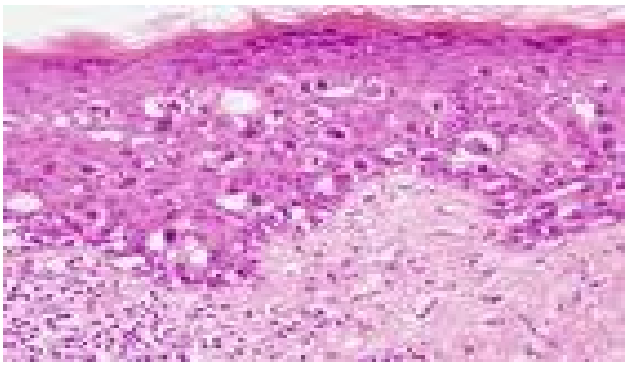

Figure: (4). Pagetoid cell

Table:(1). Histopathological assessment of patients presented with Paget's disease of breast

Histopathology	NO	Proportion
Invasive DC	13	68.4%
DCIS	6	31.6%

DISCUSSION

Paget's disease of the breast is a rare malignancy of the nipple–areola complex and results from the spread of intraductal carcinoma cells into the skin of the nipple and areola comprising 0.5–5% of all breast cancer (Nance FC, 1970; Paget, 1874; Sakorafas, Blanchard, Sarr, & Farley, 2001). In our study it comprises (2.1%). The diagnosis of nipple-areola Paget's disease requires a high index of suspicion with the incidence in patients between 50- 70 of age. This study was done at the breast clinic in 7th October hospital-Benghazi. The clinic serves the majority of breast cases in Benghazi and considered to be the sole formal breast clinic in the city. It receives the overwhelming majority of referral breast cases from other hospitals and other parts of the city and is, therefore, capable of serving as a good indicator of this condition for the whole city. A total of 897 patients had breast cancer during this period, from which Paget's disease was diagnosed in 19 patients (2.1%). This study represents a unique attempt to define the burden of Paget disease in Benghazi. The clinical features of this disease are shown in (Figure 1); the most frequent were lesions of the nipple ulceration and eczematous changes. Nipple erosion with a palpable lump of the breast is typical in the majority of the cases, the former may precede the development of a detectable mass by many years, but a careful histological examination can detect the focus (Lancer & Moschella, 1982; Nehme, 1976).

The average delay between the onset of symptoms and definitive treatment is approximately only 7 to 8 months (Gupta, Khanna, Khanna, & Gupta, 1983; Lancer & Moschella, 1982; Nehme, 1976). The importance of histologic proof was noticed by (Nehme, 1976; Satiani, Powell, & Mathews, 1977) because the differential diagnosis should include malignant melanoma and intraepithelial squamous cell carcinoma (Bowen's disease). Special stains are helpful (positively Paget's cell stains for aldehyde fuchsin and mucin, have a periodic acid-Schiff positivity and resistance to diastase digestion (Lancer & Moschella, 1982; Nehme, 1976). Radiographic studies (mammography and ultrasound) can demonstrate the presence of the speculated mass, microcalcifications or other suggestive findings of underlying breast cancer. Biopsy should be performed of the nipple Paget's disease and the underlying breast lesion if there is palpable mass or suspicious mammographic finding. Total mastectomy with ipsilateral axillary lymph nodes dissection is the treatment

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of choice in Paget's disease due to the greater incidence of invasive cancer, multifocal disease, and lymph node involvement and this was the preferred treatment in all of our patients. On the other hand for patients with Paget's disease who were presented without palpable mass and limited extent of underlying DCIS, some investigations have proposed the use of breast-conserving therapy (cone excision and radiotherapy) by the help of a more accurate and reliable imaging modality (MRI) which is necessary to select candidates for breast-conserving therapy more safely from patients with Paget's of breast (Harris, Lippman, Osborne, & Morrow, 2009; Kanitakis, 2007; Marshall et al., 2003; Sheen-Chen et al., 2001; Siponen et al., 2010).

Studies have shown, however, that patients with Paget disease of the breast who have a breast tumor and are having a mastectomy should be offered sentinel lymph node biopsy to see whether cancer has spread to the axillary lymph nodes. If cancer cells are found in the sentinel lymph node(s), more extensive axillary lymph-node surgeries may be needed (Laronga et al., 2006; Sukumvanich et al., 2007). Because many cases are missed by the general practitioners and dermatologist, it may be worthy to encourage strategies for early detection by keeping a high index of suspicion of Paget's disease for every case of eczematous breast lesion. Keeping in mind all possible limitations of our results and the small number of the study group, we want to share our experience with Paget's disease with others.

CONCLUSION

Paget's disease is a serious but usually misdiagnosed because it most often presented clinically as eczematous changes of the nipple. A thorough history and physical examination are important for every patient who is presented with skin and/or nipple changes of the breast, and physicians should maintain a high index of suspicion for Paget's disease of the breast.

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الإصابة بمرض باجيت للثدي في بنغازي– ليبيا (خلال فترة 19 عاما)

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المستخلص: الأمراض الجلدية للهالة الثديية والحلمة نادرة. ومرض باجيت للهالة وحلمة الثدي هو مرض جلدي شائع (وهي نوع من الأورام الخبيثة للثدي) والتي عادة ما تكوَّن منطقة محمرَّة (شديدة الاحمرار) محددة جيدا وأحيانا يوجد تآكل ونزف خفيف (نزوز دموي) أو منطقة جلدية متغلظة. ولأنه قد يتم الخلط بين مرض باجيت والأمراض الحميدة الأخرى للهالة وحلمة الثدي لذلك يحدث تأخر متكرر في التشخيص والعلاج. يهدف البحث لتسليط الضوء على نسبة الإصابة والتقييم السريري لهذا المرض في شرق ليبيا. تم تجميع السجلات الطبية والتقارير المرضية (تحليل العينات) لجميع المرضى الذين حضروا عيادة الثدي بمستشفى 7 أكتوبر بنغازي في فترة من يونيو 1990 إلى ديسمبر 2008. وتم أخذ البيانات الديموغرافية والصورة السريرية ونتائج الخزعات التحليلية. عدد الحالات التي تعاني من سرطان الثدي كان 897 حالة منها 19 حالة شخصت بأنها تعاني من مرض باجيت بنسبة 2.1%. وكان متوسط العمر عند اكتشاف المرض 57.3 سنة. جميع الحالات كانت إناثاً. أكثر الحالات كان لديها أكزيما في منطقة الهالة والحلمة (63.1%) وكتلة محسوسة تحت الحلمة (42.1%). أما في أكثر من ثلث الحالات فكان لديها انتقالات إلى العقد الليمفاوية الإبطية لنفس جهة الورم (36.8%). نستنتج أن مرض باجيت هو مرض خطير جدا. وتشخيصه دائما مفقود. أخذ التاريخ المرضي الشامل والفحص السريري مهم جدا لكل مريض بتغيرات جلدية في الهالة أو الحلمة. ويجب أن يحافظ الأطباء على درجة عالية من الاشتباه بمرض باجيت في الثدي وبخاصة في وجود هذه التغيرات.

الكلمات المفتاحية: مرض باجيت، أكزيما، سرطان الثدي.

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The Outcomes of Management of Necrotizing Soft Tissue- Infections in Lower Limbs of Diabetic Patients

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Abstract: Necrotizing soft tissue infection (NSTI) of the lower limb in diabetic patients (pts) is a common serious problem and is often associated with serious complications such as increased morbidity and mortality. The study aimed to assess 3 years (yrs) experience of NSTI and to review treatment and outcome in diabetic pts at Al Thoura Teaching Hospital between May 1ST 2014 –30th April 2017, over a period of 3 yrs.24 cases of NSTI of lower limbs in diabetic pts were admittedin the period of MAY 1ST 2014 –30th April 2017. Assessment and analyzing details about their presentation, clinical features, predisposing factors, treatment offered, and outcomes were performed .16 male and 8 female pts with a mean age of 56.4 yrs (range of 39- 78 yrs) were included . Majority of patients were in the age group of 50 to 70 yrs. The most important risk factors were (glycosylated HbA1c > 9.5% in 91.66%), smoking (58.33%), hypertension and hyperlipidemia (66.66% & 83.33% respectively). Neuropathy was found in 62.5% and PVD in 50%. The total involved feet are 27. The port of infection was mostly by previous unhealed feet ulcer 48.1%. The most operated surgical procedure is aggressive frequent necrectomy (51%). Multiple toes amputations and extended tarsal amputation were needed in 29.6% and 20.8% respectively. The need for grafting, flap advancement after surgical control of infection was needed in 6 cases. The failure rate of conservative surgery was 11.11%. Complete healing was in 70.37% of cases. The morbidity rate was high in all patients in this study 92.59%. The mortality rate was 3.7%. We conclude that NSTI of lower limbs is a life-threatening infective condition, common among diabetic patients, and early diagnosis with immediate and frequent surgical debridement could reduce systemic complications, morbidity, and mortality considerably.

Keywords: PVD (peripheral vascular disease), DFS (Diabetic foot syndrome), Necrotizing soft tissue infection (NSTI), patient (pts), osteomyelitis (O.M).

INTRODUCTION

Diabetes is a common disease affecting one million patients in the UK, about 2% of the whole population (Griffiths, 2002). Diabetic foot is a serious complication of diabetes mellitus and in some cases is the initial presentation of undiagnosed diabetes (Zafar, 2001). Diabetic foot syndrome (DFS) is a complex and heterogeneous disorder that affects 1 out of 5 patients with diabetes at least once in his or her lifetime with relevant consequences both on lower limb survival and general morbidity (Levin, 1995). Up to 15% of patients with diabetes will develop DFS at least once in their lives. Individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than the individuals without diabetes (Boyko & Lipsky, 1995). The Necrotizing soft tissues infection (NSTI) is a severe, serious, and maybe lethal complications of DFS. The NSTI is generally defined as any severe progressive infectious

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process affecting the skin, subcutaneous tissue, adipose tissue, superficial or deep fascia, ligaments, tendons, sheaths, joint capsules, and joints. The commonest predictor to this type of infection is a poorly controlled DM with or without previous foot ulceration. The foot ulcerations and infections associated with diabetes were the second most common cause of necrotizing fasciitis, and 15.2% of cases of necrotizing fasciitis were due to foot ulcerations and infections associated with diabetes (Elliott, Kufera, & Myers, 1996). The presence of Peripheral neuropathy which has been estimated to affect about 50% of patients, and PVD which affects as many as 40% of patients with diabetes with long duration of disease (Levin, 1995; Reiber, Boyko, & Smith, 1995), immunopathy , patient negligence to local minor trauma or mild infection, all these factors worsened the condition and lead to rapid progressive invasion threatening limb and life. The key of management is by early diagnosis and prompt surgical management that can reduce complications, morbidity and mortality rate.

The aim of this study was to analyze the outcomes of treatment of necrotizing soft-tissue infections of the lower limbs of diabetic patients and to determine factors associated with limb salvage and mortality.

MATERIAL AND METHODS:

We have conducted a prospective a study by the same surgical team for 24 patients (27 feet/legs) with necrotizing soft tissue infection cases admitted to the surgical department At El- Thoura Teaching Hospital. These emergency cases were diagnosed with severe necrotizing infection during 3 years period from 1st May 2014 to 30th April 2017. All patients were treated with multiple radical surgical debridements with a combination of broad-spectrum antibiotics and metronidazole.

All cases were collected and analyzed during this period of study. Information used in the study: demographic data, types of DM, and the underlying risk factors like smoking, hypertension, hyperlipidemia, renal impairment, and glycosylated Hb at time of presentation. The presence of neuropathy, ischemia, previous foot ulcer, mechanism of infectionentry, main operative finding, location andextent of infection, X-ray finding, types of surgery done, and the outcome of the treatment, Mortality, and hospital stay.

RESULTS

24 diabetic patients (pts) with necrotizing foot infection (NSTI) were admitted to the surgical department as an emergency during the period from May 1st, 2014 to 30th April 2017 for urgent major surgical interventions to control the infection and to save limb and life. All pts were treated primarily with immediate aggressive and frequent conservative surgery (in the form of radical excision of all devitalized/necrotic tissues). The mean age for the cases included in the study was 56.4 years, age range: 39- 78 years. Most of pts ages were in 6th and 7th decades (ages of negligence), both forming 66.6% of all cases in the study group. The male patients seem to be more affected by double than female pts (male: female ratio 2:1). The duration of diabetes mellitus (DM) ranges from 15- 30 yrs. Type II DM was almost in all cases in this series 91.66%, type I DM was less present in our study 8.33%. Risk factors among the study group are shown in (Table 1).

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Table (1). Risk factors

	Factor	NO	%
Gender	Men	16	66.66
	women	8	33.33
Age [yrs]	mean	56.4 yrs	
	range	39- 78yrs	
Types of Dm	Type I:	2	8.33
	Type II:	22	91.66
Underlying Risk/systemic factors:	HbA1C (> 9.5%)	22	91.66
	SMOKING	14	58.33
	HTN	16	66.66
	Renal impairment	5	20.83
	Hyperlipidaemias	20	83.33
Neuropathy	Sensation lost	15	62.5
	Charcot ankle	1	4.1
Ischaemia	IHD	7	29.16
	TIA, CVA	3	12.5
	PVD	12	50%
Previous Feet Ulcer/sinuses (13)(54.16%)	Underling chronic osteomyelitis	5	20.83
	Trophic/neuropathic	4	16.66
	Ischaemic	4	16.66
Previous vascular intervention: (PTA/ BY P ASS)		5	20.83

The most important risk for necrotizing infection was poorly/ uncontrolled diabetes with glycosylated HbA1c > 9.5%, which was found in most of the cases included in the study 91.66%, followed by smoking which was found only in all male pts in the study 58.33% (as Libyan females do not smoke). The other two important coexisting systemic factors are hypertension and hyperlipidemia which form 66.66% and 83.33% respectively, as the later factor is directly related to high glycosylated HbA1c. Most of renal impairment precipitated by septicemic events of severe necrotizing infections where found in about 5th of the cases included in this series. As kidneys of diabetic pts are prone to nephropathy at any stage if the DM is not under control. The presence of neuropathy, which was detected by loss of sensation,was found in 62.5% of the study group (this explains negligence minor trauma & simple infection). The lack of joint stability with no sensation of the position of a joint (Charcot ankle joint) was found in 4.1% of the cases. Half of the cases (50%) had obvious peripheral ischemic signs on both feet and legs in the form of skin changing toes, naildeformities, and feeble/ absent distal pulses. Other ischemic events (IHD, TIA, CVA) were found in 41.66% of pts. The presence of previous feet ulcer/ sinuses were found in more than half of the cases (54.16%) which were important predictors of losing limb in future. One fifth, 20.8%, of all pts in the study where exposed before to surgical/radiological vascular intervention. The total involved feet are 27 (as 3 cases in the study were a bilateral involvement). The port of infection to the feet was mostly by previous pre-existing unhealed feet ulcer, which was found in about half of the cases 48.1%, (table 2). Other sources of infections are due to feet injury from glasses, needle, nail, spikes, and blunt traumas were found in about 18.51%. The acute nail infection (acute paronychia) and neglected calluses were found in 11.11% & 7.4% respectively. 14.8% of cases had an unknown mechanism of entry of microorganisms to the soft tissues of the susceptible feet to the infections. All cases were presented clinically with a characterized offensive smell noted by family or by the patient himself, and symptoms and signs of severe in-

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flammation like fever, anorexia, vomiting, local signs of redness, swelling, crepitus, wet gangrene, and multiple skip lesions (table 2).

Table (2). Source of infections, the outcomes of the management.

	Total Involved Extremities	27	100 %
Mechanism of entry of infection (portal of entry of infection)	- Foot injury (glass, needle, nail, spike)	3	11.11
	- Acute paronychia	3	11.11
	- Previous ulcer	13	48.14
	- Callosities	2	7.4
	- Trauma	2	7.4
	- Unknown	4	14.81
Sign of severe inflammation ± offensive discharge:	- Redness, swelling, Crepitus	14	51.85
	- Wet Gangrene	6	22.22
	- Multiple Skip lesions (fig)	7	25.92
Involvement of the limb (location of infection)	- involvement of the foot	11	40.74
	- foot and ankle	8	29.62
	- Foot ,ankle, leg	6	22.22
	- Foot ,ankle, leg and thigh	2	7.40
radiographic findings :	- XRAY [osteomyelitis]	8	29.62
	- Gas bubbles	5	20.83
	- Unremarkable	14	51.85
Operative findings: (with offensive pus)	- necrotizing cellulitis	6	22.22
	- necrotizing fasciitis	15	55.55
	- myonecrosis	6	22.22
TYPES OF CONSERVATIVE SURGERY DONE:	- Aggressive frequent necrectomy (2-6 times)	14	51.85
	- 1-3 Toes amputations + open necrectomy	8	29.62
	- Extended forefoot amputation	5	20.83
	- The need for Grafting/flaps	6	22.22
	- Arthrodesis of ankle	1	3.7
Failed conservative surgery	Pt requiring radical (major) surgery (B/K OR A/K amputations)	3	11.11
Treatment outcome (Long term result & complications)	- Complete healing	19	70.37
	- Chronic sinus	1	3.70
	- Chronic ulcer	2	7.40
	- Requiring surgery after 1 yr	2	7.40
Mortality		1	3.7
Number of days in the hospital	- < 1month	25	92.59
	- >1 months	2	7.4

Feet were only affected by infection In 40.74% of patients, and about one third of cases were foot&ankle involved, and about one fifth of cases 22.22% were foot, ankle, leg involved. The entire limb involved (one side) was found in 7.4% of cases in the group. Radiography was done for all pts with an unremarkable finding in about half of the cases (51.85%), and the other half 48.14% experienced signs of osteomyelitis (O.M) and gas bubble. All pts underwent primarily emergency conservative surgery with an operative finding of spreading necrotizing cellulitis and fasciitis with offensive pus. Frank Muscle necrosis was found in 6 cases (22.22%). The most common surgical procedure is aggressive/ radical frequent debridement (necrectomy) 51% up to 6 times per case under regional/ general anesthesia which is a risk in some pts heart or brain ischemia. Multiple toes amputations and extended tarsal amputation were needed in some cases (29.6% & 20.8% respectively). The need for grafting or flap advancement after surgical control of infection on a healthy granulating wound was in 6 cases with accepted postoperative results. Fixation of an unstable an-

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kle joint by Arthrodesis was in one case (3.7%).

The failure rate of conservative surgery was 11.11%, where 3 pts require major surgery (below or above knee amputations) due to the uncontrolled rapidly spreading infection that threatening pts life, after an extensive trial of preservation surgeries. All pts were checked for more than one year with good long term results of conservative surgery. Complete healing was in 70.37% of the cases, and the remaining was ulcer & sinuses in about 11.11%.The requirement for additionalwound surgery (excision of residual O.M bone, debridement for recurrent infection) was in 2 cases (7.40%). The morbidity was high in all patients in the study. Most of pts (92.59%) stayed in hospital from 12 days to one month. In a few occasions, pts stayed for more than 4 weeks due to the need for reconstructive plastic (grafts/ flap advancement) surgeries. The mortality rate was 3.7%, one patient died because of the late presentation with worsened septicemia, acute renal shutdown and associated co-morbid conditions (ischemic heart disease and stroke).

DISCUSSION

Diabetes is increasing in prevalence, especially in developed nations. In the United States, the prevalence is estimated to be 7.3 percent of adults (Mokdad et al., 2001). Diabetic patients have always suffered from complications affecting the lower limbs. Foot infection and the subsequent amputation of a lower extremity are the most common causes of hospitalization among diabetic patients (Yönem, Cakir, Güler, Azal, & Corakci, 2001). NSTI is a severe form of foot infection and encountered a very serious and lethal conditions, characterized by an extensive necrosis of subcutaneous tissue spreading widely and rapidly to all tissues under the skin and may reach to the bone and joints. It appear normal at first skin, later it becomes affected with cellulitis with or withoutblisters, then gangrenous patches will appear as skip lesions at advanced stage. It's commonly associated with prolonged poorly controlled diabetes. It affects all ages, especially in immune-compromised pts. In elderly pts, it is a life-threatening condition if not recognized, diagnosed and managed early. Clinically NSTI is classified to necrotizing cellulitis (if subcutaneous tissue and skin affected), as necrotizing fasciitis (if the deep fascia involved), and as myonecrosis (if muscular necrosis happened). NSTI is usually caused by polymicrobial mixed infection(gram +ve cocci, gram –ve bacilli and anaerobic bacteria). The four main factors of neuropathy in the presence of callus or deformity, PVD, penetrating injuries and ill-fitting footwear (Lavery, Peters, & Armstrong, 2008) together with immunopathy and high sugar in tissues give these MO opportunities for invading, destructing and spreading rapidly in foot and limb. The presence of peripheral vascular disease (PVD) interferes with the healing process of ulcers by reducing the amount of tissue oxygen and nutrients and thus lengthening ulcer healing time,(A. Boulton, 1991, 1996; Murray & Boulton, 1995), , and the presence of diabetic peripheral neuropathy and any degree of distal ischemia act together to put these patients at high risk of limb and life complications. The Peripheral diabetic neuropathy is present in 22–24% of patients who have diabetes(Abbott et al., 2002; Tesfaye et al., 2005) The presence of previous foot ulceration is a very important portal for microorganism to soft tissues and bones. It is estimated that 15-20% of patients with diabetes will develop an ulcer on their foot at some point (A. J. Boulton, Vileikyte, Ragnarson-Tennvall, & Apelqvist, 2005). Such foot ulcers do not heal easily, are difficult to treat, and are more prone to serious infection. The usual presentation of NSTI include pain, toxic symptoms including fever, sweating, nausea, vomiting, chills, and on local examination might found swelling, redness, hotness. The Finding of Crepitus in the

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soft tissue is almost diagnostic for this condition but is not always present at the initial presentation. Blistering of the skin and soft tissue gas on radiographic examination are also highly indicative. The diagnosis can only be confirmed by immediate exploratory incision. In fact, Foot infections in diabetic pts are unpredictable, they can rapidly spread over a short period of time or even hours to involve an entire limb and thereby threatening the life. The key to a successful management of NSTI and to save limb and life is by: the first aim after early diagnosis focused on controlling the infection using a high dose of intravenous broad-spectrum antibiotics and metronidazole infusion to cover mixed aerobic and anaerobic M.O, and secondly an immediate surgical intervention by mean of Urgent, aggressive, frequent debridement with no hesitation of excising all unhealthy tissues, exposing tendons, bone, joints with local appropriate wound care which is the only way to reduce mortality rates. The delay in surgical excision of all necrotic tissues can lead to serious systemic complications which may lead to death. The Moderate-to-severe infections should receive aggressive irrigation and debridement with removal of all nonviable skin, soft tissue, and bone (Attinger, Bulan, & Blume, 2000; Frykberg, Wittmayer, & Zgonis, 2007; Wallace, 2007). The reported mortality of 30-40% reflects the inadequacy of conservative surgery in the treatment of this serious condition. The difficulties in treatment of NSTI are mainly due to a delayed diagnosis, late presentation, and delayed surgical interventions. The late presentation of patients to hospital may be due to family negligence, psychological factors, taking traditional treatment initially than early seeking medical advice. Its well-known that the foot infections in persons with diabetes are responsible for more hospital days than any other aspect of diabetes (Durham, Lukens, Campanini, Wright, & Smead, 1991; Gibbons & Eliopoulos, 1984; Nather et al., 2008), especially pts with NSTI who will stay for longer period of time for daily wound care and debridement. The thinking of reconstructingthe damaged limb should be delayed as possible until the wound becomes clean and fully granulating. Failure of conservative local debridement, lacking bone cover by healthy soft tissues, and infection is not under control or rapidly spreading are threatening patient life. The major amputations (below/ above) knees become necessary to preserve pts life, and to reduce hospital stay.

CONCLUSION

NSTI is a severe, serious infection, and often associated with high morbidity and mortality. The key of management is by early diagnosis, high dose of intravenous broad-spectrum antibiotics and metronidazole infusion together with immediate surgical aggressive debridement which shall improve the outcome.

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نتائج معالجة التهاب الانسجة الغرغريني في الارجل السكرية

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المستخلص: الالتهاب الغرغريني للأقدام السكرية هو مشكلة شائعة وخطيرة وعادة ما تكون مصحوبة بمضاعفات خطيرة مع زيادة في معدل التمرض ومعدل الوفيات. تهدف الدراسة إلى تقييم خبرة ومراجعة نتائج علاج هذا النوع من الالتهابات على مدى 3 سنوات في مستشفى الثورة التعليمي ما بين شهر مايو 2014 إلى شهر أبريل 2017. 24 حالة جمعت خلال الفترة المذكورة حيث تم أخذ البيانات وتحليلها والتي منها الصورة الإكلينيكية والعوامل المهيئة للمرض والعلاج المقدم ونتائجه. 24 حالة أدخلت المستشفى بالتهاب أنسجة غرغريني. 16 حالة ذكور و8 حالات إناث مع متوسط عمر 56.48 سنة تتراوح الأعمار ما بين 39-78 سنة. أغلبهم ما بين 50 الى 70 عاما. أهم العوامل المهيئة للمرض كان السكر التراكمي أكثر من 9.5% بنسبة 91.66% متبوعا بالتدخين (58.33%) ومرض ارتفاع ضغط الدم وارتفاع دهون الدم بنسب (66.66% و83.33% على التوالي). الاعتلال العصبي كان موجوداً في 62.5% وأمراض الشرايين الطرفية كان في 50% من الحالات. عدد الأقدام/الأرجل 27 قدم. أكثر مدخل للالتهاب هو وجود قرحة سابقة مزمنة في القدم بنسبة 48.1%. أغلب تدخل جراحة أجري هو إزالة شديدة ومتكررة للأنسجة الميتة 51%. بتر متعدد لأصابع القدم وبتر متمدد لمقدمة القدم (29.6% و20.8% على التوالي). الاحتياج إلى عمليات تجميل بعد السيطرة الجراحية على الالتهاب كان في 6 حالات. معدل فشل الجراحة التحفظية كان 11.11%. الشفاء التام كان في 70.37% من الحالات. بقاء قرحة وجيوب في 11.11%. معدل التمرض كان عالياً في جميع حالات الدراسة 92.59%. معدل الوفيات كان 3.7%. استنتجنا بأن الالتهاب النسيجي الغرغريني للأطراف السفلية هو التهاب مهدد للحياة. شائع بين مرضى السكري. التشخيص المبكر والتدخل الجراحي العاجل والمتكرر لإزالة الأنسجة الميتة يمكن أن ينقص المضاعفات العامة ومعدل التمرض والوفيات بشكل كبير.

الكلمات المفتاحية: مرض الشريان الطرفية، متلازمة القدم السكرية، الالتهاب النسيجي الغرغريني للأقدام السكرية، الالتهاب العظمي.

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Postpartum Uterine Bacterial Contamination without Clinical signs in Relation to Reproductive Performance in Dairy Cows

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Abstract: The study was performed to find out the relation between the uterine bacterial contaminations without clinical signs and postpartum (PP) reproductive performance of dairy cows. So that, uterine bacterial samples from postpartum dairy cows total (n = 44) were taken at 3rd, 15th and 30th day, and the bacterial count and score were applied. The animals were grouped to low, medium and high uterine bacterial contamination (15, 15, and 14 cows for each group respectively) according to score. Results revealed that uterine bacterial score (UBS) was decreased by the time in 3rd, 15th and 30th day (PP) for Low bacterial contamination group (5.73, 2.80, and 1.20 respectively), for Medium bacterial contamination group (7.80, 2.73, and 1.47 respectively), and for High bacterial contamination group (9.29, 6.57, and 2.21 respectively). Also, it revealed that there was a significant increase ($P<0.05$) in the duration of lochia in High than Low and Medium bacterial contamination groups. At 3rd day (PP), uterine location in all cows was represented in the abdominal cavity, but at15th day (PP), uterine involution as reaching to its normal non pregnant position in pelvic cavity was delayed in High (50%) than Low (80 %,) and Medium (53.30%) UBS groups. Moreover, at 30th day, uterine location in all cows was represented in pelvic cavity. The first estrus (PP) was significantly shorter in Medium, Low than High UBS groups. The number of services per conception showed a significant increase in High than in Low and Medium UBS groups. Also, at the 90th day (PP), the conception rate was lower in High UBS group than Low UBS group and Medium UBS group. We conclude that there was a relation between postpartum uterine bacterial contamination without clinical signs especially high contamination and reproductive performance in cows.

Keywords: uterus - bacteria – postpartum - reproductive performance and cows.

INTRODUCTION

The postpartum period is considered as a non-infectious event. The reduction in uterine size and the unidirectional flow of uterine contents, as well as gradual closure of the cervix, prevent microbial contamination. However, the reality is that the uterus invasion by microorganisms to a variable extent is depending on the animal's susceptibility and the hygienic condition of the environment(Gustafsson, Kornmatitsuk, Königsson, & Kindahl, 2004). Also during the early postpartum period, multiple bacterial species invade the uterus of cows (Rahim Ahmadi, Nazifi, & Reza Ghaisari, 2006). Uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to the establishment of uterine disease. The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria(Azawi, 2008).

A normal postpartum cow resolves uterine infection by rapid involution of the uterus

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and cervix, discharge of uterine content, and mobilization of natural host defenses, including mucus, antibodies and phagocytic cells (Azawi, 2008)**.** The uterine bacterial infection, bacterial products toxins, or the associated uterine inflammation may suppress pituitary LH secretion and disrupts postpartum ovarian follicular growth and function (I. M. Sheldon & Dobson, 2004). Series of studies confirmed that clinical and reproductive consequences are associated with these primary uterine pathogenic bacteria (Ahmed & Elsheikh, 2013; Foeldi et al., 2008) revealed that the dairy cows which suffered severe uterine bacterial infection delayed the time taken for appearance of the first dominant follicle and had a significantly increased rate of services per conception compared to the cows with mild uterine bacterial infection, also calving interval of dairy cows with severe postpartum uterine bacterial infection was significantly longer (482.50 ± 9.00 days) than that of dairy cows with mild postpartum uterine bacterial infection (407.10 ± 4.80 days). (Lewis, 1997) recorded that uterine infections have negative effects on various measures of productivity in dairy cows. Although postpartum cows develop mild endometritis, most cows are able to clear pathogenic organisms that cause endometritis before any measure of productivity is affected. This study was aimed to find out the relation between the uterine bacterial contaminations without clinical signs and postpartum reproductive performance of dairy cattle.

MATERIALS AND METHODS

Animals and samples collection: The present study was carried out at two farms in Sharkia and Damietta Provinces, Egypt during the period from January 2014 to May 2015. A total number of 44 cows aged from 3-5 years at puerperium stage (22 from each farm) were used. Uterine (endometrial swabbing) samples were taken on days 3, 15 and 30 postpartum using a transcervical guarded swab (consists of a small cotton piece wrapped around one ends of a rod sheathed in a metal guard tube). The animals were grouped to low, medium and high uterine bacterial contamination (15, 15, and 14 cows for each group respectively) according to uterine bacterial count and score.

Bacteriological examination: Uterine bacterial count was performed according to (Cain, Hanks, Weis, Bottoms, & Lawson, 2013). Sterile test tubes were labeled as follows: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and so on. Nutrient agar plates (OXOID, CM0085) were labeled as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and so on. 1:10 dilution of the sample was prepared. 9 ml of sodium chloride 0.9% w/v was inserted in each test tube. By using a new sterile pipette, 1 ml of the 10^{-1} tube was transferred into the tube labeled 10^{-2}. By using a new sterile pipette, 1 ml of the 10^{-2} tube was transferred into the tube labeled 10^{-3}, this step was repeated for the next dilutions. By using a new sterile pipette, 1 ml of the 10^{-1} tube was transferred to the agar plate labeled 10^{-1}, and the liquid was spread thoroughly and evenly over the surface of the plate using a sterile disposable spreader. By using a new sterile pipette, 1 ml of the 10^{-2} tube was transferred to the agar plate labeled 10^{-2}, and the liquid was spread thoroughly and evenly over the surface of the plate using a sterile disposable spreader, and this step was repeated for the next agar plate. Finally, agar plates were inverted and incubated at 37^{o}C for 24-48 hours. Colonies of the plate having 30 to 300 colonies were counted (Lee, 2009)**.** The Colony- Forming Unit / Milliliters (CFU/ml) can be calculated using the formula: CFU/ml = (No. of colonies x dilution factor) / volume of culture plate. A bacteriological score varying 1 to 10 was assigned to these bacteria, which corresponded respectively to table (1).

Table (1): Bacterial counts and their suggested scores

Bacterial count (CFU/ml)	Score
$< 10^{2}$	1
10^{2} to $< 10^{4}$	2
10^{4} to $< 10^{6}$	3
10^{6} to $< 10^{8}$	4
10^{8} to $< 10^{10}$	5
10^{10} to $< 10^{12}$	6
10^{12} to $< 10^{14}$	7
10^{14} to $< 10^{16}$	8
10^{16} to $< 10^{18}$	9
10^{18} to $< 10^{20}$	10

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An ascending arrangement of animals was done according to means of uterine bacterial score then grouping animals into 3groups: low (15 cows), medium (15 cows) and high (14 cows) bacterial contamination groups. The mean uterine bacterial score (UBS) for each animal was calculated as follow: (3^{rd} day UBS +15^{th} day UBS +30^{th} day UBS) / 3

Detection of postpartum reproductive performance: Anatomical location of uterus in abdominal and pelvic cavity was detected by rectal palpation in 3^{rd}, 15^{th} and 30^{th} days postpartum (Saut et al., 2011). For lochia discharge, cows were observed after parturition for recording duration of lochia. For first estrus postpartum, cows were observed for detection of estrus behavior, the interval from calving to first estrus was recorded (Habib, Bhuiyan, & Amin, 2010). Cows which come in estrus were inseminated. Non-return cows were examined for pregnancy by ultrasonography or by rectal examination 60 days after insemination. Number of services per conception was calculated by dividing the number of conceptions with the number of inseminations (Habib et al., 2010). Conception rate was calculated according to (Overton, 2009), conception rate was done at three periods, within 60 days, within 61-90 days and <90 days according to (Miah, Salma, & Hossain, 2004; Schefers, Weigel, Rawson, Zwald, & Cook, 2010).

Statistical analysis was performed using the Statistical Package for Social Sciences version 22.0 (SPSS for Windows 22.0, Inc., and Chicago, IL, USA). Data are represented in mean ± standard deviation values. Duncan's test was performed for comparing values between the groups. P<0.05 was considered to be significant

RESULTS

Uterine bacterial score (UBS): Table (2) and Fig. (1) showed that at 3^{rd} day, mean uterine bacterial score (UBS) was significantly smaller (P<0.05) in Low bacterial contamination group (5.73±0.23) and Medium bacterial contamination group (7.80±0.24) than High bacterial contamination group (9.2 9±0.22). At 15^{th} day, mean UBS was significantly smaller (P<0.05) in Low (2.80±0.20) and Medium (2.73±0.21) than in High bacterial contamination group (6.57±0.25) but there was no significant variation between Low and Medium bacterial contamination groups. At 30^{th} day, mean UBS was significantly smaller (P<0.05) in low (1.20±0.11) and Medium (1.47±0.13) than in High bacterial contamination group (2.21±0.21) but there was no significant variation between Low and Medium bacterial contamination groups.

Table (2): Uterine bacterial score (M±SE) in Low, medium, and high bacterial contamination groups.

Groups	No	Uterine bacterial score (UBS)			
		3^{rd} day	15^{th} day	30^{th} day	Means
Low bacterial contamination	15	5.73 ± 0.23	2.80± 0.20 [a]	1.20± 0.11 [a]	3.24± 0.05 [a]
Medium bacterial contamination	15	7.80 ± 0.24	2.73± 0.21 [a]	1.47± 0.13 [a]	4.00± 0.08 [b]
High bacterial contamination	14	9.29 ± 0.22	6.57± 0.25 [b]	2.21± 0.21 [b]	6.02± 0.20 [c]

The different superscript letters mean significantly differed at P < 0.05

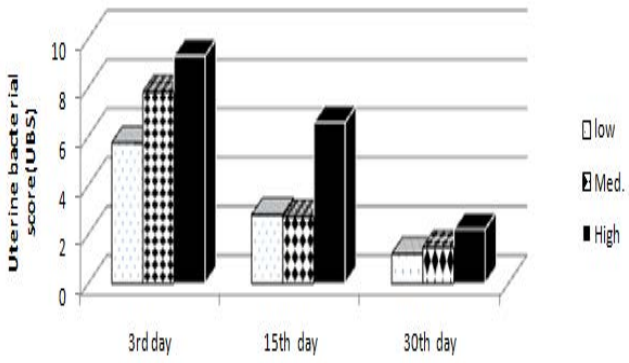

Fig.(1): Uterine Bacterial score in Low, Medium, and High bacterial contamination groups.

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Effect of postpartum uterine bacterial contamination on reproductive performance: Duration of lochia, as presented in Table (3) and Fig. (2), revealed that the duration was significantly shorter (P<0.05) in Low (12.60±0.36 days) and Medium (13.53±0.35days) than in High bacterial contamination group (17.71±0.49 days). Although Low bacterial contamination group was shorter than Medium bacterial contamination group, this difference was not significant. First estrus postpartum**,** data obtained in Table (3) and Fig. (3) revealed that the elapsed time from parturition to first estrus was significantly shorter (P<0.05) in Medium (51.07±2.00 days), Low (52.93±1.93 days) than in High bacterial contamination group (63.64±5.63 days), but there was no significant variation between Low and Medium bacterial contamination group. The results of services per conception presented in Table (3) and Fig. (4) revealed that the number of services per conception was significantly lower (P<0.05) in Low (1.67±0.19), Medium (1.93±0.15) than in High bacterial contamination group (3.14±0.21) although the number of services per conception in Low (1.67±0.19) was lower than that for Medium bacterial contamination group (1.93±0.15), this difference was not significant. Despite the uterine location of tested cows which is founded in Table (4) and Fig. (5), it was observed that at 3rd day postpartum 100% of uterine location in Low, Medium and High bacterial contamination groups was represented in abdominal cavity. However at 15th day postpartum, the uterine location in 20%, 46.70% and 50% of cows in Low, Medium, and High bacterial contamination groups, respectively were represented in abdominal cavity. While the uterine location in 80%, 53.30% and 50% of cows in the same previous groups was represented in pelvic cavity respectively at the 15th day postpartum. Meanwhile at the 30th day postpartum, 100% of uterine location in Low, Medium, and High bacterial contamination groups was represented in pelvic cavity.

Table (3): Reproductive performance in Low, Medium and High bacterial contamination groups.

Groups	No	Duration of lochia (days)	Day of first estrus	services per conception
low bacterial contamination	15	12.60 ± 0.36 [a]	52.93±1.93 [a]	1.67± 0.19 [a]
Medium bacterial contamination	15	13.53 ± 0.35 [a]	51.07± 2.00 [a]	1.93± 0.15 [a]
High bacterial contamination	14	17.71 ± 0.49 [b]	63.64± 5.63 [b]	3.14± 0.21 [b]

The different superscript letters mean significantly differed at P < 0.05.

Table (4): Percentage of abdominal and pelvic location of uterus at P.P period in Low, Medium and High bacterial contamination groups

Groups	No	Postpartum uterine location					
		3rd day		15th day		30th day	
		Abdominal	Pelvic	Abdominal	Pelvic	Abdominal	Pelvic
Low bacterial contamination	15	15 (100%)	0 (0%)	3 (20%)	12 (80%)	0 (0%)	15 (100%)
Medium bacterial contamination	15	15 (100%)	0 (0%)	7 (46.70%)	8 (53.30%)	0 (0%)	15 (100%)
High bacterial contamination	14	14 (100%)	0 (0%)	7 (50%)	7 (50%)	0 (0%)	14 (100%)

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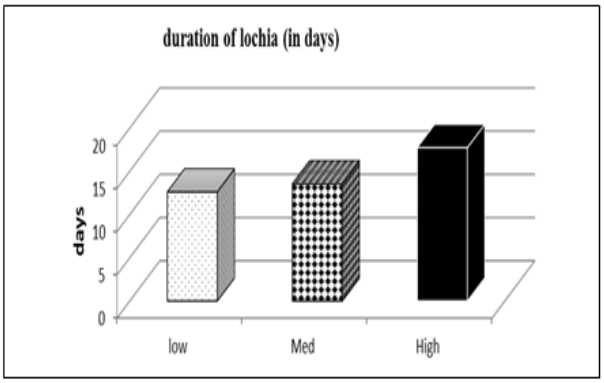

Fig. (2): Duration of lochia (in days) in Low, Medium and High bacterial contamination groups.

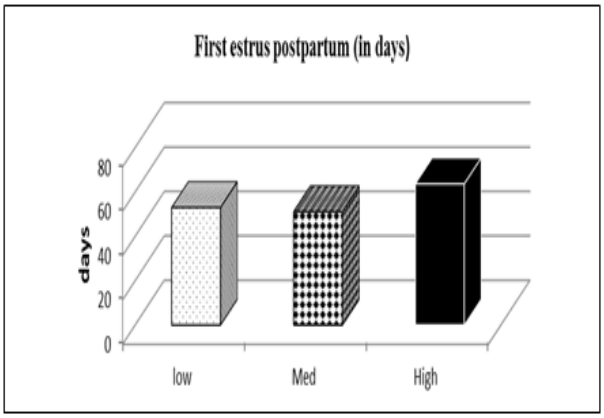

Fig. (3): First estrus postpartum (in days) in Low, Medium and High bacterial contamination groups.

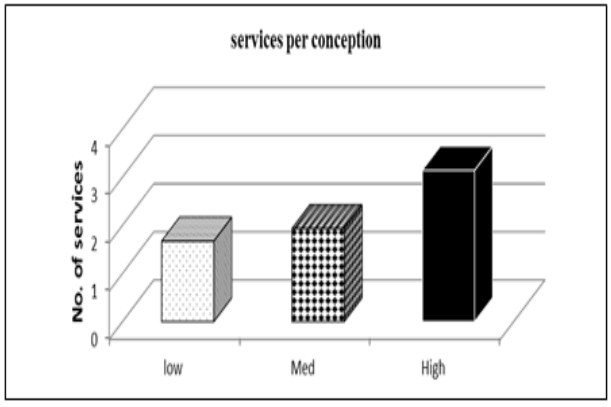

Fig. (4): Number of services per conception in Low, Medium and High bacterial contamination groups.

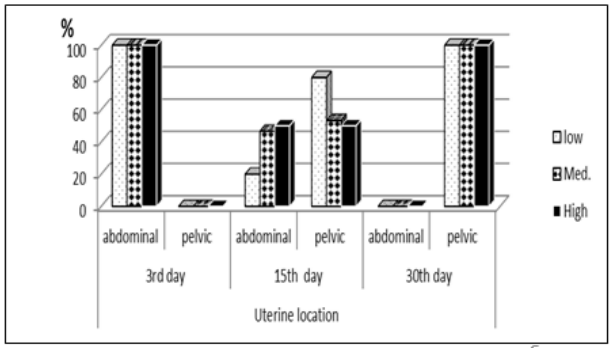

Fig. (5): Percentage of abdominal and pelvic location of uterus at P.P period in Low, Medium and High bacterial contamination groups.

46.67% of cows in Low bacterial contamination group were conceived from 1st service versus 20.00% in Medium bacterial contamination group. However, 40.00% of cows in Low bacterial contamination group, 66.67% in Medium bacterial contamination group, and 21.43% in High bacterial contamination group were conceived from 2nd service. It was observed that 13.33% of cows in Low and Medium bacterial contamination groups were conceived from 3rd service versus 42.86% in High bacterial contamination group. It was also observed that 35.71% of cows in High bacterial contamination group were conceived from 4th service. Moreover, data obtained in Table (6) and Fig. (7) revealed that within 60 days postpartum, the percentage of conceived cows was higher in Low bacterial contamination group (40%) than in Medium bacterial contamination group (20%), and there was no conception in High bacterial contamination group in this period. However, within 61-90 days postpartum, the percentage of conceived cows were (40%) in Low bacterial contamination group, (53.33%) in Medium bacterial contamination group, versus (7.14%) in High bacterial contamination group. Moreover, about (20%) of Low bacterial contamination group, (26.67%) of Medium bacterial contamination group, and (92.86%) of High bacterial contamination group required more than 90 days postpartum to be conceived.

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Table (5): Conception rate of cows in low, medium and high uterine bacterial contamination groups.

Groups	No	Conception rate %			
		1st service	2nd service	3rd service	4th service
Low bacterial contamination	15	7(46.67%)	6(40%)	2(13.33%)	0(0%)
Medium bacterial contamination	15	3(20%)	10(66.67%)	2(13.33%)	0(0%)
High bacterial contamination	14	0(0%)	3(21.43%)	6(42.86%)	5(35.71%)

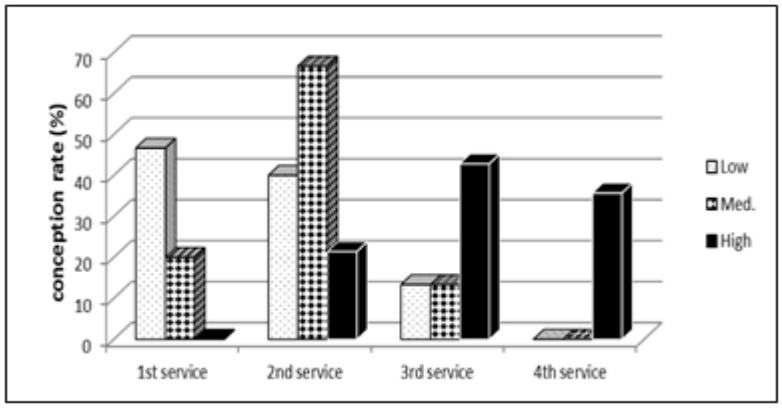

Fig. (6): Conception rate (%) of cows in low, medium and high uterine bacterial contamination groups.

Table (6): Number and percentage of conceived cows within different postpartum periods in Low, Medium and High bacterial contamination groups.

Groups	No	Number and percentage of conceived cows		
		Within 60 days	Within 61 - 90 days	<90 days
Low bacterial contamination	15	6 (40%)	6 (40%)	3 (20%)
Medium bacterial contamination	15	3 (20%)	8 (53.33%)	4 (26.67%)
High bacterial contamination	14	0 (0%)	1 (7.14%)	13 (92.86%)

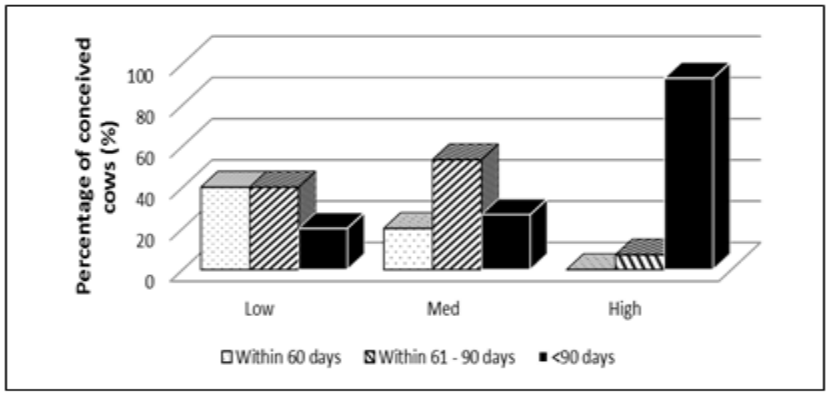

Fig. (7): Percentage of conceived cows within different postpartum periods in Low, Medium and High bacterial contamination groups.

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DISCUSSION

The reproductive performance of the breeding herd is critical due to the operation's ability to produce income. Income generation is affected not only by the absolute number of females that conceive but also by the timing and distribution of the pregnancies (Engelken, Trejo, & Voss, 2007)**.** In the present study, mean uterine bacterial score was decreased by the time in 3rd, 15th and 30th day postpartum for low bacterial contamination group (5.73, 2.80, and 1.20 respectively), medium bacterial contamination group (7.80, 2.73, and 1.47 respectively), and high bacterial contamination group (9.29, 6.57 and 2.21 respectively). Decreasing of the bacterial score by the time in our results was supported by (I. Sheldon, Noakes, Rycroft, Pfeiffer, & Dobson, 2002) who reported that the ranges of total bacterial growth scores were 0–15, 0–12, 0–10, and 0–9 for days 7, 14, 21, and 28, respectively. The postpartum period is considered as a non-infectious event. The reduction in uterine size and the unidirectional flow of uterine contents, as well as the gradual closure of the cervix, prevent microbial contamination. However, the reality is that the uterus invasion by microorganisms to a variable extent is depending on the animals susceptibility and the hygienic condition of the environment (Gustafsson et al., 2004). Also during the early postpartum period, multiple bacterial species invade the uterus of cows (Rahim Ahmadi et al., 2006). A normal postpartum cow resolves uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defenses including mucus, antibodies, and phagocytic cells (Azawi, 2008). Series of studies confirmed that clinical and reproductive consequences are associated with these primary uterine pathogenic bacteria (Foeldi et al., 2008).

Effect of postpartum bacterial contamination on reproductive performance in dairy cows, duration of lochia**,** and the greatest flow of lochia occurs during the first 2–3 days; by 8 days it is reduced, and virtually disappeared by 14–18 days postpartum (**Noakes *et al*., 2001).** Data revealed that duration of lochia was significantly shorter ($P < 0.05$) in low and Medium groups than that reported in high bacterial contamination group. First estrus postpartum is the time between the date of calving to the date of first subsequent estrous, this period is required for resumption of ovarian activity and uterine involution. It refers to the reproductive efficiency of an individual because the shortest the post-partum heat period the highest the calf production in their lifespan (Habib et al., 2010).

Our finding revealed that the elapsed time from parturition till the appearance of first estrus was significantly ($P < 0.05$) shorter in Medium and Low than that found in High bacterial contamination group. The results were confirmed by (Ahmed & Elsheikh, 2013) who stated that the sever postpartum uterine bacterial infection in dairy cows significantly extended the time taken for the resumption of the first estrus compared to the dairy cows suffered mild postpartum uterine bacterial infection. In addition, (I. Sheldon et al., 2002) observed that uterine bacterial infection, bacterial products toxins or the associated uterine inflammation may suppress pituitary LH secretion and disrupt postpartum ovarian follicular growth and function. In the same line, (Ahmed & Elsheikh, 2013) stated that a severe postpartum uterine bacterial infection in dairy cows significantly delayed the time taken for the appearance of the first dominant follicle as compared to dairy cows suffered mild postpartum uterine bacterial infection.

The study revealed that the number of services per conception was significantly ($P < 0.05$) lower in low bacterial contamination group and Medium bacterial contamination group than that found in high bacterial contamination group. These results came in the same line with (Ahmed & Elsheikh, 2013) who reported that

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the mean rate of service per conception for the dairy cows with sever postpartum uterine bacterial infection was significantly higher than that of the dairy cows with a mild uterine bacterial infection. Number of services per conception (all cows) is a better index for evaluating conception in the herd because it includes all inseminations, whether the cow finally became pregnant or not (Fodor & Ózsvári, 2015) Uterine location and involution as measured by the time taken to reach the uterus to non- pregnant size, position and consistency was completed by day 24.1 ± 4.7 (range - 18 to 34 days). (Abeywansa, Abeygunawardena, & Jayatilaka, 1991) results agree with our finding which revealed that at 3rd day uterine location for each cow in all groups was represented in abdominal cavity while its location at 30th day was represented in non-pregnant position in pelvic cavity. Also (Saut et al., 2011) reported that at 3rd day postpartum, uterine location for each cow was represented in abdominal cavity, and 58% of the cows evaluated by rectal palpation was located within the abdominal cavity at 14^{th} day pp while at 28^{th} day pp the uterus of all cows was within the pelvic cavity. Also, our results at 15^{th} day postpartum indicated that the uterine location in 80 %, 53.30%, and 50 % of cows in Low, med, and High bacterial contamination groups were represented in pelvic cavity, respectively. These results indicate that high bacterial growth density delay uterine involution, and delaying involution by the effect of bacteria was supported by (Dobson-Hill, 2009) who reported that most cows are able to eliminate bacterial contamination during puerperium. However, 10 to 17% of cows are unable to do this. In these cows, the bacteria persist, cause infection and inflammation, and delay uterine involution.

Conception rate represents the ratio of the number of conceptions to the number of services and is expressed as a percentage (Habib et al., 2010) Reproductive efficiency can be increased in farm animals by decreasing the interval from parturition to conception (Wettemann, 1980). Data revealed that at first service, 46.67% of cows in Low bacterial contamination group were conceived versus 20 % in Medium bacterial contamination group while there was no conception for High bacterial contamination group. Also, it was observed that by third service all animal in Low bacterial contamination group and Medium bacterial contamination group were conceived, while by the fourth service all animal in High bacterial contamination group were conceived.Also, results revealed that within 60 days postpartum the percentage of conceived cows was higher in Low bacterial contamination group than in Medium bacterial contamination group. At the day 90 postpartum, most of the animals in Low bacterial contamination group (80%) and Medium bacterial contamination group (73.33%) were conceived versus only 7.14% in High bacterial contamination group, and the highest percentage of these animals (92.86%) need more than 90 days to conceive. This finding agreed with (I. M. Sheldon & Dobson, 2004) who stated that In cattle, postpartum contamination of the uterine lumen is ubiquitous, and persistence of pathogenic bacteria commonly causes clinical disease. The consequences are subfertility associated with delayed ovulation after parturition, a persistence of the corpus luteum once it forms, and lower conception rates. Also, (Gitonga, 2010) recorded that management and biological factors that may prolong the calving to conception interval are including; delayed resumption of postpartum ovarian activity, poor heat manifestation or detection, and presence of postpartum problems.

It was concluded that there was a relation between the high contaminations without postpartum clinical signs appearance and reproductive performance, so farms need more hygienic conditions or methods of treatment to maintain dairy farms economically viable.

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التلوث البكتيريّ الرحميّ بعد الولادة دون علامات سريرية وعلاقتها بالأداء التناسلي في الأبقار الحلوب

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المستخلص: أجريت الدراسة لمعرفة العلاقة بين الملوثات الجرثومية الرحمية دون وجود علامات سريرية والأداء التناسلي بعد الولادة (PP) في الأبقار الحلوب. تم أخذ مسحات رحميّة من الأبقار بعد الولادة (ن = 44) في اليوم الثالث والخامس عشر والثلاثين، وتم تطبيق عدد البكتيريا والنتيجة. تم تقسيم الحيوانات حسب التلوث البكتيريّ الرحميّ إلى منخفض ومتوسط وعالٍ (15، 15، و14 بقرة لكل مجموعة على التوالي) وفقاً لدرجة التقييم. أظهرت النتائج أن درجة البكتيريا الرحمية (UBS) انخفضت بحلول الوقت في اليوم الثالث والخامس عشر والثلاثين (PP) لمجموعة التلوث الجرثومي المنخفض (5.73، 2.80 و 1.20 على التوالي)، لمجموعة التلوث المتوسطة (7.80، 2.73 و 1.47على التوالي) وللمجموعة عالية التلوث (9.29 ، 6.57 و 2.21 على التوالي). كما أظهرت النتائج زيادة معنوية ($P < 0.05$) في مدة نزول النفاسة lochia في مجموعات التلوث الجرثومي العالية والمنخفضة والمتوسطة في اليوم الثالث (PP) وكان موقع الرحم (الانكماش الرحميّ) في جميع الأبقار في تجويف البطن. ولكن في اليوم الخامس عشر (PP) تأخر انكماش الرحم (%) للوصول إلى وضعه الطبيعي ما قبل الحمل في تجويف الحوض في مجموعة عالية (50 ٪) والمنخفضة (80 ٪) والمتوسطة (53.30 ٪) مجموعات UBS. لكن في اليوم الثلاثين لوحظ موقع الرحم في جميع الأبقار في تجويف الحوض. كان أول شبق (PP) أقصر بكثير في مجموعتين UBS المنخفضة والمتوسطة عنه في المجموعة عالية UBS. ووجد أن عدد التلقيحات لكل حمل به زيادة معنوية في مجموعة عالية UBS عنه في مجموعتين منخفضة ومتوسطة UBS. في اليوم 90 (PP) كان معدل الحمل أقل في مجموعة UBS عالية من مجموعة UBS المنخفضة ومجموعة متوسطة UBS. نستنتج من هذه الدراسة، وجود علاقة بين التلوث الجرثومي الرحمي بعد الولادة دون علامات سريرية وخاصة التلوث العالي وتأثيره على الأداء التناسلي في الأبقار.

الكلمات المفتاحية: رحم – بكتيريا – فترة بعد الولادة – الأداء التناسلي – أبقار.

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Prevalence of Pulp Stones in Libyan Subpopulation: A Panoramic Radiographic Study

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Abstract: The aims of this study were to evaluate the prevalence of pulp stones in permanent posterior teeth of a group of adult Libyan dental patients using digital panoramic radiographs. Materials and Methods: Panoramic radiographs of 1200 adult patients (600 females and 600 males) were examined retrospectively to determine the prevalence and distribution of the pulp stones. All posterior teeth were investigated except third molars, and the data obtained were recorded as present or absent according to gender, tooth types, dental arches, sides and dental status (intact, restored, or carious). The Chi-square test was used for difference comparisons ($P<0.05$). Results: Of the 1200 patients, 363 (30.2%) had one or more teeth that contained pulp stones. Pulp stones were detected in 1402 of the 16738 teeth, to give a tooth prevalence of 8.4%. Pulp stones were discovered in 9.2% of females and 7.5% of males, with a highly significant difference between the genders ($P<0.0001$). Pulp stones were more prevalent in the maxillary arch (10.3%) than in the mandibular arch (6.5%), with a significant difference between arches. Pulp stones were most frequently occurring in right maxillary first molars (24.6%) and least occurring in left maxillary first premolars (0.1%). Pulp stones prevalence was significantly more common in the first molars than second molars, in the molars than premolars in each dental arch, and in the first maxillary molar than first mandibular molars. Pulp stones were detected more frequently in the intact teeth than in carious and restored teeth. Conclusion: The occurrence of pulp stones in this study was significantly higher in females than males, in maxillary teeth than in mandibular teeth, molars than premolars, first maxillary molars than mandibular molars and in intact teeth than restored and carious teeth.

Keywords: Panoramic radiographs; Pulp stone; Prevalence; Libyan subpopulation.

INTRODUCTION

Pulp stones are calcified discrete masses that occur in the dental pulp. They are found in healthy, diseased, and even unerupted teeth (Arys et al., 1993; Moss and Hendricks, 1988; Sener et al., 2009; Tamse et al., 1982). Pulp stones can be found in both primary and permanent teeth (Sisman et al., 2012). Pulp stones may be observed in the coronal part of the pulp or may be even in the radicular pulp, as free, attached, or embedded calcified bodies in the dentine (Colak et al., 2012). According to their structure, pulp stones are classified as true, false, and diffuse. The size of pulp stones range from small microscopic particles to large masses that almost obliterate the pulp chamber (Johnson et al., 1956).

The aetiology of pulp stone is not exactly known, several factors have been involved in pulp stones formation like caries, deep restoration (Goga et al., 2008), chronic Inflammation, interaction between pulp tissue

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and, epithelium (Moss and Klyvert, 1983), circulatory disturbance in pulp (Sundell et al., 1968), age (Hillmann and Geurtsen., 1997), genetic predisposition (VanDenBerghe et al., 1999), and orthodontic tooth movement (Stenvik and Mjör, 1970). They are usually identified during radiographic examination as radiopaque areas of variable sizes, number, and shapes (Langeland et al., 1974). Pulp stones observed on the radiographs as a definite round or ovoid radiopacities within the coronal or radicular portion of the pulp or they may extend from the pulp chamber into the root canals.

Pulp stones occur in all tooth types but most frequently in molars (Colak et al., 2012; Goga et al., 2008; Ranjitkar et al., 2002; Sener et al., 2009; Sisman et al., 2012; Tamse et al., 1982; White and Pharoah, 2009). Previous studies reported that the prevalence of pulp stone based on radiographic examination various percentages ranging from 8–90% depending on the study type, design, and radiographic technique used (Arys et al., 1993). Histological method of evaluation is reported to yield higher values than radiographic method (Ranjitkar et al., 2002).

Pulp stones can complicate endodontic treatment by obstructing access to root canals and their subsequent cleaning and shaping (Ibarrola et al., 1997). The aims of this study were to evaluate the prevalence of pulp stones in the permanent posterior teeth of a group of adult Libyan dental patients using digital panoramic radiographs and to determine possible associations between pulp stones and gender, tooth type, dental arches, side, and dental status, also to compare the results with published data. This will provide information to the dental practitioner about the types of teeth which are more likely to reveal technical difficulties associated with the endodontic treatment of such teeth.

MATERIALS AND METHODS

The study was granted the approval by Elaml dental center at Benghazi, Libya. In this retrospective cross-sectional study, a total of 1200 Digital panoramic radiographs (OPG) were randomly selected from the records of dental patients who attended the Elaml dental center at Benghazi, Libya for routine dental treatment. Digital panoramic radiographs were taken by using Owandy digital x-ray unit system machine (Italy). This study composed of 600 males and 600 females. A total of 16738 teeth were studied. Digital panoramic radiographs were examined by one examiner. The radiographs of children and patients with mixed dentition and Teeth with crown or bridge which will obstruct the proper evaluation of pulp were excluded. To evaluate pulp stones, radiographs were digitalized and then pulp chambers were examined by one examiner. Molars and premolars in the maxillary and mandibular arches in both sides were examined for the absence or presence of pulp stones, except for third molars. Definite radiopaque bodies observed inside the pulp chambers were described as pulp stones and were scored as present or absent according to genders in both arches, on the left and right side, and according to tooth type in both arches (Figure 1). The dental status of each tooth was categorized as intact, restored and carious. The shape, size and number of pulp stones were not evaluated. The examiner was calibrated by reading 50 of radiographs containing different cases of pulp stones before the investigation began. To check the reliability of the radiographic examination**,** a sample of 100 radiographs was re-examined by the same examiner two weeks later, and an agreement of 100% was obtained. Statistical analyses were performed using (SPSS 18.0, Chicago, USA) The Chi-square test was used to compare the frequency of pulp stone occurrence between genders, tooth types, dental arches and sides. Differences were considered as significant when $P < 0.05$.

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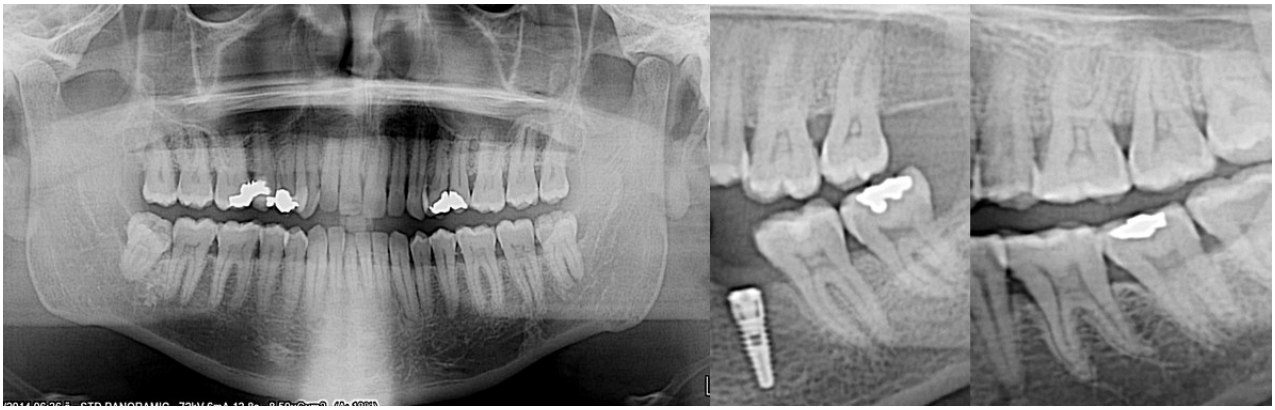

Figure (1): Panoramic radiographs showing radiopaque pulp stones in the pulp chamber of molars

RESULTS

This study consists of 1200 patients (600 females and 600 males). The distribution of patients and teeth having pulp stones according to gender is shown in (Table 1). Of the 1200 patients, 363 (30.2%) had one or more teeth that contained pulp stones, which comprised of 199 (33.2%) females and 164 (27.3%) males with a significant difference between genders P<0.05. Pulp stones were detected in 1402 of the 16738 teeth, to give a tooth prevalence of 8.4%. Pulp stones were detected in 9.2% (777/8410) of teeth in females and in 7.5% (625/8328) of teeth in males, with a highly significant difference between the genders (P<0.0001).

The distribution of pulp stone according to dental arches and genders is represented in (Table 2). Pulp stones were detected in 10.3% (847/8183) of teeth examined in the maxilla, and in 6.5% (555/8555) of teeth examined in the mandible with a significant difference between arches (P<0.05). The prevalence of pulp stones in the maxilla was higher in females 11.8% (488/4123) than in males 8.8% (359/4060). Also, its prevalence in the mandible was higher in females 6.7% (289/4287) than in males 6.2% (266/4268) with a highly significant difference between arches and genders (P<0.0001).

Table (1): Distribution and frequency of pulp stone according to genders.

Pattern	Female (%)	Male (%)	Total (%)	P
Patients with pulp stone	199(33.2%)	164 (27.3%)	363 (30.2%)	
Patients without pulp stone	401(66.8%)	436 (72.7%)	837 (69.8%)	0.02
Total patients	600(50%)	600 (50%)	1200	
Teeth with pulp stone	777 (9.2%)	625 (7.5%)	1402 (8.4%)	
Teeth without pulp stone	7633(90.8%)	7703 (92.5%)	15336 (91.6%)	0.000
Total teeth	8410(50.2%)	8328 (49.8%)	16738	

Table (2): The distribution of pulp stone according to dental arches and genders.

Location	Female		Male		Total		
	No. of teeth examined	No. of teeth with pulp stones	No. of teeth examined	No. of teeth with pulp stones	No. of teeth examined	No. of teeth with pulp stones	P
Maxilla	4123	488 (11.8%)	4060	359 (8.8%)	8183	847 (10.3%)	
Mandible	4287	289 (6.7%)	4268	266 (6.2%)	8555	555 (6.5%)	
Total	8410	777 (9.2%)	8328	625 (7.5%)	16738	1402 (8.4%)	

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According to the arch side, the statistic study shows that pulp stones were more prevalent on the right side 8.6% (717/ 8384) than on the left side 8.2% (685/8354). No significant difference was found between the right and the left side (P=0.4, >0.05) (Table 3).

According to tooth type, the statistic study shows that pulp stones were most frequently detected in right maxillary first molars (24.6%) and least detected in left maxillary first premolars (0.1%) (Table3).

Pulp stones were detected in 1.4% (118/8587) of the premolars, and in 15.8% (1284/8151) of the molars teeth examined.The prevalence of pulp stones in the molars was significantly higher than that in premolars (P<0.0001). The occurrence of pulp stones was higher in the first molars 19.6 % (751/3833) than in the second molars 12.3 %(533/4318) with a highly significant difference ($P < 0.0001$). The prevalence of pulp stones was higher in the second premolars 1.9 %(81/4192) than in the first premolars 0.8 % (37/4395) in each dental arch with a highly significant difference ($P < 0.0001$) (Table 4).

The prevalence of pulp stones was higher in the maxillary molars (first molars 23.9% and second molars 15.8%) than in the mandibular molars (first molars 15.04% and second molars8.9%) in all arch sides and both genders with highly significant difference (P < 0.0001). However, in premolar teeth, the prevalence of pulp stones was higher in the mandibular premolars (first premolars 1.4% and second Premolars 2.3%) than in the maxillary premolars (first premolars 0.2% and second premolars 1.5%) in all arch sides and both genders with a highly significant difference (P < 0.0001) (Table 5). Regarding the dental status, the teeth with pulp stones were examined for intact, restored, and carious. 618(44.1%) of intact (sound) teeth, 271(19.3%) of restored, and 513 (36.6%) of carious teeth had pulp stone (Table 6). The results are presented in figure 2.

Table (3): Distribution and frequency of pulp stone according to arches, sides and tooth types.

	Right side		Left side		P
	No. of teeth examined	No. of teeth with pulp stone	No. of teeth examined	No .of teeth with pulp stone	
Maxilla					
First premolar	1041	3 (0.3%)	1025	1 (0.1%)	
Second premolar	989	14 (1.4%)	987	16 (1.6%)	
First molar	972	239 (24.6%)	993	231 (23.3%)	
Second molar	1087	172 (15.8%)	1089	171 (15.7%)	
Mandible					
First premolar	1168	19 (1.6%)	1161	14 (1.2%)	
Second premolar	1113	30 (2.7%)	1103	21 (1.9%)	
First molar	963	142 (15.2%)	932	139 (14.9%)	
Second molar	1078	98 (9.1%)	1064	92 (8.6%)	
Total	8384	717(8.6%)	8354	685 (8.2%)	0.4

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Table (4): Pulp stone distribution according to tooth type for both dental arches.

	No. of teeth examined	No. of teeth with pulp stone	P
First premolar	4395	37 (0.8%)	
Second premolar	4192	81(1.9%)	0.000
Total premolars	8587	118 (1.4%)	
First molar	3833	751 (19.6%)	
Second molar	4318	533 (12.3%)	0.000
Total Molars	8151	1284 (15.8%)	

Table (5): The distribution of pulp stone according to dental arches, genders, and location.

Location	Female		Male		
	Right (%)	Left (%)	Right (%)	Left (%)	Total (%)
Maxilla					
First premolar	1(.2%)	1(.2%)	2 (0.4%)	0 (.0%)	4 (0.2%)
Second premolar	8 (1.6%)	8 (1.6%)	6 (1.2%)	8 (1.6%)	30 (1.5%)
First molar	131(26.8%)	140 (27.7%)	108 (22.4%)	91(18.7%)	470 (23.9 %)
Second molar	103 (18.3%)	96 (17.2%)	69 (13.1%)	75 (14.1%)	343 (15.8 %)
Mandible					
First premolar	11 (1.9%)	7 (1.2%)	8 (1.4%)	7 (1.2%)	33 (1.4%)
Second premolar	16 (2.9%)	10 (1.8%)	14 (2.5%)	11(2.0%)	51(2.3%)
First molar	71 (15.4%)	67 (14.4%)	71 (14.9%)	72 (15.4%)	281(15.04%)
Second molar	57 (10.4%)	50 (9.2%)	41 (7.8%)	42 (8.0%)	190 (8.9%)

Table (6): Prevalence of pulp stones in teeth with different crown status.

Number of intact teeth with pulp stone	Number of restored teeth with Pulp stone	Number of Caries teeth with pulp stone
618 (44.1%)	271 (19.3%)	513 (36.6%)

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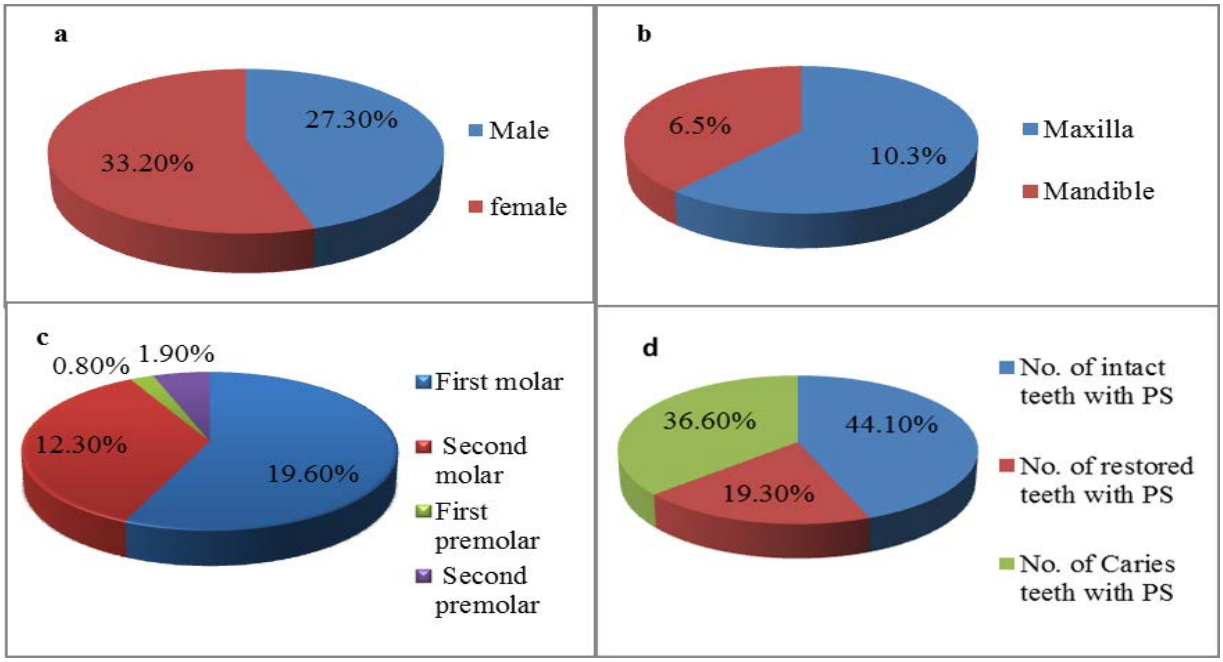

Figure (2): Distribution and frequency of pulp stones according to (a) Genders. (b) Arches. (c) Tooth type of both arches (d) Dental status.

DISCUSSION

Previous studies have evaluated the prevalence of pulp stones using radiography. However, the true prevalence is likely to be higher because pulp stones with a diameter less than 200 µm cannot be detected on radiographs (Goga et al., 2008; Sener et al., 2009). To assess the prevalence of pulp stones, (Baghdady et al., 1988; Colak et al., 2012) used bitewing radiographs, (Al-Hadi Hamasha and Darwazeh, 1998; Ravanshad et al., 2015) used periapical and bitewing radiographs (Al-Ghurabi and Najm, 2012; Kalaji et al., 2017; Turkal et al., 2013) used panoramic radiographs in their study. Panoramic radiographs can show all teeth on both arches by using one radiograph, and panoramic images are excellent screening for pulpal calcifications as all teeth can be assessed using the same image (Bains et al., 2014; Nayak et al., 2010).

Furthermore, digital panoramic images may be evaluated using enhancing software that enhances detection. This study evaluated the pulp stone with a digital panoramic radiograph so that posterior teeth involved with calcification in both the maxilla and mandible can be detected. Recently, cone-beam computed tomography (CBCT) has been used to evaluate the prevalence of pulp stones, this technique provides accurate anatomical details in three dimensions offering the possibility to view an individual tooth in axial, sagittal and coronal views (Da Silva et al.,2017; Hsieh et al., 2018; Patil et al., 2018; Rodrigues et al., 2014). The incidence of pulp stones has been reported to be from 8% to 95% in the permanent dentition (Gulsahi et al., 2009; Sisman et al., 2012; Tamse et al., 1982; Turkal et al., 2013).

The result of this study on a group of Libyan dental patients has shown an overall prevalence of 30.2% for individuals and 8.4% for all examined teeth. This prevalence was higher than the results of previous studies by (Gulsahi et al., 2009) 5% and by (Turkal et al., 2013) 2.1% in Turkish population, (Kalaji et al., 2017) 3.99% in Yemeni population, (Al-Ghurabi and Najm, 2012) 7.3% in Iraqi population and less than the studies by (Renjitker et al., 2002) 10.3% in Australian population, (Al-Hadi Hamasha and

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Darwazeh,1998) 22% in Jordanians, (Baghdady et al., 1988) 14.8% in Iraqi population, (Al-Nazhan and Al-Shammrani , 1991) 10.2% in Saudi population, (Kannan et al.,2015) 15.7% in Malaysian population, (Bains et al.,2014) 9.09% in Indian population, and (Ravanshad et al.,2015) 11.25% in Iranians population. These differences in prevalence in different populations and different geographic areas may be attributed to the variation of conditions related to the studied population, such as ethnicity, dental care, and dental habits. The differences may be also due to the differences in sample size and in the methods used.

In the present study, the prevalence of occurrence of pulp stones was higher in females than males in each tooth type and both arches, and these differences were statistically highly significant ($P < 0.0001$). This finding is in agreement with other studies previously reported by (Bains et al., 2014; Colak et al., 2012; Kannan et al., 2015; Ravanshad et al., 2015; Sisman et al., 2012; Tamse et al., 1982; Turkal et al., 2013). However, some studies have shown that pulp stones were more prevalent in males than in females, and other studies have reported no significant differences between genders (Al-Nazhan and Al-Shammrani, 1991; Baghdady et al., 1988; Kalaji et al., 2017; Ranjitkar et al., 2002). A possible explanation for this difference may be attributed to the bruxism which is more prevalent in females. Bruxism causes longstanding irritation on the dentition (Sener et al., 2009; Sisman et al., 2012).

The prevalence of pulp stones occurrence in this study was significantly higher in the maxillary arch than in the mandibular arch ($P<0.0001$). Pulp stones were detected in 10. 3% of the examined maxillary teeth and 6.5% of mandibular examined teeth. This finding is consistent with other studies previously reported (Kalaji et al., 2017; Ranjitkar et al., 2002; Sisman et al., 2012; Turkal et al., 2013). Although in other studies, significant differences were not detected between arches (Al-Ghurabi and Najm, 2012; Al-Hadi Hamasha and Darwazeh, 1998; Kannan et al., 2015; Patil et al., 2018).

In this study, the pulp stones were more slightly detected on the right side than on the left side, but there were no significant differences between sides ($P=0.4$, >0.05). These findings were in compliance with other studies previously reported on the Turkish population (Colak et al., 2012) and the Saudi population (Patil et al., 2018). According to the findings of other studies, significant differences were detected between sides on Yemeni population (Kalaji et al., 2017) and on Turkish population (Turkal et al., 2013). However, another study by (Sisman et al., 2012) on Turkish population, and a study by (Ranjitkar et al., 2002) on Australian population reported that the prevalence of pulp stones occurrence on the left side was higher than that on the right side.

In both genders, in all dental arches and sides, the prevalence of pulp stone occurrence in molar teeth was significantly higher than that in premolar teeth ($P<0.0001$). The maxillary first molar teeth exhibited the highest occurrence of pulp stone (23.9%). This finding is in agreement with other previous studies (Ranjitkar et al., 2002; Sisman et al., 2012; Tamse et al., 1982) but according to another study, the prevalence of pulp stones occurrence is to be more in the mandibular first molar teeth (Al-Hadi Hamasha and Darwazeh, 1998).

The prevalence of pulp stones occurrence was significantly higher ($P<0.0001$) in the first molar teeth (19.6%) than in the second molar teeth (12.3 %) in all dental arches, sides, and in both genders. This finding is consistent with previous studies (Al-Hadi Hamasha and Darwazeh, 1998; Baghdady et al., 1988; Colak et al., 2012; Gulsahi et al., 2009; Kalaji et al., 2017; Kannan et al., 2015; Patil et al., 2018; Ranjitkar et al., 2002). A possible explanation of this result is that the early eruption time of

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the first molar teeth will expose them to more irritants or degenerative changes, therefore, confirming that pulp calcification increases with age. Another proposed factor is that the molar teeth are the largest teeth in the dental arches, and their pulp tissues possess rich blood supply which may cause the precipitation of calcium in the pulp chamber and bear the strongest mastication forces in the arch. This may lead to greater precipitation of calcification; (Al-Ghurabi and Najm, 2012; Al-Hadi Hamasha and Darwazeh, 1998; Kalaji et al., 2017; Sisman et al., 2012).

In both genders, in all dental arches and sides, the prevalence of pulp stones occurrence in second premolar teeth was significantly higher than that in the first premolar teeth ($P<0.0001$). However, other previous studies reported that the prevalence of pulp stones is more frequent in the first premolar teeth than in second premolar teeth (Al-Ghurabi and Najm, 2012; Gulsahi et al., 2009; Sisman et al., 2012).

The incidence of pulp stones was comparatively high in the intact teeth (44.1%) when compared to the carious (36.6%) and restored teeth (19.3%), Although these findings disagree with other studies (Baghdady et al., 1988; Kannan et al., 2015; Patil et al., 2018; Ranjitkar et al., 2002; Ravanshad et al., 2015; Sener et al., 2009) that reported restored and carious teeth exhibited higher prevalence of pulp stone than intact molar teeth. Some researchers did not notice any significant differences between prevalence of pulp stones in intact or non-intact teeth (Baghdady et al., 1988; Gulsahi et al., 2009; Ravanshad et al., 2015; Tamse et al., 1982).

The exact aetiology of pulp stones formation is still unclear; many controversies exist regarding its aetiological factors. Previous studies have reported that pulp calcifications are more common in patients with cardiovascular diseases (Edds et al., 2005; Nayak et al., 2010). Another study demonstrated the association between coronary atherosclerosis and pulp calcifications (Maranhao et al., 1987). In contrast, Horsley et al., 2009 reported that there is no significant correlation between carotid calcification and pulp stones. Clinically pulp stones have no significance other than possibly causing difficulties during endodontic treatments, such as obstructing canal location and negotiation (Gutmann, 1997).

The present study has some limitations. The limited sample size which included only adolescents and the samples were obtained from only one center. Incidence of pulp stones in anterior teeth and detailed configurations of pulp stones were not assessed in this study. Age-related changes and the relationship between systemic disorders and pulp calcifications were also not evaluated.

CONCLUSION

The occurrence of pulp stones in this study may provide additional information regarding the dental morphological features of Libyan adults. The occurrence of pulp stones in this study was significantly higher in females than males, in maxillary teeth than in mandibular teeth, in first molars compared with other posterior teeth, first maxillary molars than mandibular molars, and in intact teeth than restored and carious teeth.

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انتشار حصاة اللبية في عينة من الليبيين: دراسة إشعاعية بانورامية

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المستخلص: هدف هذه الدراسة هو تقييم مدى انتشار حصاة اللبية في الأسنان الخلفية الدائمة لمجموعة من مرضى الأسنان الليبيين البالغين باستخدام صور الأشعة البانورامية الرقمية. المواد والطرق: تم فحص صور الأشعة البانورامية من 1200 مريض بالغ (600 أنثى و600 ذكر) لتحديد انتشار وتوزيع حصاة اللبية. تم فحص جميع الأسنان الخلفية فيما عدا الأضراس الثالثة، وتم تسجيل البيانات التي تم الحصول عليها على أنها موجودة أو غير موجودة وفقاً للأجناس، أنواع الأسنان، فك الأسنان العلوي والسفلي، جوانب الفك وحالة الأسنان (سليمة، منخورة، أو مرممة). تم استخدام اختبار كا^2 لمقارنة الفروق. النتائج: من بين 1200 مريض، كان 363 (30.2٪) أسنان واحدة أو أكثر تحتوي على الحصاة اللبية تم اكتشاف الحصاة اللبية في 1402 سن من أصل 16738سن بنسبة 8.4 ٪، وأظهرت النتائج انتشار الحصاة اللبية في أسنان الإناث بنسبة 9.2 ٪ و7.5 ٪ في الذكور مع فرق كبير للغاية بين الجنسين. كانت الحصاة اللبية أكثر انتشارًا في الفك العلوي (10.3٪) عن الفك السفلي (6.5٪) مع اختلاف كبير بين الفكين. كانت الحصاة اللبية أكثر انتشارا في الأضراس الأولى في الفك العلوي الأيمن بنسبة (24.6 ٪) وأقل نسبة في الضواحك الأولى في الفك العلوي الأيسر (0.1 ٪). كانت نسبة انتشار الحصاة اللبية أكثر شيوعا في الأضراس الأولى من الأضراس الثانية وفي الأضراس أكثر من الضواحك في كل فك وفي الأضراس الأولى للفك العلوي أكثر من الأضراس الأولى للفك السفلي. ونسبة انتشار الحصاة اللبية في الأسنان السليمة أكثر من الأسنان المنخورة والمرممة. الخلاصة: كان ظهور الحصاة اللبية في هذه الدراسة أعلى بكثير في الإناث من الذكور، في أسنان الفك العلوي من أسنان الفك السفلي، الأضراس من الضواحك، الأضراس الأولى للفك العلوي من أضراس الفك السفلي والأسنان السليمة من الأسنان المنخورة والمرممة.

الكلمات المفتاحية: صور الأشعة البانورامية، الحصاة اللبية، انتشار، الليبيون.

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Effect of Intraperitoneal Instillation of Bupivacaine on the Pain Scores post operation of Laparoscopic Cholecystectomy

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Abstract: Laparoscopic cholecystectomy is now an established form of treatment for patients with symptomatic gall stones as it is an excellent mean to minimize the trauma and agony of the patient following surgery, although recent studies have shown that patients still experience considerable pain after this surgery. In our current study, we aim to assess the effectiveness of intraperitoneal instillation of bupivacaine in the reduction of post-operative pain after laparoscopic cholecystectomy. 40 patients were randomly allocated in two groups; a study group that received 50 ml of bupivacaine (50%) instilled intraperitoneally into the gall bladder bed and under the surface of diaphragm, and control group which received 50 ml of 0.9% normal saline instilled in the same way. Data recorded from patients in pre-designed format and enrolled in a randomized double-blind prospective study showed a significant decline in post-operative pain scores in the study group between 1st and 4th hours as compared to the control group and, consequently, consumption of analgesics was lower in intergroup comparison. Discharge after surgery was significantly earlier in the study group (75%) one-day admission, while only (35%) of group B discharged after one-day hospitalization. We conclude that routinely intraperitoneal instillation of bupivacaine in laparoscopic cholecystectomy is a simple and safe method to minimize postoperative abdominal pain and analgesic requirements, which enhances early mobilization and discharge, and may become a routine practice.

Keywords: Postoperative pain; Bupivacaine, intraperitoneal; Laparoscopic cholecystectomy.

INTRODUCTION

Laparoscopic cholecystectomy is now an established form of treatment for patients with symptomatic gall stones. As it is an excellent mean to minimize the trauma and agony of the patient following surgery.

Although thought to result in less postoperative pain, recent studies have shown that patients may experience considerable pain after laparoscopic cholecystectomy procedures (Joris et al., 1992). However there still remains some challenges to minimize the post-operative pain in patients, the pain reaches a maximum level within 6 hours of the procedure and then gradually decreases over a couple of days (Bisgaard, Klarskov, Rosenberg, & Kehlet, 2001).

The etiology of pain is complex, including damage to abdominal wall structures, the induction of visceral trauma and inflammation, and peritoneal irritation because of CO2 entrapment beneath the hemidiaphragms, neuropraxia of the phrenic nerve caused by distention of the diaphragm during gas insufflation, and/or acid milieu created by the dissolution of CO2 (Alexander & Hull, 1987).

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Being a relatively new procedure there is no general agreement on effective postoperative pain control modalities have been proposed to relieve postoperative pain after laparoscopies like Non-steroidal anti-inflammatory drugs /opioids, intraperitoneal local anesthetics, and port site infiltration of local anesthetics.

Local anesthetics are widely used, have a good safety profile, and are available in long-acting preparations, they provide the benefit of anesthesia without the systemic side effects, local anesthetics block the generation, and propagation of action potentials in nerve and other excitable tissues in a reversible manner, probably at the level of the passive sodium channels.

Bupivacaine is a long acting amide-type local anesthetic, released for clinical uses in 1996. When ropivacaine is given intraperitoneally, it starts acting within 10 to 20 minutes, and the duration of action lasts for four to six hours. Intraperitoneal instillation of local anesthetics using 20 ml of 0.5% bupivacaine results in less postoperative pain as in some studies were carried out with variable results in patients undergoing laparoscopic cholecystectomy (Chundrigar, Hedges, Morris, & Stamatakis, 1993; Joris et al., 1992; Pasqualucci et al., 1996; Rademaker, Ringers, Odoom, Kalkman, & Oosting, 1992).

In our current study, we aim to assess the effectiveness of intraperitoneal instillation of bupivacaine in the reduction of postoperative pain after laparoscopic cholecystectomy.

MATERIALS AND METHODS

A total of (40) ASA I and II patients between 26- 45 years of age scheduled for laparoscopic cholecystectomy were enrolled in a randomized double-blind prospective study after taken written informed consent.

Inclusion Criteria the study included all patients regardless of gender, with chronic cholecystitis and in ages between 20 to 60 years.

Exclusion Criteria the study excluded patients who received opioids or tranquilizers for more than one week prior, or when the operation was converted from Laparoscopic to open cholecystectomy. All patients were worked up with detailed history, clinical examination, and baseline pre-operative investigations,

The visual analogue scale (VAS) scoring system Figure1 (Breivik et al., 2008) was explained to all patients on the evening before surgery.

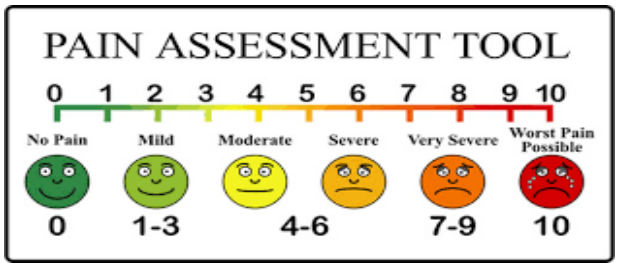

Figure (1). (VAS) The visual analogue scale of pain

The patients were randomly allocated in two groups A & B, by a lottery method.

Group A was a study group who received 50 ml of bupivacaine (50%) instilled intraperitoneally into the gall bladder bed and under the surface of the diaphragm. **Group B** was a control group and received 50 ml of 0.9% normal saline instilled intraperitoneally into the gall bladder bed and under the surface of the right diaphragm.

The visual analogue scale scoring system: was assessed at 1, 2, 4, 8, 12, 24 hours postoperatively, and blood pressure and heart rate were assessed at 120 min intervals as well as the need for analgesia frequency and dose were recorded precisely.

A conventional balanced general anesthesia was administrated,, the induction protocol was standard for all patients and anesthesia was maintained with a mixture of nitrous oxide and oxygen, ventilation was adjusted to maintain end-tidal carbon dioxide between 35 and 40 mmHg. Patients were placed in anti-

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Trendelenburg position during laparoscopy, and intraabdominal pressure was maintained between 12 and 14 mmHg. Patients were randomized into one of the two groups by the closed envelope technique.

A doctor, who had not participated in the surgery, prepared a drug solution and the drug was filled in preceded syringes and given to the surgeon. The surgeon and the assistants were unaware of the treatment for which the patient was randomized.

At the end of the procedure, those patients who were allocated to group A received 50 ml of bupivacaine (50%) intraperitoneally instilled on the operative site and inferior aspect of diaphragm via the lateral port site with patient in supine position (after peritoneal wash and suctioning), and those allocated to group B received 50 ml of 0.9% normal saline solution as (placebo), and was instilled in the same pattern. CO_2 was then evacuated from the peritoneal cavity and skin incision was sutured. Operative details such as bile, blood spilled, washout, drain, operation duration were recorded in predesigned patient format and the degree of postoperative pain was assessed at intervals 1, 2,4, 8, 12, 24 hours post-operative using the VAS score. When the score was high, patients were given an injection of Diclofenac sodium (75 mg Intramuscular), the time of the first analgesic and total analgesic requirements during the 24-hour post-op period were recorded, and the occurrence of adverse events was also recorded.

Statistical analysis: was done using SPSS software for Windows version 16.0. For non-continuous data, Chi-square test was used. The mean and the standard deviation of the parameters studied during observation period were calculated for the two treatment groups and compared using Student's t-test.

The critical value of 'p' indicating the probability of significant difference was taken as < 0.05.

RESULTS

The two groups were comparable for age, sex and preoperative vital signs (Table 1).

Table (1). Demographic data

Mean (SD)		
	Group A (n=20)	Group B (n=20)
Age (yrs.)	33.1±7.0	35.2+6.0
Sex (F: M)	18:2	18:2
PR (beats/min)	85.5(6.83)	88.30 (6.03)
SBP (mmHg)	121.50(8.84)	121.41(6.80)
DBP (mmHg)	81(7.18)	81.6(4.66)
MBP (mmHg)	94.5(7.03)	94.9(4.17)
RR (/min)	17.55(2.86)	16.50(2.82)

Abbreviation: PR pulse rate, SBP-systolic blood pressure DBP-diastolic blood pressure, MBP-mean blood pressure, RR-respiratory rate.

Inter group comparison of mean VAS scores showed a significant decline in A study group between 1st and 4th hour as compared to Placebo group and the pattern of change in mean VAS score overtimes was significantly different (P value <0.05) (Table 2).

Table (2). the pattern of change in mean VAS score over postoperative times

VAS post-operative pain score overtimes

Post-operative time in hours	VAS Pain Score Bupivacaine group	VAS Pain Score Placebo group	P value
1st	2.42 ± 0.71	3.78 ± 1.18	0.010
2nd	1.73± 0.71	4.08 ± 1.05	0.008
4th	2.13 ± 0.56	4.12 ±1.13	0.005
8th	3.27 ± 0.78	3.52 ± 1.22	0.055
12th	3.88 ± 0.85	3.85 ± 0.88	0.488
24th	2.61 ± 1.08	2.55 ± 1.33	0.744

Consumption of analgesics was also lower in patients of group A (20%) 4 patients out of 20, while it was (85%)17 patients out of 20 in-group B. (Figure2)

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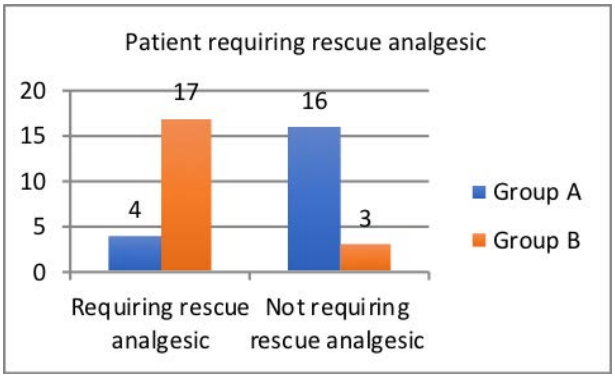

Figure (2). Patient requiring rescue analgesic

Discharge after surgery was significantly earlier in-group A (75%) one-day admission while only (35%) of group B discharged after one-day hospitalization (Figure3).

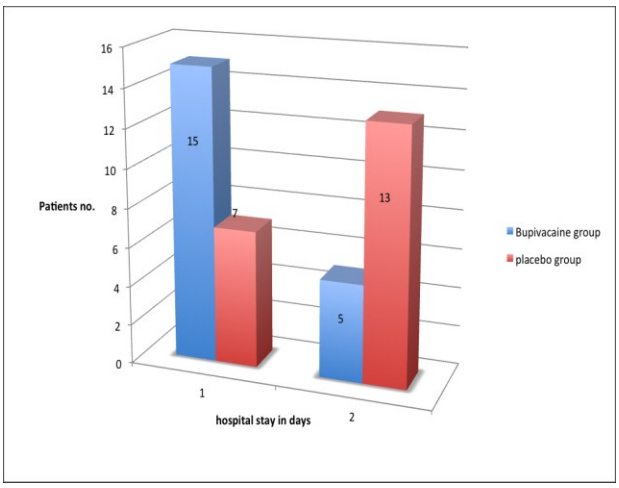

Figure (3). Hospital stay in the two groups

DISCUSSION

Although laparoscopic cholecystectomy pain is less intense and lasts for a short amount of time than open surgery, it remains a problem and may delay discharge of the patient. Therefore, adequate early postoperative relief of pain after LC is an essential goal to enable the patient to go home early with little pain and in stable condition (Lepner & Goroshina, 2003; Refaie & Khatab, 2005).

In this study, intraperitoneal instillation of bupivacaine was found to be beneficial in reducing the intensity of abdominal pain in the early few hours after the operation, which may enhance mobilization and early discharge after surgery, likewise reduction of analgesic requirement in comparison with the control group in patients underwent LC. Our findings are in agreement with other studies done by (Elhakim, Elkott, Ali, & Tahoun, 2000; Refaie & Khatab, 2005) who found a reduction in the intensity of pain and analgesic requirements, by using bupivacaine after LC.

On the other hand, there were studies that failed to demonstrate any pain reduction with intraperitoneal instillation bupivacaine in patients undergoing LC as (Rademaker et al., 1992; Ure et al., 1993).

The difference between our study and these studies may be attributed to the different responses of patients to the bupivacaine, or differences in its amount or concentration used in these studies or may be attributed to variations in patient selection criteria, some intraoperative events, or techniques used in each study.

In the present study, we compared bupivacaine group with the control group and found that bupivacaine group had good control of abdominal pain in early postoperative before 6 hours as compared with the control group. These results are consistent with that of (Lepner & Goroshina, 2003).

The pain score for both groups has the highest intensity after 6 hours postoperative, with the biggest difference between the two groups at before 6hours intervals, after that it declined to a comparable VAS values up to 24 hours.

Therefore, the main effect of bupivacaine in this study seems to reduce the pain during the early few hours after LC. (Kucuk, Kadiogullari, Canoler, & Savlı, 2007). This is the period in which the pain is in its highest intensity and the patients need adequate pain relief (Sharan et al., 2018).

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Although the half-life of bupivacaine is approximately 2.7 hours, its beneficial effect in soft tissue is up to 12 hours (Refaie & Khatab, 2005).

CONCLUSION

Routinely intraperitoneal instillation of bupivacaine in laparoscopic cholecystectomy is a simple and safe method to minimize postoperative abdominal pain and analgesic requirements, which enhances early mobilization and discharge, and may become a routine practice.

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ETHICS

All patients involved in this study were pre-informed about the nature and steps of the study and signed a written consent that was taken from each without cognizance about which group he/she belongs to (double-blind trial) and were attached to each patient format sheath.

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تأثير تقطير المخدر الموضعي (البوبيفاكين) داخل الغشاء البريتوني (الصفاق) على الشعور بالألم بعد الجراحة في حالات استئصال المرارة بالمنظار

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المستخلص: يعتبر الآن استئصال المرارة بالمنظار العلاج الافتراضي والخيار الأول للمرضى الذين يعانون من حصوات بالحويصلة المرارية وبالرغم من أنه وسيلة ممتازة للحد من معاناة المريض بعد الجراحة إلا أن الدراسات الحديثة قد أظهرت أن المرضى لا يزالون يعانون من آلام ملحوظة حتى مع هذا النوع من الجراحات الحديثة. في هذه الدراسة نهدف إلى تقييم فعالية تقطير عقار البوبيفاكايين داخل الصفاق في الحد من الآلام ما بعد عملية استئصال الحويصلة المرارية بالمنظار، حيث تم تحديد أربعين مريضا قسموا عشوائيا إلى مجموعتين متماثلتين في العدد والعمر والجنس والحالة الصحية الأولى، مجموعة الدراسة تشمل أولئك الذين تلقوا 50 مل من عقار بوبيفاكايين (50 ٪) داخل الصفاق والثانية مجموعة الشاهد وتشمل أولئك الذين تلقوا 50 مل من المحلول الملحي المشابه بنسبة (0.9 ٪) بنفس الطريقة للتمويه. أظهرت البيانات المسجلة من المرضى في النموذج المعد مسبقًا وتحليلها من خلال دراسة استطلاعية عشوائية مزدوجة التعمية انخفاضًا ملحوظًا في درجات الألم بعد العمليات الجراحية في مجموعة الدراسة وبخاصة بين الساعة الأولى والساعة الرابعة مقارنةً بمجموعة الشاهد وبالتالي كان استهلاك المسكنات أقل عند المقارنة بين المجموعتين ونتج عن كل ذلك أن الخروج من المستشفى بعد الجراحة أصبح بعد يوم واحد من الإيواء لـ (75 ٪) من المرضى في مجموعة الدراسة مقارنة لـ (35 ٪) في مجموعة الشاهد بذلك من الممكن أن نخلص إلى أن تقطير عقار البوبيفاكيين داخل الصفاق في عملية استئصال الحويصلة المرارية بالمنظار هو طريقة بسيطة وآمنة لتخفيف الألم بعد الجراحة وتحد بشكل كبير من طلب المريض للعقاقير المسكنة في فترة النقاهة وبالتالي لعدم التعرض إلى آثارها الجانبية، كل ذلك يعزز من إمكانية العودة المبكرة للبيت والعمل قد يصبح تقطير عقار البوبيفاكايين داخل الصفاق ممارسة روتينية شرط خضوعها للمزيد من الدراسة والبحث.

الكلمات المفتاحية: الألم بعد العملية الجراحية، بوبيفاكين، داخل الصفاق، استئصال المرارة بالمنظار.

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The Effect of Salinity on Wheat Genotypes during Germination Stage

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Abstract: Salinity is a major abiotic stress that adversely affects wheat production in many regions of the world. Salinity stress limits wheat growth, development, and yield. Identification of salinity tolerant genotypes is critical for yield improvement. Therefore, a series of control environment experiments were carried out to evaluate the response of two spring wheat and two winter wheat cultivars (*Triticum aestivum* L.) to different levels of salinity. The experiments were designed in a randomized complete block design (RCBD) with five replications. Twenty seeds of each genotype were placed on pre-moistened filter paper in Petri dishes and placed in an incubator at 20 °C. The seeds were subjected to 4 levels of salinity 0, 50, 100, and 150 mM NaCl. Seedlings were harvested after 8 days, and data on final germination percentage, rate of germination, mean daily germination, shoot and root length, and seedling fresh and dry weight were recorded. The results indicated that winter and spring wheat genotypes differed significantly for germination percentages, rate of germination, mean daily germination, shoot and root lengths, and seedling fresh and dry weight. The results showed that salinity did not affect final germination percentage until salinity level reached to 100 mM NaCl; whereas seeds subjected to 100 and 150 mM NaCl retarded germination by 1 and 2 days of spring wheat, and 2-3 days of winter wheat respectively, as compared with 0 and 50 mM NaCl treatment. The data also showed that increasing salinity level significantly decreased shoot and root length, however, the study found that salinity affected root growth more severely than shoot growth of seedlings. Significantly, root length and dry weight of root ranked genotypes in the same order as their salt-tolerance. Therefore, the study concluded that the measurements of root growth would be effective criteria for screening wheat genotypes for salt tolerance at seedling stages.

Keywords: *Triticum aestivum* L, germination index, salinity tolerance index, seedling vigor index.

INTRODUCTION

Salinity is a major abiotic factor reducing plant growth and productivity throughout the world. It is estimated that over 800 million ha will be affected by salinity soon (Rengasamy, 2006; FAO, 2008, Shahbaz and Ashraf, 2013). Salinity affects more than 40% of soils in the Mediterranean regions (Nedjimi, 2014). Recent tendency and future demographic predictions propose that it is important to produce more crops which require effective utilization of salt-affected land and saline water resources. (Qadir *et al.* (2008) found that at least 20 percent of the world's irrigated land is salt-affected and/or irrigated with saline water. About two million additional ha of cropping lands are affected by salinity every year (Rengasamy, 2006; Tuteja, 2007, Jamil *et al.,* 2011). Irrigated agriculture is a major human activity, which often leads to secondary salinization of land and water resources in arid and semi-arid conditions (Shrivastava and Kumar, 2015). Because of salinization increase in agricultural lands, it is expected that about 50 % of cropland will be lost by the middle of the 21st century (Wang *et al.,* 2007). Saline soils are

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found where rainfall is low and in coastal regions where saline water has entered the soil (Tanji, 1990). Still, even in regions with sufficient rainfall, salt can be accumulated in poor drainage soils. Another source of soil salinity is the substantial use of fertilizers (Plaut *et al.,* 2013).

Wheat (*Triticum aestivum* L.) is the world's most widely grown cereal crop for food (Carte, 2002). It is an important staple food source for 30% of humans all over the world and its farming is important for global food security (Muthusamy *et al*., 2017). Wheat is a generally grown in irrigated, dry, and high rainfall areas and from temperate, humid to dry and cold conditions (Dubcovsky and Dvorak 2007). Growing of human population and reduction in agriculture land availability are two threats for agricultural sustainability (Shahbaz and Ashraf, 2013). Considering the importance of wheat on an economic basis, the demand for wheat is expected to increase in the future with the increase in global population (Barnes and Shields, 1998). Global wheat production is projected to be about 735 million tons in 2016-17 (FAO, 2013). Currently, about 65 % of the wheat crop is used for food, 17 % for animal feed, and 12 % in industrial applications, including biofuels (Oleson, 1994; FAO, 2013). To meet the demand, 40 % more grain in 2020 is required (Andersen *et al*., 1999). Increases in the cultivated area are expected to contribute only about one-fifth of the global cereal production between 1995 and 2020 (Andersen *et al.,* 999). Therefore, improvements in crop yields will be required to bring about the necessary production increases.

Germination of seed is vital for the seedling establishment for ensuing plant stand. Salinity can affect germination and seedling growth by producing an osmotic pressure that prevents or reduces water uptake. Also, salinity may affect germination due to Na and Cl ions toxicity (Munns, *et al.,* 2006). Wheat has a moderate tolerance to salinity (Acevedo *et al*, 2002). Francois *et al*. (1986) found that salinity level of > 4.5 dSm^{-1} electrical conductivity of the saturation extract decreases the percentage of plants establishment per unit area, and at 8.8 dSm^{-1} the wheat plants emergence decreased to 50 percent (Francois *et al.,* 1986). Salinity stress symptoms include, reduced seed germination, plant growth, and plant yield. However, plant species and genotypes within species show differential responses to salinity stress (Djanaguiraman and Prasad, 2013, Setter *et al.,* 2016). To our knowledge, screening spring and winter wheat germplasms to salinity stress and understanding the genetic variability for the seedling characteristic was not studied in details. Therefore, the objective of this study is to evaluate spring and winter wheat genotypes for salinity tolerance at the germination stages and to determine seedling growth traits associated with salinity tolerance.

MATERIAL AND METHODS

Plant Materials: Four genotypes of wheat *(Triticum aestivum* L.) were used in this study. Two genotypes of spring wheat (H0800310 and SD4279) and two genotypes of winter wheat (OK04111 and TX06A001263) were obtained from crop physiology research laboratory at Kansas State University, Manhattan KS. USA

Experimental and Treatment Conditions: The experiments were conducted during spring 2014 in a controlled environment at the Department of Agronomy, Kansas State University, Manhattan, KS, USA. A split plot experiment based on randomized complete block design (RCBD) with five replications was employed. The main plots were allocated to salinity levels, whereas the sub-plots were assigned for wheat genotypes. Three different concentrations of a saline solution prepared with deionized water (50, 100 and 150 mM NaCl with electric conductivity [EC] value of 5.6, 10.6 and 16.2 dSm-1) were used for salinity treatments and deionized water was

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used as a control solution (0 mM NaCl). Healthy seeds of each genotype were surface sterilized with sodium hypochlorite solution (5 %) for five minutes, and rinsed with sterilized distilled water, air dried and used for the experiment. A set of 20 seeds were placed in a petri dish with Whatman no. 1 filter paper discs; and it was moisturized 5 mL of the different saline solutions (50, 100 and 150 mM NaCl) and a control solution (0 mM NaCl). The filter paper was moisturized on a daily basis till the end of experiment and filter papers were changed once in every two days to prevent salt accumulation due to evaporation. The seeds moisturized with deionized water instead of NaCl solution saved as an absolute control treatment for the experiment. A total of five replications used for control and NaCl treatments. All the Petri dishes were placed in the dark throughout the germination period (total of 8 days) at 20±2°C in an incubator (Low temperature Illuminated incubator, Thermo Scientific Model 818, USA). Seeds were considered germinated (Feekes 0.9) when both shoot and root extended more than 2 mm from the seed (Islam *et al*., 2012). The following traits were recorded during the germination period, and 8 days after sowing:

Germination traits: Germination percent (*G %*) was expressed according to Nasri *et al*., 2011. The following formula was used to calculate *G%*:

$$G\ \% = (NSG \div TNSS) \times 100$$

Where *NSG* is the number of seeds germinated at the end of the experiment (8 days after sowing). *TNSS* is the total number of seeds sown.

Germination index (*GI %*) in each NaCl treatment was calculated according to the equation given by Karim *et al*. (1992). The following formula was used to calculate GI %:

$$GI\ \% = (\%\ GNaCl \div \%\ GC) \times 100$$

Where *% GNaCl* is germination percent at different NaCl treatments. *% GC* is germination percent in control treatment.

The germination rate (GR) was calculated according to the equation given by Rubio-Casal,*et al* (2003). The count of germinated seeds was recorded at 24 hours interval from sowing till the end of experiment (8 days after sowing) and used to calculate GR. The following formula was used to calculate GR:

GR=(n1t1)+ (n2t2)+⋯+ (nxtx)÷TNGS

Where n_1 is the number of seeds germinated on the first day of germination, t_1 is the number of days taken for the first germination, and *TNGS* is the total number of seeds germinated.

Mean daily germination (MDG) was calculated as per Gairola *et al*. (2011). The following formula was used to calculate MDG:

$$MDG = TNGS \div TNDG$$

Where *TNGS* is the total number of germinated seeds and *TNDG* is the total number of days taken for final germination.

Early seedling traits: Thereafter, morphological traits *viz*., shoot and root length, and fresh and dry weight were subsequently measured from 5 uniform seedlings from each replication at an early seedling stage (Feekes 1). Selected seedlings were dissected and shoot, and root length were recorded. The length from the seed to the tip of the root and leaf blade was calculated and expressed in cm to measure the root length and shoot length, respectively, using a digital vernier caliper. The fresh weight of shoot and root was recorded using a weighing balance (Salter Brecknell, ESA-600, China) and then dried in an oven maintained at 70 °C till it attains stable weight. After that, shoot and root dry weights were recorded. Using the morphological traits, the salinity tolerance index (STI) and Seedling vigor index (SVI) were calculated.

The following formula was used to calculate STI (Tsegay *et al*., 2014):

$$STI = (SdwNaCl \div SdwC) \times 100$$

Where *SdwNaCl* is the dry weight of seedling

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from NaCl treatment. *SdwC* is the dry weight of seedling from control treatment.
The following formula was used to calculate SVI (Abdoli *et al.* 2013):

$$SVI = (SL \times G\%)100$$

Where *SL* is seedling length and *G%* is germination percent.

Experiment Design and Data Analysis: The experiment design was a randomized complete block design (RCBD) with five replications. The analysis of variance of the data and the comparison of the means were done using least significant difference (LSD) using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Analysis of variance results showed that salinity, genotype, and salinity x genotypes interaction had a significant effect ($P<0.0001$ or $P < 0.05$) on germination %, germination index, mean daily germination, germination rate, and seedling vigor index. However, salinity x genotypes interaction had no significant effect on mean daily germination (Tables 1 and 2). Also, the results showed that salinity, genotype, and their interaction had a significant effect ($P<0.0001$ or $P < 0.05$) on all seedling characteristics studied (Tables 1 and 2). These four genotypes were ranked based on the seedling salinity tolerance and vigor index (Fig 3 a and b), such that those with the smallest and largest reduction percent over the control were ranked respectively as the most and least tolerant genotype at 150 mM NaCl. These genotypes were (1) tolerant to salinity at germination stage (SD4279 and H0800310) (2) moderately tolerant to salinity at germination stage (TX06A001263) and (3) susceptible to salinity at germination stage (OK04111).

Table (1). Probability values of effects of salinity (S), genotype (G) and salinity x genotype interaction on germination and early seedling traits in experiment 1 at germination stage (Feekes 0.9).

Traits	Salinity(S)	Genotype(G)	SxG
Germination %	<.0001	<.0001	0.0013
Germination index (%)	<.0001	<.0001	0.0013
Mean daily germination	<0.0001	0.0478	0.1691
Germination rate (d^{-1})	<0.0001	< 0.0001	<.0001
Shoot length (cm)	<0.0001	< 0.0001	0.0340
Root length (cm)	<0.0001	< 0.0001	0.0123
Seedling length (cm)	<0.0001	< 0.0001	0.0022
Seedling fresh weight (g)	<0.0001	< 0.0001	<.0001
Seedling dry weight (g)	<0.0001	< 0.0001	0.0233
Salt tolerance index	<0.0001	< 0.0001	0.0160
Seedling vigor index	<0.0001	< 0.0001	0.0358

Table 2 Effect of salinity stress on germination and seedling traits of two spring wheat and two winter wheat genotypes. Individual datum is the mean of four replications. Means that have the same letter in each trait are not significantly different ($p \leq 0.05$) from each other.

Traits	NaCl levels (mM)			
	0	50	100	150
Germination %	100 ^{a}	96.8 ^{b}	84.0^{c}	73.0^{d}
Germination index (%)	100^{a}	96.8^{b}	84.0^{c}	73.0^{d}
Mean daily germination	8.3 ^{a}	7.6^{a}	4.4^{b}	3.1^{c}
Germination rate (d^{-1})	2.0^{c}	2.1^{c}	2.6^{b}	3.5^{a}
Speed of germination	9.9^{a}	9.5^{b}	7.0^{c}	4.6^{d}
Shoot length (cm)	8.3^{a}	8.1^{a}	6.8^{b}	5^{c}
Root length (cm)	6.5^{a}	6.3^{b}	5.3^{c}	2.9^{d}
Seedling fresh weight (g)	0.18^{a}	0.17^{b}	0.15^{c}	0.13^{d}
Seedling dry weight (g)	0.08^{a}	0.07^{b}	0.06^{c}	0.04^{d}
Salt tolerance index	100^{a}	93.0^{b}	73.7^{c}	53.6^{d}
Seedling vigor index	14.8^{a}	14.0^{b}	10.1^{c}	5.9^{d}

Germination Parameters: Germination percentage (G %) of wheat genotypes was significantly ($P < 0.0001$) reduced by increasing salinity level. Genotypes SD4279 and H0800310 were tolerant to salt stress, whereas genotypes OK04111 and TX06A001263 were sensitive to

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salt stress at 150 mM NaCl. Mostly, an increasing level of salinity stress resulted in a decrease in germination percentage over control. However in these two genotypes, the germination percentage was not affected at 50 mM NaCl but decreased by20% at 100 mM NaCl, and by 30 and 33% at 150 mM NaCl over the control (0 mM NaCl; Fig. 1a).

The results showed that salinity significantly reduced germination index (GI) at moderate and high NaCl concentrations with the largest decrease at 150 mM NaCl. The results showed a decline by 33 % in the germination index in OK04111 genotype under a high level of salinity 150 mM NaCl. However, under the same level of salinity, genotype SD4279 had about 20 % decline in term of germination index (Fig. 1b--). In addition, the analysis of variance showed that salinity significantly increased (P < 0.0001) germination rate (GR) (Tables 1 and 2). Figure 1d showed that increasing salinity concentration resulted in a dramatic increase in germination rate. Genotypes OK04111 and TX06A001263 showed an increase in germination rate over the control by 77 % and 95 %, respectively at 150 mM NaCl, whereas genotype SD4279 had a percent of increase of 36 % at the same level of salinity 150 mM NaCl over the control. Mean daily germination (MDG) was strongly decreased with salt stress in all genotypes. The results showed that, in some genotypes, moderate salinity decreased mean daily germination by a lesser extent and severe stress decreased. Genotypes OK04111 and TX06A001263 showed a decline over the control by 67 and 69 % respectively, whereas genotype SD4279 showed a decline over the control by 44 %, (Fig 1c).

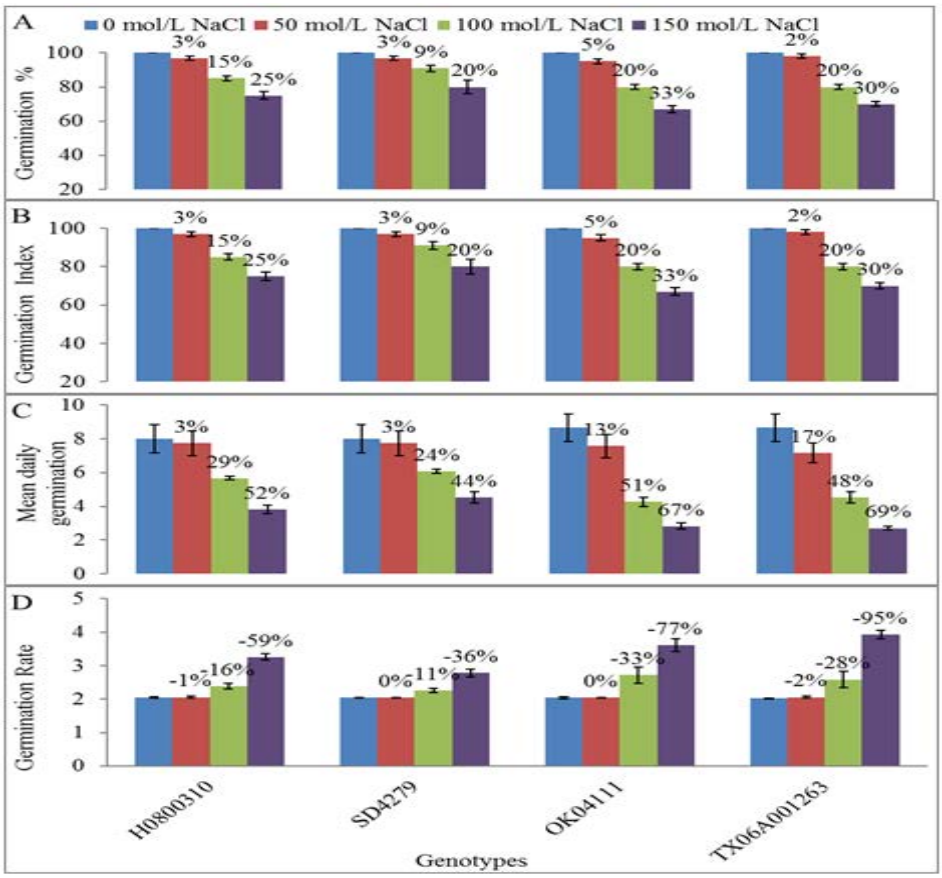

Figure (1). The effects of different salinity levels (0, 50, 100, and 150 mM NaCl) on (A) germination percentage (B) germination index (%) (C) mean daily germination and (D) germination rate (d) of two spring wheat and two winter wheat genotypes. Percent reduction in all traits due to level of salinity (50, 100, and 150 mM NaCl) as compared to control is indicated. Vertical lines on top of bars indicate standard error of means (n = 5).

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Seedling Parameters: All seedling parameters decreased with increasing salinity level (Tables 1 and 2). Under non-saline conditions (0 mM NaCl), genotypes showed no significant differences in terms of shoot length. However, under moderate and high levels of NaCl condition, there were significant differences in the response of genotypes to salinity levels (Fig. 2a). Under high level of salinity, 150 mM NaCl, the genotypes OK04111 and TX06A001263 had the greatest decrease in shoot length, and genotypes SD4279 and H0800310 had the lowest decrease in shoot length. Similarly, there were significant differences among genotypes in terms of root length in response to salinity stress. Increasing NaCl level resulted in a significant decrease in root elongation as compared to the control. Increasing salinity levels inhibited the root length of wheat genotypes. In fact, root length was more affected by salt stress than shoot length. Genotypes OK04111 and TX06A001263 showed a percent decline of above 60 % (Fig. 2 b). In addition, increasing salinity level consistently reduced the growth and biomass production of almost all wheat genotypes used in this study. In comparison with control, the maximum reduction in seedling fresh weight was observed in OK04111 and TX06A001263 with a reduction percentage of 30 and 30 %, respectively. Seedling dry weight was also decreased with increasing salt concentrations (50 to 150 mM NaCl; Fig. 2c). The seedling dry weight was decreased to a higher level than fresh weight under a high level of salinity 150 mM NaCl. The highest decline of fresh weight was by 33 % in OK04111, while the dry weight declined by 57 % in the same line (Fig.2 c and d). Results regarding salt tolerance (ST) of different wheat genotypes showed that genotype SD4279 was tolerant to salinity stress at the germination stage. However, genotypes H0800310 and TX06A001263 were moderate to salinity stress, and genotype OK04111 was sensitive to salinity stress. Based on tolerance at the germination stage, genotypes were grouped as tolerant, moderate, and sensitive genotypes based on salinity tolerance index. The results showed that genotype SD4279 had a reduction percentage of 33 % over control. Therefore, this genotype was more tolerant to salinity stress, and genotypes H0800310 and TX06A001263 had reduction percentages of 47, 49% respectively, and therefore these genotypes were moderate to salt stress at germination stages, though the genotype OK04111 had a reduction percentage of 56% and therefore this genotype was sensitive to salt stress at germination stages (Fig. 3a). Increasing salinity concentrations from 50 to 150 mM NaCl gradually decreased seedling vigor index. The highest seedling vigor index was observed in control, while salinity at 50, 100, and 150 mM NaCl significantly decreased seedling vigor index. A significant decrease was observed at 150 mM NaCl salinity in genotype OK04111. Data showed that the genotype OK04111 had 69 % decline over control, while the SD4279 had 50 % decline over control (Fig. 3b).

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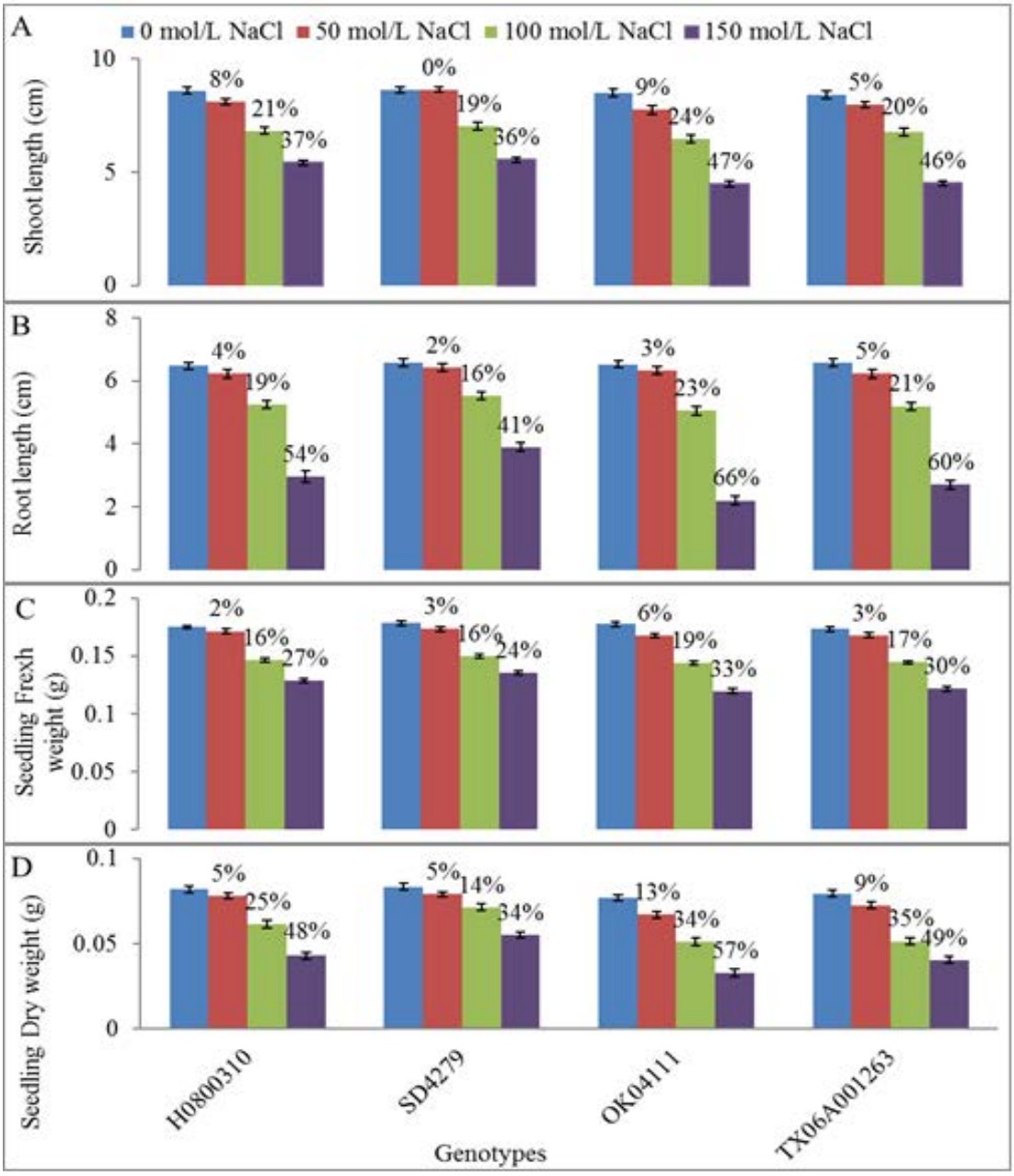

Figure (خطأ! لا يوجد نص من النمط المعين في المستند.**).** Effect of different salinity levels (0, 50, 100, and 150 mM NaCl) on (A) shoot length (cm), (B) root length (cm), (C) Seedling Fresh Weight, (D) seedling dry weight (g), (D) salt tolerance index, and (E) seedling vigor index of two spring wheat and two winter wheat genotypes. Percent reduction in all traits due to the level of salinity (50, 100, and 150 mM NaCl) as compared to control is indicated. Vertical lines on top of bars indicate standard error of means (n = 20).

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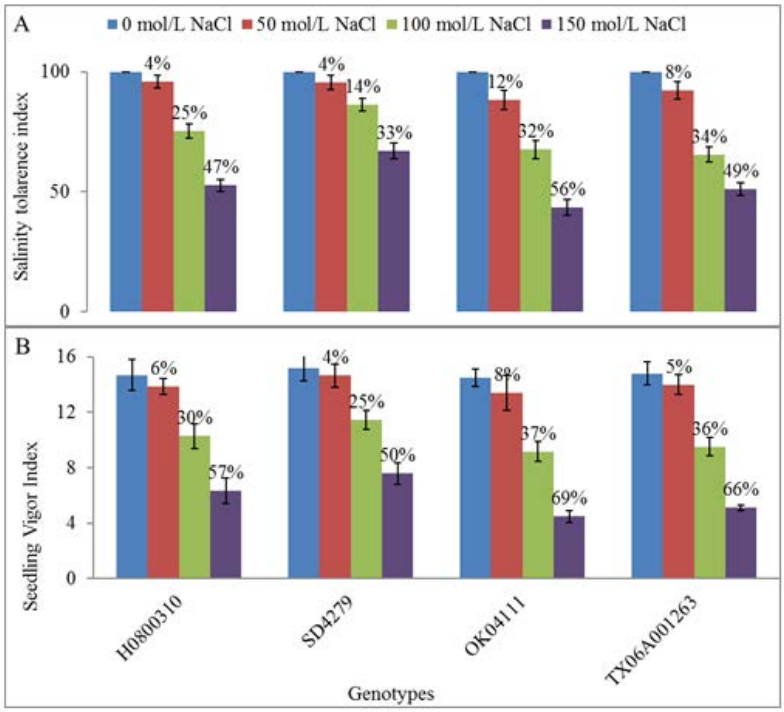

Figure (3). Effect of different salinity levels (0, 50, 100, and 150 mM NaCl) on (A) salt tolerance index, and (B) seedling vigor index of two spring wheat and two winter wheat genotypes. Percent reduction in all traits due to the level of salinity (50, 100, and 150 mM NaCl) as compared to control is indicated. Vertical lines on top of bars indicate standard error of means (n = 20).

DISCUSSION

The results showed that by increasing NaCl concentrations the germination in wheat genotypes was delayed and decreased, also the germination percentage, germination index, germination rate, and mean daily germination were significantly ($P < .0001$) decreased by salinity stress. Similar results were reported by Rahman *et al.* (2008); Khayatnezhad, and Gholamin, (2010); Nasr *et al*. (2012); Kumar *et al*. (2012); and Hussain *et al*. (2013). These studies reported that there exists genetic variability among wheat genotypes for salinity tolerance based on seed germination percentage and seedling growth. Salinity affects germination in two ways: (1) a high concentration of salt in the growth medium decreased the osmotic potential to a level that prevented water uptake and reduced utilization of nutrients essential for germination, and (2) Na^+ and Cl^- ions are toxic to the embryo (Kayani *et al*., 1990; Munns,*et al*. 2006). Winter wheat genotypes responded differently to the salinity level. It appears that at concentrations up to 150 mM NaCl in the growth solution, the water potential of the seeds is still sufficiently low to bring an adequate amount of water for the several metabolic processes that lead to germination. Other studies reported that the difficulty of growth under salinity stress may result from decreased water potential of the seeds (Rahman *et al*., 2008; Muhammad and Hussain, 2012). The results of this study are analogous to those described by other researchers (Catalan *et al*., 1994; Kazemi and Eskandari, 2011; Muhammad and Hussain, 2012). Physiologically, salinity stress has a negative impact on many processes however the most significant effect is reducing cell division and expansion, which result in decreasing shoot and root length. With increasing NaCl concentration, it affected seedling fresh and dry weight. Reduction of seedling dry weight relatively depended on shoot and root lengths and branches. The results obtained in this study were consistent with previ-

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ous findings that have indicated significant differences in the salt tolerance of wheat genotypes and their differential responses to increased salt concentrations (Catalan *et al*., 1994; Rahman *et al*., 2008; Adjel *et al*., 2013). In addition, the results showed that the most sensitive growth characters to salinity were root length, which agreed with a previous study by (Akbarimoghaddam *et al.* 2011), and dry matter production while germination percentage was the least sensitive under salinity. Nevertheless; the genotypes which had higher germination percentages also had higher root length, shoot length, and dry matter production. For this reason, seedling length and dry weight are considered as selection criteria for salinity tolerance. It is estimated that in addition to higher dry weight, longer shoots and roots development will allow more successful selection for high salt tolerance. Yet, root length and dry weight can be considered as selection criteria only when there is a high germination percentage. For these reasons, the seedling vigor index, which is a function of both germination percentage and seedling length, was determined to be a more consistent selection criterion. Genotypes such SD4279 and H0800310 were considered as salinity tolerant genotypes.

CONCLUSIONS

In conclusion, this investigation was carried out to inspect winter and spring wheat genotypes for salinity tolerance and to evaluate the effects of salinity on germination and seedling growth of 2 spring and 2 winter wheat genotypes. Genotypic variability for salt tolerance was found among different wheat genotypes. Seedling vigor index is a good parameter for evaluating salinity tolerance at germination stages. According to that, the genotypes were ranked based on the seedling vigor index, such those with the smallest and largest reduction percentages over the control were ranked respectively as the most and least tolerant genotypes at 150 mM NaCl. According to that genotypes were divided into three categories (1) tolerant to salinity at germination stage (SD4279 and H0800310), (2) moderately tolerant to salinity at germination stage (TX06A001263), and (3) susceptible to salinity at germination stage (OK04111). Overall, it can be determined that under control (0 mM NaCl) conditions, all wheat genotypes had good germination and growth attributes. However, wheat genotypes showed a differential response at higher levels of salinity. Yet, salinity reduced all germination traits of wheat genotypes. These results indicate that genetic variation exists among those wheat genotypes in terms of germination under salinity stress condition. Further studies are needed to see the effect of salt stress on the germination and seedling growth of these germplasms under field conditions.

Salinity tolerance and seedling vigor indexes were the best germination traits that can be used as a selection criterion for salinity tolerance in wheat. Based on those genotypes, SD4279 and H0800310 were identified as tolerant genotypes to salinity stress at germination stages. These investigations showed the existence of significant genetic variability in spring and winter wheat lines for salinity stress at germination stages. Therefore, additional research might be directed to develop a new screening technique to identify a large germplasm collection for salinity tolerance during germination and seedling stages of development. This study focused on the effect of salinity stress at germination stages, therefore, further research on the effects of salinity stress during the booting, flowering and post-flowering stages, grain filling, and seed development are needed to evaluate the effect of salinity stress at later stages of wheat growth and development.

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تأثير الملوحة على أصناف مختلفة من القمح أثناء مرحلة الإنبات

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المستخلص : تعتبر الملوحة من أهم العوامل البيئية التي تؤثر سلبًا على إنتاج محصول القمح في العديد من مناطق العالم. حيث إن الاجهاد الملحي يحد من نمو محصول القمح وتطوره، وبالتالي يؤثر على الناتج النهائي للمحصول. لهذا أصبح تحديد وإيجاد الأصناف النباتية الوراثية المتحملة للملوحة أمراً بالغ الأهمية لتحسين الناتج النهائي للمحصول. لذلك تم إجراء سلسلة من التجارب المعملية لتقييم استجابة اثنين من أصناف القمح الربيعي واثنين من أصناف القمح الشتوي (*Triticum aestivum* L) لمستويات مختلفة من الملوحة. نفذت التجارب وفق تصميم القطاعات العشوائية الكاملة بخمسة مكررات. تم زراعة 20 بذرة من كلٍّ من التراكيب الوراثية في أطباق بتري تحتوي عل ورقة ترشيح. وتم معاملة البذور بتراكيز مختلفة من المحلول الملحي (0، 50، 100، و150 مل مول من كلوريد الصوديوم). حفظت الأطباق في الحاضنة عند 20 م درجة مئوية لمدة 8 أيام. تم خلال هذه الفترة التأكيد من إضافة الماء أو المحلول الملحي لكل الأطباق وحسب التركيز المطلوب. كما تم خلال هذه الفترة تجميع البيانات الخاصة بالأنبات. بعد ثمانية أيام من الزرع تم إنهاء التجربة وتم تجميع البيانات الخاصة بنسبة الإنبات، معدل الإنبات، متوسط الإنبات اليومي، طول السويقة وطول الجذور، وزن البادرات الطازج والجاف. أشارت النتائج إلى أن الطرز الوراثية للشتاء والربيع اختلفت بشكل كبير لنسب الإنبات، ومعدل الإنبات، ومتوسط الإنبات اليومي، وطول الجذور، وأوزان الجذور الطازجة والجافة. أظهرت النتائج أن الملوحة لم تؤثر على نسبة الإنبات النهائية حتى وصل مستوى الملوحة إلى 100 مل مول من كلوريد الصوديوم. في حين إن البذور المعاملة بملوحة بتركيز 100 و150 مل مول تأخرت بها عملية الإنبات ليوم أو يومين على التوالي بالنسبة لبادرات القمح الربيعي ومن يومين إلى ثلاثة أيام على التوالي بالنسبة لبادرات القمح الشتوي، بالمقارنة مع 0 و50 مل مول من كلوريد الصوديوم. وأظهرت البيانات أيضا أن ارتفاع مستوى الملوحة، صاحبه انخفاض كبير في طول النبات وطول الجذر، ومع ذلك وجدت الدراسة أن تأثير الملوحة على نمو الجذر كان أكثر حدة من تأثير الملوحة على نمو الساق بشكل ملحوظ، وتؤكد الدراسة على أن طول الجذر والوزن الجاف للجذور صنفت النباتات بنفس الترتيب من حيث تحملها للملوحة. ولذلك، خلصت الدراسة إلى أن قياسات نمو الجذور ستكون معايير فعالة لفحص التراكيب الوراثية للقمح من أجل تحمل الملوحة في مراحل الإنبات.

الكلمات المفتاحية: الإنبات، مؤشر الإنبات، الملوحة، مؤشر تحمل الملوحة، مؤشر قوة البادرة.

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