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Breastfeeding Practices During Neonatal Period in Benghazi

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Abstract: Breastfeeding is the safest and healthiest infant feeding method; exclusive breastfeeding for the first 6 months of infant life is strongly advised. This study was conducted to assess infant feeding practice and maternal determinants of infant feeding during the first month of life. In a hospital-based cross-sectional survey at the neonatal unit and OPD of Benghazi Pediatric Hospital, mothers were selected by reason of infant age not exceeding 4 weeks. A sample of 306 mothers was collected, and the alarming results are that about (31.8%) of the mothers are either not breastfeeding their babies or practicing inappropriate early weaning (mixing). Moreover, inadequate milk production (25%) was reported as the most common cause of these practices. In addition, the role of the trustworthy education channel about breastfeeding is minimal, if not there, such as mother and child health care. Also, providers are the source of knowledge for only 2.38%. Operative delivery is associated with a marked delay in breastfeeding initiation (Pearson Chi-square =13.2, P=0.001), and older age group mothers are associated with a greater probability of operative delivery, hence it is an in-direct mediator of delayed breastfeeding initiation. These results reflect poor maternal knowledge and attitude about breastfeeding that ultimately resulted in poor practice and low rate of exclusive breastfeeding, in addition to the limited awareness of healthcare providers about the importance of early initiation of breastfeeding to encourage the mothers and guide them to breastfeed their babies immediately after birth.

ممارسات الرضاعة الطبيعية خلال الشهر الأول من حياة الرضع بنغازي

الكلمات المفتاحية : الرضاعة الطبيعية الحصرية، بدء الرضاعة الطبيعية، الولادة الجراحية، عمر الأم، معرفة الأم.

المستخلص : الرضاعة الطبيعية هي الطريقة الأكثر أمانا وصحة لتغذية الرضع، وينصح بشدة بالرضاعة الطبيعية الحصرية لأول 6 أشهر من حياة الرضع. أجريت هذه الدراسة لتقييم ممارسة تغذية الرضع ومحددات الأمهات لتغذية الرضع خلال الشهر الأول من الحياة. تم اختيار الأمهات وفقا لعمر الرضيع الذي لا يتجاوز 4 أسابيع، وذلك من خلال مسح مقطعي في المستشفى في مستشفى بنغازي للأطفال. جمعت عينة من 306 من الأمهات، والنتائج المقلقة هي أن حوالي 3/1 (31.8٪) من الأمهات: إما لا يرضعن أطفالهن، أو يمارسن الفطام المبكر غير المناسب (الخلط) بالإضافة إلى ذلك، فإن دور قناة التثقيف الجديرة بالثقة حول الرضاعة الطبيعية محدود للغاية إن لم يكن موجودا، مثل الرعاية الصحية للأم، والطفل، ومقدمي الرعاية الصحية هي مصدر المعرفة لـ 2.38 ٪ فقط. وترتبط الولادة الجراحية بتأخير ملحوظ في بدء الرضاعة الطبيعية (مربع بيرسون تشي = 13.2 ، P = 0.001)، وترتبط الأمهات الأكبر سنا من الفئة العمرية باحتمال أكبر للولادة العملية، وبالتالي فهي وسيط غير مباشر لبدء الرضاعة الطبيعية المتأخرة. وتعكس هذه النتائج ضعف معرفة الأمهات ومواقفهن بشأن الرضاعة الطبيعية التي أدت في النهاية إلى سوء الممارسة، وانخفاض معدل الرضاعة الطبيعية الخالصة، ومحدودية وعي مقدمي الرعاية الصحية بأهمية البدء المبكر في الرضاعة الطبيعية لتشجيع الأمهات، وتوجيههن لإرضاع أطفالهن رضاعة طبيعية مباشرة بعد الولادة.

INTRODUCTION

Breastfeeding is the ideal method of infant nutrition that should be started immediately after birth and be exclusive during the first four to six months of life, after which a complementary diet of foods to be given, with the continuation of breastfeeding until the age of two years. It is the optimal scheme of infant feeding that is strongly recommended by the World Health Organization (WHO) (WHO, 2003).

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Breastfeeding is the single most efficient and cost-effective activity in reducing early childhood morbidity and mortality worldwide. Breastfeeding saves the lives of 800,000 children and about 200 mothers annually. Besides being a nutrition source, breast milk contains immune-related components and various biologically active substances that contribute to efficient nutrient utilization and give the child active and passive protection against infections (Victora et al., 2016). Breast milk cannot be duplicated by any artificial means; it is unique in its composition and function, and no infant formula can even resemble a mother's milk. Moreover, breast milk changes over time, and even over the course of a day, to meet the changing needs of the growing child (Martin et al., 2002). In addition to its innumerable immediate benefits, breastfeeding has long-term advantages as it enhances school achievement, increases intelligence in adulthood, and has other social benefits as it raises productivity and earning capability (Hayatbakhsh et al., 2012). Furthermore, breastfeeding comprises plenty of benefits to mothers: it reduces the risk of breast cancer and ovarian cancer as data suggest that women who do not breastfeed their baby face a higher risk of breast and ovarian cancer, obesity, type II diabetes, metabolic syndrome, and cardiovascular diseases (Stuebe, 2009). Although the benefits of breastfeeding are well documented by many organizations, institutes, and dozens of studies (Jones et al., 2003; Nabulsi et al., 2014; Roberts et al., 2013; WHO, 2000), exclusive breastfeeding rate and early initiation of breastfeeding have not reached the desired level in many countries all over the world. Therefore, it is an area of interest for healthcare providers and researchers (Emmanuel, 2015; Jebena & Tenagashaw, 2022).

In Libya, there are some studies addressing different aspects of breastfeeding, such as the one done in Benghazi assessing the baby's correct position, attachment, and effective suckling during breastfeeding as practiced by mothers attending hospitals. They concluded that a mother aged less than 20 years and primipara mothers showed the more frequently wrong practice of breastfeeding and recommended that mothers should be observed at the onset of breastfeeding and, if needed, subsequent counselling should be given accordingly (Goyal et al., 2011). In Middle Eastern countries, much research investigated the effect of socio-demographic factors on breastfeeding. For example, in Jordan, a study concluded that breastfeeding was independent of the mother's age, father's education level, and child's gender. In addition, they found mother characteristics associated with less chance of breastfeeding are higher education, employed mothers, and mothers delivered by cesarean section (Khassawneh et al., 2006).

In developing countries such as African countries, breastfeeding can be the difference between life and death for several reasons, including poor hygiene and lack of clean water (Hörnell et al., 2013). Even though breastfeeding is reasonably common practice, in Kenya, for instance, the practice is thought to be sub-optimal, with an initiation rate of 58% within the first hour of birth. Exclusive breast feeding (EBF) is not common, as only 32% of infants below the age of six months are exclusively breastfed. Kenya National Bureau of Statistics (KNBS, 2010). In Ghana, mother's exclusive breastfeeding was affected by a few mother-related social factors, for example, mothers who work in the informal sector of employment were able to exclusively breastfeed their infants and breastfeed more than eight times in the previous 24 hours of data collection, as compared to mothers in the formal sector of employment. Moreover, family support, bed-sharing, flexible work schedules, and cultural beliefs are key factors in exclusive breastfeeding and breastfeeding frequency (Nkrumah, 2017). In Cameroon, some cultural taboos and beliefs

have a negative impact on breastfeeding practices, as traditional beliefs in that community influence mothers to practice mixed feeding. These include pressure by village elders and families to supplement due to the belief that breast milk is an incomplete food and does not increase the infants' weight (Kakute et al., 2005). Despite all the efforts encouraging mothers to lactate their babies; the lack of progress made in improving breastfeeding rates globally over a decline in breastfeeding in many communities was noted by the WHO. That is why more awareness of mothers regarding breastfeeding benefits should be considered and addressed, which will help in providing women and their children with the support and protection they are entitled to (Li et al., 2002). The aim of study were to investigate maternal characteristics regarding adherence to breastfeeding of their infants during the neonatal period, and to know the impact of socio-demographic, obstetrical, and perinatal characteristics on breastfeeding.

MATERIALS AND METHODS

Study design: cross-sectional study Sampling and settings hospital-based convenience samples were drawn during the period from March 2016 to September 2017.

Data collection tool: A questionnaire was used to collect the data from mothers visiting the neonatal outpatient department of Benghazi Paediatric Hospital. The questionnaire contains about 17 questions. Part of them inquires about mother characteristics like age, education level, health, and medications. Another part asks about early infant feeding practices such as time of starting breastfeeding, frequency of feeding, and giving of other feedings besides breast milk, and the last part is informing about delivery related criteria like mode of delivery, gestational age and birth order.

Inclusion criteria:

1- Libyan nationality of the mother.

2- The baby's age is less than 4 weeks.

Statistical software and analysis: The data was entered into SPSS version 21 for analysis, and quantitative data were summarized to list the descriptive statistics in the form of a range, mean, and standard deviation for continuous variables and count and percentages for discrete variables with plotting of graphs and curves when necessary. Inferential statistics in the form of correlation assessment and Pearson chi-square were used to find the explanatory variables (factors) that might influence the variation in breastfeeding practice of the studied sample.

Ethical issue: Verbal permission was confirmed prior to data collection from all participants, the study holds no risk or harmful procedure for the study sample, and their privacy is secured as the data is entered anonymously.

RESULTS

Maternal characteristics: This study included data from 306 mothers. The age distribution of the sample is of three age categories; 22.2% of them are less than 25 years, 49% are of age from 25 to 35 years, and 28.8% are above 35 (figure 1). Most of the mothers (81.7 %) are with high education level, followed by primary education (15.4%), and the least (2.9%) for illiterate mothers. Some mothers (22.5%) are experiencing motherhood for the first time. 21.9 have three children followed by 19.6 have two children, and only 13 mothers have seven children or more.

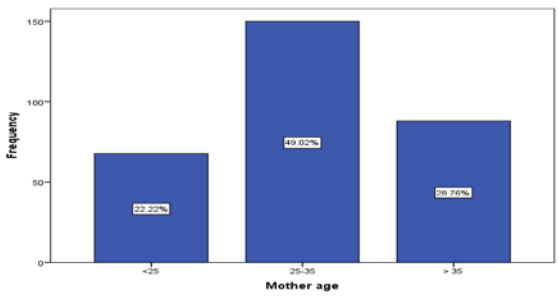

Figure: (1). Mother age distribution of the study sample.

Mothers with previous experience of maternity were asked about breastfeeding their youngest infants (not the current one), their answers were 155 (65.1 %) mothers gave a history of breastfeeding, 62 (26.1%) said that they mix breastfeeding with other feedings, and only 21 (8.8%) said that they did not breastfeed their infants. About 68% of the mothers breastfed their current infants, while 25.9 % gave breastfeeding besides other feedings, and only 5.9% of them are not lactating. In addition, 65% of them breastfed their infants at a regular frequency (figure 2). The time of breastfeeding initiation after delivery is variable. 64.59% of the mothers started breastfeeding after more than one hour, and the remaining 35.41% started correctly within the first hour of giving birth of their babies (figure 3).

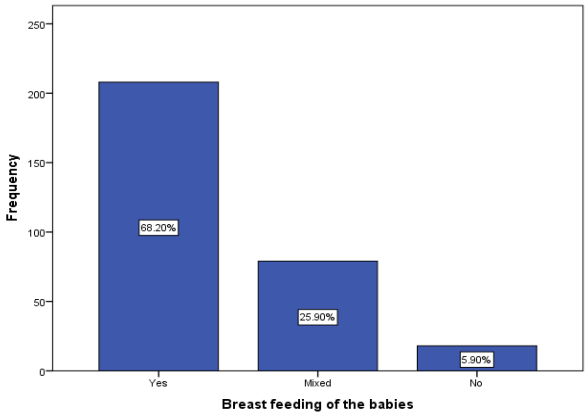

Figure: (2). Mode of infant feeding exclusive breastfeeding, mixed, or no breastfeeding

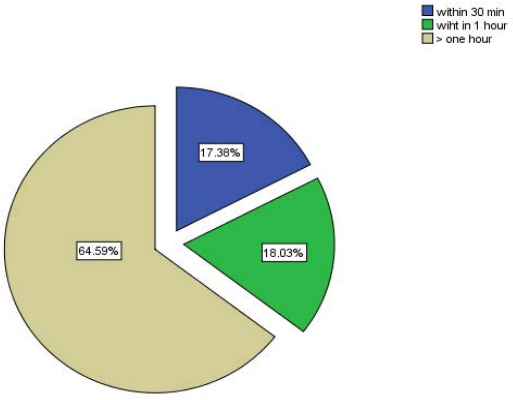

Figure: (3). Time of starting breastfeeding immediately after the birth of the infants

Some mothers included in this study (5.9%) quit breastfeeding their infants, one quarter (25%) of the mothers stopped breastfeeding because of not producing enough milk, another 25% stopped due to infant illness and admission, and 18.75% said that their infant refused to breastfeed (figure 4).

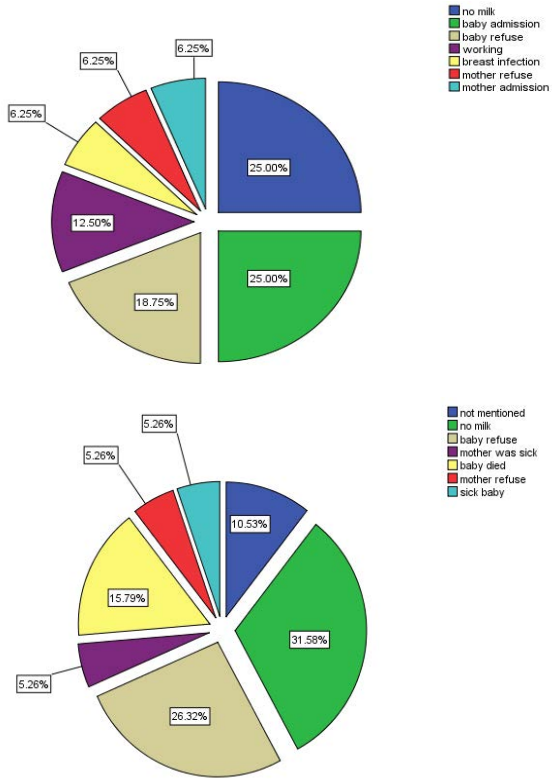

Figure: (4). Causes of breastfeeding stopping for the current baby (left) and previous baby (right)

While the mothers who continued breastfeeding along with other food (early and inappropriate weaning) asserted different causes of mixing, the most commonly reported cause is “not producing enough milk” as it was reported by 38 mothers (48.1 % of those who mixed). Mother working was the second most common cause of mixing (17 mothers, 21.5%). Other reasons might be related to the baby, such as infant illness and hospital admission (4 cases), twin infants (3 cases), baby refusing or not accepting breast milk (2 cases), and cleft lip and palate (1 case). Mother-related factors for mixing are cracked or retracted nipples (4 mothers) and mothers with one breast (2

mothers). Few cases mentioned no reason (Figure 5).

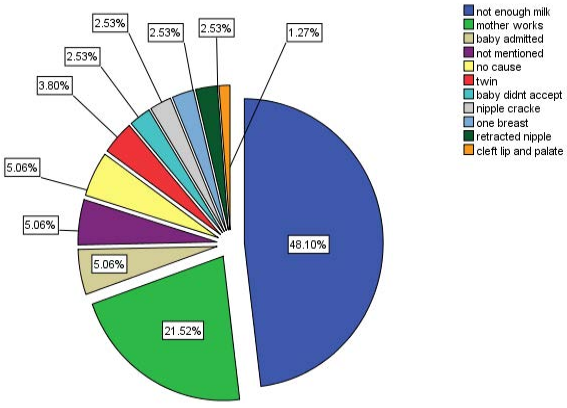

Figure: (5). Causes of mixing feeding of the current baby of the study sample

To compare the practice of breastfeeding of the mothers with other infants, data about the causes of stopping (figure 4) of the previous infant feeding were recorded. Generally, there is a very large similarity between the causes of stopping or early weaning between the current and previous baby, as 31.58% of the mothers quit breastfeeding the previous baby because of not producing enough milk, followed by 26.32% reporting the baby refusing. Whereas the top causes of mixing of previous baby feeding were not producing enough milk and mother work in percentages of 33.33% and 31.67% respectively.

Most of the previously studied (96.1%) mothers have knowledge about the importance of breastfeeding from different sources: the community, their mothers, sisters, mothers-in-law, etc. The other sources of knowledge include their school education, books, obstetricians, pediatricians, and media.

Infant's characteristics: The infants of this study are of age 4 weeks or less. In addition, the great majority of the infants of the mothers included in this study are full term and delivered with good health, as they represent 92.8%. Preterm infants are only 6.9% of the study sample and were not healthy at birth. The mode of delivery is either normal vaginal in 54.9% or cesarean in 44.8 of the study sample. Moreover, 72.2 % of the infants had a birth weight between 2.5-4 kg, see (Table 1).

Table: (1). Infants characteristics of the study sample

Characteristic		Frequency	Percent (%)	Missing (count & %)
Maturity	Full term	284	92.8	1 (0.3%)
	Preterm	21	6.9	
Mode of delivery	Vaginal	168	54.9	1 (0.3%)
	Cesarean	137	44.8	
Birth Weight (kg)	1-1.4	7	2.3	1 (0.3%)
	1.5-2.4	64	20.9	
	2.5-4	223	72.9	
	>4	11	3.6	
Vac-cination	Yes	228	74.5	1 (0.3%)
	No	77	25.2	
Use of tradi-tional therapy	Yes	14	4.6	1 (0.3%)
	No	291	95.7	

Correlation and chi-square assessment: Bivariate correlation was done to determine the factors affecting breastfeeding initiation. The time of starting breastfeeding is significantly associated with the mode of delivery ($p<0.001$). Mothers who delivered by cesarean section had a significant delay in breastfeeding initiation (chi-square = 13.2, degree of freedom =2, p= 0.001, see Table 2 and Figure 6).

Table: (2) Cross tabulation for breastfeeding initiation according to a mode of delivery

Mode of delivery	Time of breastfeeding initiation				Chi-square
	Within 30 min	Within 1 hour	>One hour	Total	Pearson Chi-square = 13.17 Degree of freedom= 2 p-value 0.001**
Normal vaginal	38	36	94	168	
Caesarean	15	18	103	136	
Total	53	54	197	304	

** Highly significant result (P value < 0.05).

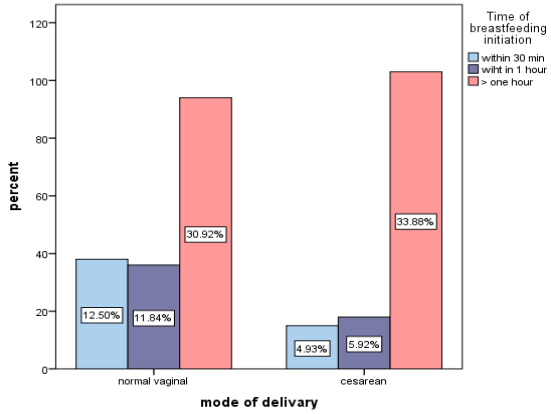

Figure: (6). Time of breastfeeding initiation according to the mode of delivery of the study sample

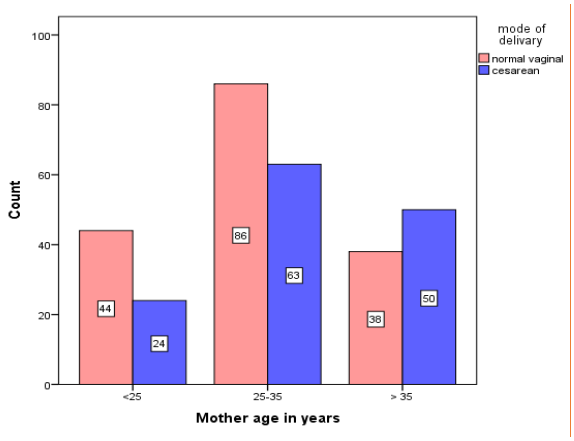

Figure: (7). Distribution of mode of delivery according to the mother age of the study sample

Whereas the mode of delivery is related to the mother's age ($p<0.001$). Therefore, the mother's age is indirectly related to the time of breastfeeding initiation through its correlation with a mode of delivery. Consequently older mothers tend to deliver by cesarean section more than younger mothers, a cesarean mode of delivery is associated with late breastfeeding initiation (Table 3 and Figure 7). Other factors such as maternal age, mother education level, infant weight, and infant gestational age were tested to find if they have any effect on the time of initiation of breastfeeding and all were found to be insignificant determinants.

Being a new mother or having other children is not significantly associated with a time of breastfeeding initiation, mode of delivery, or mode of infant feeding.

Table: (3) Cross tabulation of a mode of delivery according to maternal age

Mother's age (years)	Mode of delivery			Chi-square
	Normal vaginal	Caesarean	Total	Pearson Chi-square = 8.001
< 25 y	44	24	68	
25-35	86	63	149	
>35	38	50	88	Degree of freedom= 2
Total	168	137	305	p-value 0.018*

*Significant result.

DISCUSSION

Mother characteristics associated with successful breastfeeding practice are an important area to be studied. This study investigated maternal factors determining the initiation of lactation after delivery and the causes of mixing and stopping breastfeeding. About two-thirds of the study sample (around 65) reported late starting of breastfeeding more than one hour after giving birth. The demographic characteristics of the sample of mothers, such as maternal education level, showed an insignificant impact on the time of breastfeeding initiation post-delivery. Obstetrical factors such as mode of delivery are found to be an important determinant of breastfeeding initiation as cesarean section. The mode of delivery is significantly associated with late initiation of lactation compared to normal vaginal delivery. Conversely, a study done in the USA found that cesarean delivery was not associated with breastfeeding initiation. However, they concluded that breastfeeding practices after a first birth are a significant predictor of breastfeeding in subsequent births (Sutherland et al., 2011).

According to the result of the present study, parity has been found to be insignificantly associated with breastfeeding initiation. However, a previous study found that a first-time mother is less likely to practice

exclusive breastfeeding compared with her multiparous counterpart, suggesting that previous breastfeeding experiences have an important role in shaping the current feeding practice, (Februhartanty, 2008) Not producing enough milk is the most frequently reported cause of cessation of breastfeeding among the sample of this study. This result was similar to that of a study in Saudi Arabia, as they found that 49.6% of the studied population reported that inadequate breast milk is the most common cause of mixing or stopping breastfeeding (Al-Hreashy et al., 2008).

These results reflect the reality of wrong knowledge and practice of mothers as it is well known that milk production during breast-feeding is increased by breastfeeding frequently due to the effect of infant suckling in the stimulation of milk production so that the more frequently breastfed infant is associated with more milk production and vice versa. Agreeing with this fact, a study in the United Kingdom showed that exclusively breastfed infants were required to be fed more frequently than infants who were bottle or mixed-fed (Casiday et al., 2004). Mother work is another frequently mentioned obstacle against the good practice of breastfeeding among the sample of this study, as they need to mix infant feeding due to their absence while they are at work (Kamal et al., 2021).

In Ghana, a study done to find the effect of maternal work on exclusive breastfeeding practice concluded that mother work has been identified as one of the factors that negatively impact exclusive breastfeeding in the first six months of life. They found that mothers engaged in the formal sector of employment are unable to exclusively breastfeed after maternity leave because facilities at their workplaces and conditions of work do not support exclusive breastfeeding (Nkrumah, 2017) Another reported cause of infant feeding mixing is multiple births which is a known cause of inappropriate infant feeding. A study revealed that insufficient milk for the twins and time for breastfeeding are common causes of early cessation of breastfeeding among mothers of twins (Damato et al., 2005). Though most of the mothers (96%) included in this survey have some knowledge of breastfeeding, it is inadequate, and for others, not correct because the source of knowledge might be not reliable, as more than 68% of mothers rely on the surrounding community, as their mothers, sisters, mothers-in-law, etc., while the role of a formal reliable channel of health education seemed to be very limited, as obstetricians or antenatal care providers represent the source of knowledge for only 2.4% of the sample, this result reflects poor knowledge and subsequently, incorrect practice of breastfeeding.

Searching the previous studies demonstrates that few studies have been done on sources of information and the influence of maternal knowledge on the practice of breastfeeding. Generally maternal prenatal intention to breastfeed has an impact on infant feeding practices, so high intention and self-efficacy increase the likelihood of breastfeeding for 6 months. Therefore, all women should be guided to plan for breastfeeding their children in the antenatal period (Donath et al., 2003) The basic criteria of the sample of this study show an insignificant association with breastfeeding initiation. However, maternal age was found to be indirectly associated with breastfeeding initiation through its association with the mode of delivery. Consequently, older age mothers of this sample were predominantly delivered operatively compared to younger age ones, and mothers with operative delivery showed a significant delay in breastfeeding initiation for more than one-hour post-delivery. This result partly agreed with the available literature on this concern, as it is generally suggested that the relationship between maternal age and infant feeding practices differs according to the place of study. For example, one study found that older age is associated with

low rates of exclusive breastfeeding compared to younger mothers (Brown et al., 2011). On the other hand, other studies demonstrated that older maternal age is associated with exclusive breastfeeding and longer duration of breastfeeding (Bolton et al., 2009).

CONCLUSION

To sum up, maternal knowledge, attitude, and practice about breastfeeding is an extremely important domain of mother and child health in any community. This study showed poor results regarding breastfeeding practice during the first 4 weeks of infant life, many infants are fed incorrectly, as those who were breastfeeding represent only about two-thirds of the sample, and the others either have mixed feeding or are not breastfeeding, these figures during infancy are greatly disappointing.

Most mothers know about breastfeeding. However, the source of knowledge is not trusted. Therefore, the clearly wrong practice of infant feeding was detected within this sample. Operative labor is the single detected cause of late breastfeeding onset, whereas older mothers are more frequently delivered by cesarean section. Therefore, older mothers might show a later onset of breastfeeding due to operative delivery.

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Author contributions: Contribution is equal between authors.

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Rehabilitation Followings Surgical Fixation of Bimalleolar Fractures

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Abstract: Fractures of the ankle are the commonest type of lower limb fractures and one of the most not unusual type of fractures all over the world. Purpose was to ascertain how powerful and safe are interventions that are used for rehabilitation following surgical treatment of bimalleolar fractures. This was a prospective study conducted on 60 individuals who were subjected to surgical fixation of bimalleolar fractures within the period of 2015 to 2017 at Aljalaa trauma hospital in Benghazi - Libya. The patients were randomly divided according to the sort of rehabilitation protocol into; Group A: who commenced early weight bearing (EWB) by below knee orthosis, and Group B: who began non-weight bearing (NWB) exercises by means of axillary crutches. Olerud Molander Ankle score (OMAS) was used to assess clinical results. Group A had higher OMAS rating than group B but out a significant statistical difference, and there was no significant difference regarding SF-36 scores. No significant difference between early weight bearing rehabilitation and non-weight bearing one as regard functional outcomes. Considering patient's comfort, early functional improvement, and the low complication rate, early postoperative weight bearing and range of motion (ROM) exercises are recommended in subjects with surgically treated ankle fractures.

التأهيل بعد العلاج الجراحي لكسور الكاحل

الكلمات المفتاحية:
مقيـ . . . .اس Olerud Molander للكاحـ . . ل؛ تحميل بدون الوزن؛ إعادة تأهيل؛ كسور الكاحل.

المستخلص: كسور الكاحل هي النوع الأكثر شيوعًا لكسور الأطراف السفلية ومن بين أكثر أنواع الكسـ ور شيوعًا في جميع أنحاء العالم. وكان الهدف هو تحديد فعالية وسلامة التدخلات المستخدمة لإعادة التأهيل بعد الترجيع المفتوح والتثبيت الداخلي لكسور الكاحل. حيث كانت هذه تجربة عشوائية محكومة مرتقبة أجريـ ت على 60 مريضًا خضعوا لترجيع مفتوح وتثبيت داخلي لكسور الكاحل ثنائية القطب في الفترة بين 2015 م –2017 م في مستشفى الجلاء للحوادث بنغازي – ليبيا. تم تقسيم المرضى حسب طريقة إعادة التأهيل إلى؛ المجموعة (أ): الذين بدأوا في التحميل المبكر للوزن (EWB) باستخدام دعامة أسفل الركبـ ة، والمجموعـ ة (ب): الذين بدأوا التمارين بدون تحميل للوزن (NWB) وذلك باستخدام العكازات الإبطية. وتـ م اسـ تخدام مقياس Olerud Molander للكاحل (OMAS) لتقييم النتائج السـ ريرية. وكـ ان OMAS أعلـ ى فـ ي المجموعة (أ) مقارنة بالمجموعة(ب) ولكن بدون فرق معتد به إحصائيًا، ولم يكن هناك فرقـ ا كبيـ را فيمـ ا يتعلق بنطاقات SF-36. لا يوجد فرق كبير بين إعادة التأهيل بتحميل الوزن المبكر وبين إعادة التأهيل بدون تحميل الوزن فيما يتعلق بالنتائج الوظيفية. وبالنظر الى راحة المريض، والنتائج الوظيفية المحسّنة مبكـ رًا، ونقص معدل المضاعفات المتزايد، نوصي بتمارين تحميل الوزن في وقت مبكر بعد الجراحة ومدى الحركة (ROM) في المرضى الذين يعانون من كسور الكاحل المعالجة جراحيًا.

INTRODUCTION

Fractures of the ankle are the commonest type of lower limb fractures and one of the most not unusual types of fractures everywhere with occurrence between 100 and 150 per 100,000 person/y and rising. More than half occurs during sport activities, revealing a healthy patient population (Reiner et al., 2013). The charges

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are high for the patients and community. The burden of these injuries is associated with surgery, hospitalization, and the time wasted in occupational incapacity. To minimize these prizes, early functional return is important (Del Buono et al., 2013). The preliminary non-surgical or surgical treatment of bimalleolar fracture serves to maintain the ankle anatomy and stability. Immobilization may assist to offer ideal conditions for fracture to heal. It contributes to decrease chance of malunion or nonunion. However, immobilization additionally increases the threat of joint stiffness, weakness, swelling and residual pain. Whilst immobilized, load-bearing is either limited or not allowed, with resultant generalized decrease of movements and activity. Rehabilitation is geared toward enhancing involvement in job and luxurious hobbies, regaining movement and minimizing pain and other disabilities (Canton et al., 2021).

The surgical indications are determined by the conformity of the joint. If joint is incongruent, fractures are usually managed by surgical methods to provide stability to the joint. The postoperative management protocols range notably from strict confinement in a plaster cast without load-bearing lasting for many weeks to prompt post-operative guarded mobilization. Immediate post-operative unprotected weight-bearing (WB) as permitted has been advised with caution (Güngör et al., 2022).

Many systemic audits were posted on special perspectives of postoperative management protocols, all failed to obtain distinct decision for the postsurgical regimen of bimalleolar fractures as they vary with respect to protection, ankle motion, and bearing weight. Non-randomized studies were implicated in these reviews and meta-analysis to potentiate displaying how effective and safe are the interventions which might be applied for rehabilitation following internal fixation of ankle fractures. Moreover, certain perspectives of the post-operative protocols were put in comparison.

MATERIALS AND METHODS

This study is a prospective randomized controlled trial was conducted on a total of 60 individuals who were subjected to open surgical treatment of bimalleolar ankle fractures in the period between 2015 to 2017 at Al Jalaa Trauma Hospital in Benghazi - Libya. Inclusion criteria were: mentally and psychologically well and physically active patients, non-osteoporotic bone, body mass index (BMI) around 26, and closed fractures treated by open reduction and rigid internal fixation. Exclusion criteria were: geriatric and pediatric age groups, all open injuries, pilon fractures, multi trauma patients, trimalleolar fractures, bimalleolar fractures associated with foot fracture, bilateral ankle fractures, and patients with neuropathic and inflammatory joint diseases. There was 49 males and 11 females with median age of 35 years. To compare postoperative outcome, patients were assigned randomly to 2 patches; group A who started early weight bearing (EWB) by below knee orthosis, and group B who started non-weight bearing (NWB) exercises by using axillary crutches.

Patients were evaluated using clinical scoring system called modified Olerud and Molander ankle score modified from original score (OMAS) in 1984, which has the following scale:

Pain: no pain 25, mild (weather liable) 20, while sporting 15, when walking on normal ground 5, fixed and relentless 0.

Stiffness: none 10, morning stiffness 5, and constant stiffness 0.

Swelling: none10, evening time only 5, and persistent 0.

Stair-ascending: no problems10, affected 5, and not possible 0.

Sports: normal10, affected 5, and not possible 0.

Supports: no support 10, bandaging 5, and walking aid 0.

Ordinary activity and job: not changed 25, loss of speediness 20, shift to less demanding job 10, and severe work impairment 0.

The results were collected, and arranged in tables and subjected to statistical analysis by means of SPSS 22.0 for windows. Statistics testing for normal distribution was achieved using the Shapiro Walk test. Frequencies and relative percentages represented the qualitative variables. The difference between qualitative items was determined by $\chi 2$ test and Fisher test. The mean ± SD presented the quantitative parametric variables, and the median and range expressed the non-parametric variables. The difference for quantitative parametric items was extracted using independent t test and for quantitative non-parametric items Mann Whitney test was applied. To statistically compare the results P-value was used for level of significance, when P-value ≤ 0.05 this means significant, if p <0.001 it means highly significant difference but when P > 0.05 there is non-significant difference.

Nature of the intervention: Many interventions for rehabilitation are applied to manage the complications of bimalleolar fracture and immobilization. Rehabilitation can start while patient is immobilized, where the patient may begin early passive or active movements, or early weight bearing. Instead, rehabilitation can commence after the period of immobilization, where interference may involve exercise and manual therapy. Manners of immobilization entail casts (made from plaster of Paris or synthetic substances), half casts in form of back slabs, and orthotic devices (braces), and these were recognized as a part of the rehabilitation for our study where they may enhance or limit the initiation of other interventions during the immobilization period, like walking with weight bearing (WB exercise) (Deng et al., 2022).

RESULTS

The mean age of patients in group A was 40.51 ± 10.45 years, 24 patients (80%) were males, and 6 (20%) patients were females with mean BMI was 26.12 kg/m^2. Meanwhile, the mean age of patients in group B was 37.64 ± 9.35 years, 25 patients (83.3%) were males, and 5 (16.7%) were females with mean BMI was 25.74 kg/m^2. Thirty-two patients came from rural areas and 28 were residents in urban areas. There was no significant difference between the groups regarding demographic data (Table 1).

Group A included 3 (10%) diabetic, and 4 (13.3%) hypertensive patients, with 13 (43.3%) were smokers. Group B had, 5 (16.7%) diabetic, and 3 (10%) hypertensive patients, with 11 (36.7%) were smokers. However, no significant difference was seen (Fig.1).

Table: (1). Socio-Demographic characteristics

		Group A (n=30)	Group B (n=30)	t / χ^2	P
Age (years) Mean ± SD		40.51 ± 10.45	38.64 ± 9.35	0.730	0.468
Gender	Female	15 (50%)	15 (50%)	0.127	0.639
	Male	15 (50%)	15 (50%)		
Residence	Rural	15 (50%)	17 (56.7%)	0.268	0.605
	Urban	15 (50%)	13 (43.3%)		
BMI (kg/m^2) Mean ± SD		26.12 ± 3.57	25.74 ± 2.96	0.449	0.655

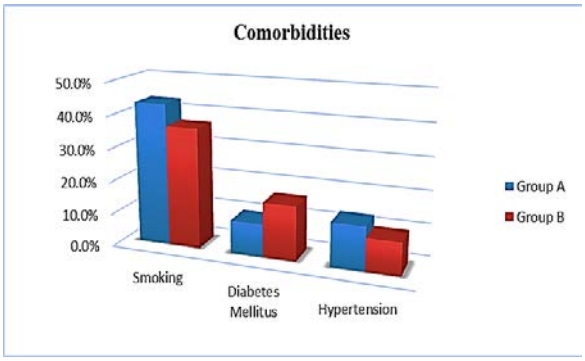

Figure: (1). Comorbidities distribution

Regarding group A, 21 patients (70%) had right side affected, and 9 patients (30%) had left side, with cause of fracture was direct fall in 10 patients (33.3%) and RTA in 7 patients (23.3%) and sports trauma in 13 patients (43.3%). In group B, 18 patients (60%) had

right side affected, and 12 patients (40%) had left side, with fracture caused by direct fall in 9 patients (30%), RTA in 6 patients (20%) and sports trauma in 15 patients (50%). However, P value stwo study groups (Table 2).

Table: (2). Fracture characteristics

Side	Group A (n=30)	Group B (n=30)	χ^2	P
Rt.	21 (70%)	18 (60%)	0.417	0.659
Lt.	9 (30%)	12 (40%)		
Cause				
RTA	7 (23.3%)	6 (20%)		
Fall	10 (33.3%)	9 (30%)	0.272	0.873
Sports	13 (43.3%)	15 (50%)		

According to Lauge-Hansen classification, group A had 13 patients (43.3%) with supination-external rotation (SER), 3 patients (10%) with supination-adduction (SA), 9 patients (30%) with pronation-adduction (PA), and 5 patients (16.7%) with pronation-external rotation (PER), while group B had 12patients (40%) with SER, 5 patients (16.7%) with SA, 10 patients (33.3%) with PA, and 3 patients (10%) with PER. No significant difference was found between the groups.

Group A had higher OMAS rating than group B but out a significant statistical difference (Table 3). No statically significant difference was seen between the two groups with respect to SF-36 scores (Table 4).

Table: (3). Clinical evaluation using Olerud Molander ankle score (OMAS)

OMAS	Group A (n=30)	Group B (n=30)	t	p
After 6 weeks Mean± SD	52.1 ±20.56	43.51 ± 21.75	1.57	0.121
After12weeks Mean± SD	70.24 ±14.73	65.83 ± 18.47	1.02	0.311
After 6 months Mean± SD	87.2 ± 13.54	80.49 ± 17.67	1.65	0.104

Table: (4). Postoperative SF-36

	Group A (n=30)	Group B (n=30)	t	p
Physical functioning Mean ± SD	76.28 ± 16.73	74.63 ± 21.54	0.331	0.742
Role-physical functioning Mean ± SD	28.67 ± 36.85	24.27 ± 35.61	0.471	0.640
Bodily pain Mean ± SD	81.22 ± 17.3	76.32 ± 18.31	1.07	0.291
General health perception Mean ± SD	79.44 ± 18.62	81.73 ± 14.61	0.530	0.598
Fatigue Mean ± SD	75.61 ± 15.45	74.55 ± 16.29	0.259	0.797
Social functioning Mean ± SD	86.57 ± 22.34	84.9 ± 20.26	0.303	0.763
Emotional functioning Mean ± SD	13.78 ± 24.37	10.92 ± 32.68	0.384	0.702
Mental health Mean ± SD	84.64 ± 12.18	81.57 ± 15.34	0.858	0.394

Complications seen in group A included one patient (3.3%) with superficial infection, and in group B included 3 patients (10%) with superficial infection, one patient (3.3%) with reflex sympathetic dystrophy (RSD), and one patient (3.3%) with deep venous thrombosis (DVT). However, P value did not show significant difference among the two groups.

With respect to the type of fixation in lateral or medial malleolus P value did not show significant difference between the group A and group B (Table 5 and Fig.2).

Table: (5). Fixation types of lateral and medial malleolus

Lateral malleolus fixation:	Group A (n=30)	Group B (n=30)	χ^2	p
2x screws fixation	3 (10%)	2 (6.7%)	0.311	0.856
Screw fixation and neutralization plate	19 (63.3%)	17 (56.6%)		
Neutralization plate	6 (20%)	4(13.3%)		
Dorsolateral buttress plate	2 (6.7%)	7(23.3%)		
Medial malleolus fixation:				
1x screw fixation	2 (6.7%)	2 (6.7%)	2.75	0.601
1x screw fixation and K. wire	20(66.6%)	23 (76.6 %)		
2x screws fixation	2 (6.7%)	1 (6.7%)		
Not applicable/no fixation required	6 (20%)	4 (13.3%)		

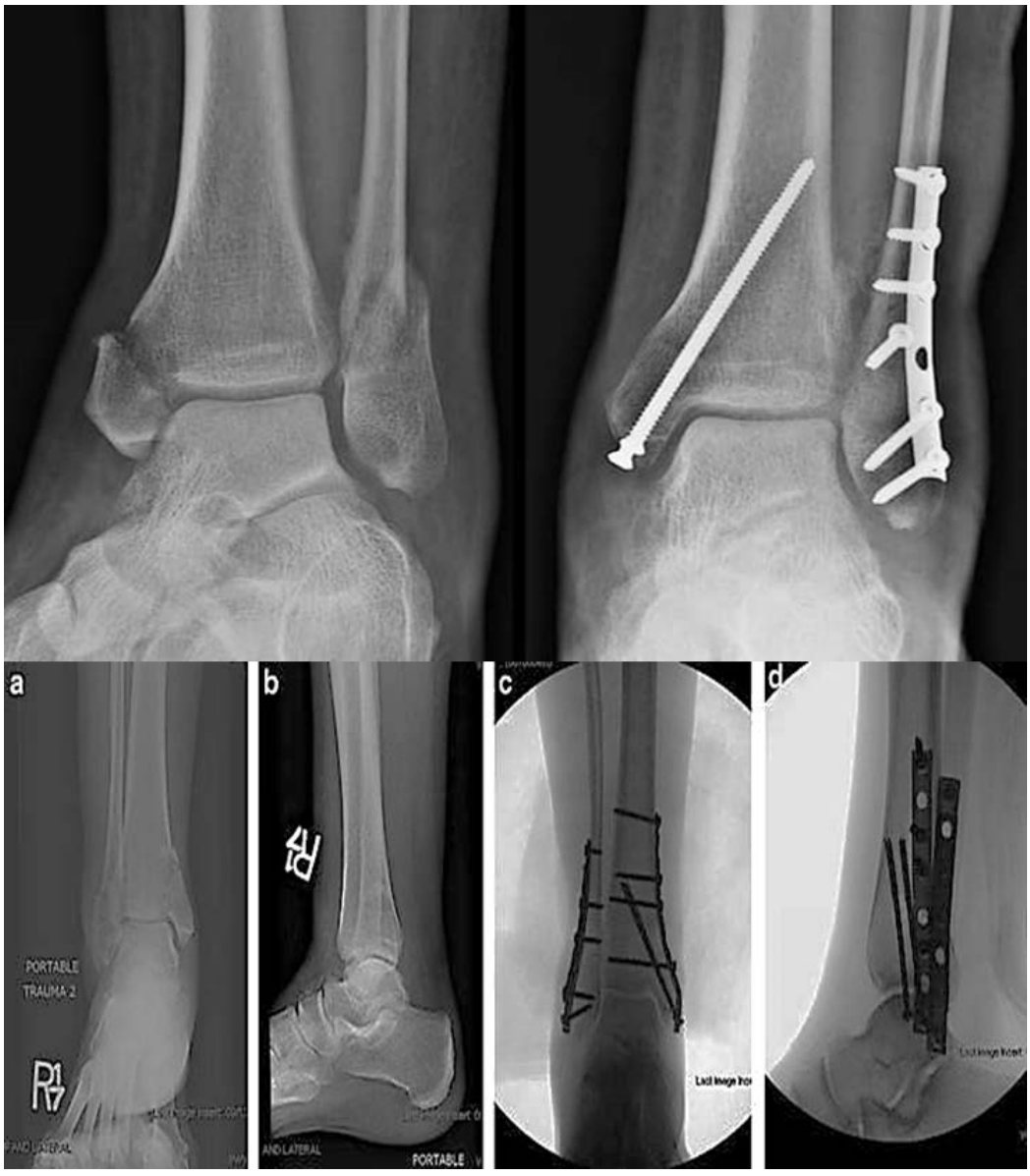

Figure: (2). Bimalleolar fractures treated by open reduction and internal fixation using isolated screws or plate with screws for medial malleolus, and plate and screws for lateral malleolus.

DISCUSSION

In this study, we aimed to decide how powerful and safe are interventions that are used for rehabilitation following internal fixation of bimalleolar fractures. Majority of patients with bimalleolar fractures, whether surgically fixed or dealt with non-operatively, have a tendency to bitch of stiffness, edema and pain which is referred to as 'fracture disease'. This might be attributed to pain and the deprivation of joint from normal physiological stresses, which ends by stiffness. It has been seen that as much as 77% of affected individuals suffer from ankle stiffness following elimination of cast in bimalleolar fractures. Most of these sequelae take place in the first two of weeks of immobilization. Even though the evidence is conflicting, most researches point to the fact that early rehabilitation following ankle fractures might prevent these sequelae in the short period. Prolonged inspections did not reveal significant differences regarding early mobilization and immobilization following bimalleolar fractures. Nevertheless, most studies that favor early active rehabilitation program as superior relate the results from subjects treated in cast postoperatively relative to subjects who adopted early range of motion exercises program. The knowledge is scarce on the importance and effects of different standardized rehabilitation programs.

In a recent retrospective-controlled trial of multicenter origin conducted by Smeeing et al. to study three interventions for rehabilitation: unprotected NWB, protected WB as tolerated and unprotected WB after surgical fixation of ankle supination external rotation injury only. The published post hoc analysis embraced 115 participants in the three interventions. They concluded that the unprotected WB mobilization regimen enhanced short-term functional results without increasing complication rate (Smeeing et al., 2015). This study was included at interim analysis, and reported that the mean age of the involved subjects is 39.0 (± 14.4) years, with range of 18 - 65 years, 61 of which (53.0%) are males. This is compatible with our results. In comparison with our findings, the trial of Latham et al. intended to know if exercise done at home with minimal ground contact supervised by physiotherapist increased the hip score after termination of conventional hip fracture rehabilitation program, and showed that at recruitment, the value for mean age of subjects is 78 years (SD ± 9.9 years); 69% are females. Based on age, baseline function, or gender there were no significant differences between individuals who disappeared to follow-up (53 pts.; 27 belong to control and 26 to intervention group) and individuals who finished the follow up at 9 months. Those disappeared from follow up constituted 21.6% of the randomized sample (Latham et al., 2014).

In line with our findings is Zeng's study of 160 ankle fracture patients (58 males and 102 females, aged 41.71 ± 14. 51 years) undergoing open reduction and internal fixation, grouped into the non-enhanced recovery after surgery or enhanced recovery after surgery based on the treatment procedure. The statistical data (sex, age, fracture type, dislocation, and associated diseases) were similar between both cohorts with p value >0.05 (Zeng et al., 2022). Current preferences in early rehabilitation challenge with conventional NWB concept and no strong consensus to clarify the optimum intervention. This perhaps is reflected by the confronting publications referring to the evaluation of WB programs after bimalleolar fracture surgical treatment (Swart et al., 2015). Some studies have investigated three types of interventions after bimalleolar fracture surgical fixation: non weight bearing early mobilization, early weight-bearing mobilization and immediate weight-bearing mobilization, a few reported encouraging results, while others warned about elevating complication levels (Kearney et al., 2021). In our study, OMAS was higher in group A (WB group) compared to group B (NWB group) but without statistically significant difference. Earlier trials involving even younger population demonstrated improved performance

when subjected to OMAS a year following the fracture in contrast to the findings in the current research. A mean of 91 points was found by Tropp in a collection of 30 patients having unimalleolar/ bimalleolar/ or trimalleolar fractures with an average age of 26 (Tropp & Norlin, 1995). Egol and Nilsson disclosed that patients older than 40 have extended possibility of less functional score after one year of surgery and even three years later (Egol et al., 2000; Nilsson et al., 2003). The results of OMAS for ages more than 40 years were almost similar to what we found in our study. These results promote the findings that younger ages achieve superior outcome and that adult having identical fractures better not be considered as a congruent group. The age must be regarded as an important issue when evaluating consequences after ankle fracture.

In this study, there was no significant difference between the groups regarding the SF-36 scores. Segal reported that SF-36 ratings showed significant differences among 3 ankle fracture groups (uni/bi/tri malleolar) and normal individuals in all 8 sub-test groups and in the summarizing scores (physical health summary; PHS and mental health summary; MHS) but no significant variations among ankle fracture groups in all SF-36 sub-test groups (Segal et al., 2014). This goes in harmony with our findings. In his systemic review Black revealed significantly better results for many parameters including improved early dorsiflexion, time to FWB, early come back to work and briefer hospitalization (patient aged <60 y.) in the early WB group (Black et al., 2013). This is in accord with our results. Also, in line with our findings, Latham reported that no critical negative events encountered that were attributed to the intervention. One negative occurrence that might be attributed to the interventional procedure is mild to moderate pain. Pain is regarded as an adverse event when it continued longer than 48 hours and when candidates had to adjust their daily activities or have medicine. Twenty-three patients in the intervention group experienced pain of mild to moderate severity. Both groups had numerous health issues that were irrelevant to interventions included in the study, this is compatible with high level of comorbid conditions in this community (Latham et al., 2014). A study conducted by Dehghan showed that no difference in return to work (RTW). At 6 weeks postoperatively, EWB subset had notably increased ROM of ankle (41 against 29, with $P < 0.0001$); OMAS (45 against 32, with $P = 0.0007$), and SF-36 ratings on the objective (51 against 42, with $P = 0.008$) and intellectual (66 against 54, with $P = 0.0008$) elements. However, no differences were seen related to infections or surgical site problems, and no failure of implants or redisplacement were encountered. The patients who started delayed WB got more sessions of scheduled or executed surgery for implant extraction as a result of plate irritation (19% against 2%, with $P = 0.005$) (Dehghan et al., 2016). This augments our conclusion in the present study. Vioreanu reported that the patients who started earlier movement with a portable cast got improved clinical score ratings (OMAS and American Orthopedic Foot and Ankle Score; AOFAS) at 9 and 12 weeks postoperatively, and resumed work activity earlier (after 67 days) compared with patients who started NWB in the below - knee cast (after 95 days), with $p < 0.05$. No statistical difference is seen among the two groups at 6 months regarding life quality (SF-36 Survey). The early mobilization cohort had postoperative infection rate of 10% (one superficial and two deep) (Vioreanu et al., 2007).

CONCLUSION

This study revealed no significant difference between WB rehabilitation and NWB one as regard functional outcomes. Considering patient's comfort, early functional improvement, and the low complication rate, early postoperative WB and ROM exercises are recommended in subjects with surgically treated ankle fractures. However, both the surgeon and the patient have to be alert of

the potential hazard of wound problems linked to this method of treatment, so the accelerated rehabilitation protocol should be tailored to patient. Further studies on larger sample size and large geographical scale are suggested to emphasize our findings.

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Complications of ventilation tube insertion in otitis media with effusion

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Abstract: Otitis media with effusion (OME) is the accumulation of fluid in the middle ear space without any symptoms of inflammation. It leads to decreased hearing and delayed speech in children. It is treated by ventilation tube insertion. These tubes may have complications itself like otorrhea, eardrum atrophy, perforation, serous otitis media, and tympanosclerosis. The aim of this study is to evaluate the complications of ventilation tube insertion in 100 cases done at specialty surgical center of otolaryngology/Benghazi over a period of one year (January 2021 to December 2021). Complications was seen in 42% of the cases, the most frequent complication was otorrhea followed by tympanosclerosis. the least encountered complication was tympanic membrane atrophy. Serious complications may be seen in some cases like cholesteatoma but it was not reported in this series. There was no statistically difference between male and female in terms of post-operative complications. It is advised to follow up these operated cases regularly to diagnose any complication and treat it early and properly.

مضاعفات زراعة انابيب التهوئة في حالات ارتشاح الاذن الوسطي

الكلمات المفتاحية :
ارتش . . . اح الاذن الوسطي،
زراع . ة انابي . ب التهوئة،
مضاعفات.

المستخلص : ارتشاح الاذن الوسطي هو تجمع سائل في تجويف الاذن الوسطي دون وجود علامات التهاب به ا. يصيب الكبار والصغار ولكنه أكثر شيوعا عند الأطفال ويؤدي الي نقص في السمع وبالتالي تأخر النط ق عند الاطفال. تحتاج هده الحالات الي تصريف السائل جراحيا وزراعة انابيب تهوئة في الاذن الوسطي لمنع تجمع ه مرة اخري، هدا الاجراء لا يخلو من مضاعفات مثل افرازات الادن المزمن، تصلب غشاء الطبلة، ترقق غش اء الطبلة وربما ثقبها أيضا. الغرض من الدراسة هو تقييم هده المضاعفات وأثرها على المريض مستقبلا. أجري ت الدراسة على 100 حالة في فترة سنة في مركز الجراحات التخصصية لجراحة الانف والاذن والحنجرة ببنغازي. كانت نسبة المضاعفات 42% أشهرها افرازات الاذن المتكررة عند 14 % من الحالات واقلها ترقق الطبلة 5%. من المضاعفات النادرة الحدوث هي تسوس النتوء الحلمي ويحدث بنسبة ضئيلة جدا، لم نسجل أي إصابة من هده المضاعفات في دراستنا، كما لم يكن هناك فرق بين الذكور والاناث من حيث حدوث مضاعفات عندهم من عدمها. ننصح بمتابعة مثل هده الحالات بصفة دورية للتعرف على المضاعفات مبكرا ومعالجها

INTRODUCTION

OME is characterized by the accumulation of fluid in the middle ear space without acute inflammation symptoms. It is indeed a common condition in children, especially between the ages of two and five years. In fact, it is estimated that approximately 80% of children experience at least one episode of OME before the age of ten, as supported by the study by (Khanna et al., 2008).

When OME is left untreated, it can lead to various complications, including: (1) Hearing loss due to decreased sound wave transmission in the liquid media of the middle ear that characterize otitis media with effusion. (2) Perforated tympanic membrane by the increased accumulation of fluid in the middle ear space. (3) Prolonged fluid accumulation and negative pressure in the middle ear causes thinning and retraction of the ear drum which predisposes to adhesive

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otitis media.(4)Tympanosclerosis: due to formation of calcium deposits or scarring on the eardrum or middle ear bones, which can affect hearing. (5)Sever retraction and adhesive otitis media may predispose to cholesteatoma in rare occasions.

Early diagnosis and appropriate management of OME are important to prevent or minimize the risk of these complications. Prolonged deprivation of hearing can result in language, speech, and cognitive development disorders, as well as academic difficulties especially in children (da Costa Monsanto et al., 2016). mechanical obstruction of Eustachian tube by enlarged adenoids or oedema secondary to allergy or infection can also cause eustachian tube dysfunction and contribute to otitis media with effusion (Kamrani K et al., 2008; Shaffer et al., 2018)

One of the most effective treatments for OME is to aerate the middle ear through the insertion of ventilation tubes (Yang et al., 2012). There are two main types of ear tubes: short-term and long-term tubes (Heidemann, 2015). each type of these tubes have complications and benifets. Reports have indicated several complications associated with ventilation tube insertion, such as otorrhea, tympanosclerosis, persistent perforation, eardrum stretching, cholesteatoma, and ventilation tube granuloma (Branco et al., 2017; Nogan et al., 2017). In many cases, it can be challenging to distinguish between complications resulting from the disease itself and those resulting from the treatment. Otorrhea, is the most common complication of ventilation tube insertion and can occur in 3% to 50% of cases (van Dongen et al., 2015).

Aims of the study: The aim of this study is to determine the complications associated with ventilation tube (VT) insertion surgery conducted at the specialized surgical center (SSC) for Otolaryngology in Benghazi, Libya.

MATERIALS AND METHODS

Study design: This study is cohort study design of one hundred patients who have been surgically treated by ventilation tube insertion at SSC. Demographic questionnaire, which gathers information on age, sex, reasons for surgery, and date of surgery has been applied to collect the data. Additionally, patient medical records will be examined to gather data on complications and symptoms. Follow-up will be conducted over a period of 2 months to 1 year to assess any complications.

Study settings: Data collection from January 2021 to December 2021. The final sample size will be compared to existing literature, and the incidence of postoperative complications related to myringotomy and ventilation tubes will be approximately calculated.

Inclusion criteria:
OME that required myringotomy and ventilation tube insertion,nsertion of bilateral or unilateral ventilation tubes and placement of the grommet or T-tube style of tube,

The exclusion criteria:
Craniofacial anomalies such as cleft lip or palate, patients requiring repeat ventilation tube insertion and in cases where concurrent diseases that affect the eardrum are present or there are incomplete medical records, additional considerations will be taken into account.

In order to obtain informed consent, the study population and parents will be provided with comprehensive information about the purpose and procedure of the study. They will be informed about the potential risks and benefits of participating in the study. Clear explanations will be given regarding the collection and use of their medical data for research purposes. Participants will be invited for re-examination and interviews to gather more detailed information about their medical history, concurrent dis-

eases, and any relevant factors that may impact the study outcomes. These interviews will help in ensuring that accurate and comprehensive data is obtained for analysis. Privacy and confidentiality of the participants' information will be maintained throughout the study process.

Risk factors for OME (Otitis Media with Effusion) include passive smoking, bottle feeding, day-care nursery attendance, and atopy. Both children and adults can develop OME, but the underlying causes may differ between these age groups. In children, OME is more common due to anatomical factors. The Eustachian tube, is positioned more horizontally in younger children. As children grow up and develop into adults, the Eustachian tube elongates and angles caudally, reducing the risk of OME. Therefore, the prevalence of OME is higher in children compared to adults (Nemade et al., 2018).

Developmental anomalies related to the palate, palate muscles, decreased muscle tone in the palate muscles, and variations in bone development can increase the risk of OME in children. Conditions such as cleft palate and Down syndrome, which affect the structure and function of the Eustachian tube and mucociliary clearance, are associated with a higher susceptibility to OME. These factors has an effect on the accumulation of fluid in the middle ear.

OME seen more common between the ages of one and six years. The highest age group affected is at two years. The incidence of OME tends to decrease after the age of five. young children are particularly vulnerable to OME, likely due to factors such as the immaturity of the Eustachian tube and increased susceptibility to upper respiratory infections.

OME is more prevalent during the winter months, which corresponds to a higher incidence of upper respiratory infections. Upper respiratory infections can lead to inflammation and congestion of the Eustachian tube, impairing its normal function and contributing to the development of OME. Early detection, treatment, and regular follow-up are essential in children at increased risk of OME to prevent complications and mitigate the impact on hearing and development. Children with OME may exhibit signs of inattention or decreased academic performance Esposito S et al. The presence of fluid in the middle ear can affect hearing, leading to difficulties in understanding speech and potentially affecting a child's ability to focus and learn.

Large adenoids, may cause obstruction of the Eustachian tube can lead to poor ventilation of the middle ear, which leads to the development of OME. The adenoids can also serve as a reservoir of bacteria that enter the Eustachian tube, leading to the growth of biofilms and inflammation. This inflammation can further contribute to blockage and the accumulation of fluid in the middle ear. (Searight et al., 2023). The mucociliary defense system in the Eustachian tube is the first line of defense in the middle ear. Immunoglobulins which are produced by the mucosa play a role in this defense system. In OME, there is a significant elevation of these immunoglobulins in the effusions, suggesting that the defense system may stimulated (Schilder et al., 2016) treatment of allergic rhinitis, which is often associated with OME, may be beneficial for patients. Allergies can contribute to the development of OME, and addressing allergic symptoms may help alleviate OME in these cases.

Hearing loss is a common complaint in patients with OME. Patients or parents may notice communication difficulties, withdrawal, and lack of attention. During examination, clinicians may observe impaired speech and language development. Patients may also experience intermittent otalgia (ear pain), aural fullness (sensation of pressure or blockage in the ear), or a sensation

that the ear is popping (Skoner et al., 2009).

Childhood hearing loss can indeed have an impact on language development, and hearing aids are often considered a non-invasive option for treating OME. However, for cases of persistent OME where long-term changes in the middle ear and tympanic membrane occur, resulting in permanent hearing loss, ventilation tubes may be recommended (Gan et al., 2017; Schilder et al., 2016). The insertion of ventilation tubes aims to improve the ventilation of the middle ear, equalizing its pressure with the atmosphere, which can lead to improved hearing (Esposito et al., 2021).

There are two main types of ventilation tubes: short-term and long-term. Short-term tubes are smaller and typically remain in place for six months to a year before eventually coming out on their own. On the other hand, long-term tubes tend to stay in place for a longer duration, ranging from 20.6 to 35 months. However, it is worth noting that around 20% of ears that undergo ventilation tube insertion may require additional procedures within two years of the initial insertion (Ferlito et al., 2020).

(Ferlito et al., 2020; Vlastarakos et al., 2007) had studied the potential morbidity and complications associated with ventilation tube insertion (VTI). According to them, VTI, despite being a routine procedure, can be associated with significant morbidity. Complications related to VTI were reported to occur in 17% to 80% of operated ears, depending on specific circumstances of children. It is important to note that the complications and their frequencies can vary from patient to anther due to different factors such as the patient population, surgical technique, follow-up protocol, and other individual characteristics. While VTI is generally considered a safe and effective intervention for certain cases of otitis media with effusion (OME), the potential complications should be taken into account. Some of the reported complications associated with VTI may include otorrhea, tube obstruction or extrusion, persistent perforation of the tympanic membrane, tympanosclerosis, and granulation tissue formation.

Appropriate postoperative care and follow-up aremandatory to monitor and manage any complications that may arise. It can be challenging to differentiate between the sequelae resulting from the disease itself and those arising from the treatment. VTs are advised for the treatment of OME to reduce complications of the condition. OME can have significant consequences, including hearing loss, which can impact speech and language development in children. By inserting VTs, normalization of pressure in the middle ear and drainage of fluid can help reduce these adverse effects and improve hearing.

Furthermore, severe complications, such as cholesteatoma, can potentially arise from untreated or recurrent OME but this is very rare complication of the disease and the insertion of the tube itself by implanting squamous epithelium in the middle ear. Regarding the complications associated with VT insertion, otorrhea is the most common complication reported in children. Otorrhea can occur due to infection or inflammation around the ventilation tube, and it typically resolves with appropriate treatment. Regular follow-up and proper care of the VT site, keeping the ear dry, can help minimize the risk and manage otorrhea effectively. The incidence of early otorrhea varies from 7% to 34%, while delayed otorrhea occurs in 12-24% of cases. Recent studies have reported a higher percentage, reaching 47.3%. However, in Ferlito's series, the incidence of early and late otorrhea is lower (3.4% and 8.6%) compared to the literature.

According to the study by (Esposito et al., 2021), several factors have been noticed as

potential risk factors of postoperative otorrhea. These factors include:

1. Younger children may have a higher risk of postoperative as differences in immune response, anatomy, and susceptibility to infections are more in younger children.
2. Mucoid effusion can provide a favorable environment for bacterial growth and increase the risk of infection.
3. Adequate antibiotic treatment can help prevent or manage infections and reduce the incidence of otorrhea especially in the post-operative period..
4. Contamination of the external auditory canal during or after the surgery may introduce bacteria that can cause otorrhea. Sterile techniques and proper postoperative care can help minimize this risk.
5. Bleeding at the operation site may contribute to the development of otorrhea. As blood in the middle ear can serve as a medium for bacterial growth and increase the risk of infection.
6. Concurrent upper respiratory tract infections, such as the common cold, can increase the likelihood of postoperative otorrhea as it spreads to the middle ear.

However, the study by (Esposito et al., 2021). suggests that gastric reflux does not appear to play a significant role in the pathogenesis of post-tympanostomy otorrhea. Gastric reflux occurs when stomach acid flows back into the esophagus, and it was not found to be a major contributing factor to postoperative otorrhea following VTI.

The use of local antibiotic or antibiotic-steroid drops after VTI is considered useful by some authors, while others consider it ineffective or contraindicated. The low incidence of otorrhea could be attributed to strict water precautions following VTI (Skoner et al., 2009).

Adenoidectomy performed in conjunction with VTI may be a factor contributing to a reduction in the rate of otorrhea. Some authors have reported that VTI with adenoidectomy was associated with a lower number of otorrhea episodes (Ferlito et al., 2020). Adenoidectomy has been found to reduce the need for additional VTI, and previous data suggest that post-operative otorrhea significantly increases both the rate of tube extrusion and the need for subsequent VTI (Monasta et al., 2012).

Medial migration of VTs refers to the movement of the tube from its original position towards the middle ear cavity has been rarely reported in the literature from 0% to 1.1%, indicating its infrequent nature.

Medial migration of VTs is considered the fifth most common indication for tympanostomy tube removal as it is no longer serve its intended purpose of ventilation and to prevent middle ear complications. This complication can be due to technical errors during the insertion or may occur later due to factors such as persistent negative middle ear pressure. The migration of tubes into the middle ear space can contribute to severe middle ear pathology, and some authors recommend their prompt removal (Cunningham et al., 1993; Schilder et al., 2016).

Tympanostomy tube blockage has also been observed, with rates ranging from 11% to 12%. The use of pre-operative eardrops has been reported to significantly reduce the rate of VT blockage from 13.1% to 1.6%. However, obstruction of the VT can also occur due to concretions of ototopical medications. This blockage can influence the probability of early extrusion of the VT (Bassim & Drake, 2005). Regular follow-up can contribute to a low incidence of tympanostomy tube blockage as early identification helps prompt management .

According to the study by (Barati et al., 2012), long-term ventilation tube insertion can lead to eardrum atrophy in the area

where the tube is inserted. Eardrum atrophy refers to the thinning and weakening of the tympanic membrane in that specific region over time, this can result in atrophy, which may manifest as thinning, retraction, or other structural changes in the eardrum. Eardrum atrophy has a potential predisposition to permanent perforation. It is important to monitor the condition of the eardrum during long-term ventilation tube placement and assess for any signs of atrophy or other structural changes. In cases where eardrum atrophy is observed or a permanent perforation occurs, further intervention or surgical treatment may be required to address the issue and restore normal middle ear function..

Granulation tissue is a predisposing factor for otorrhea. In cases where granulation tissue is present, the use of antibiotic-corticosteroid drops has been reported to eliminate the tissue, making tube removal unnecessary. Tympanic retraction is considered a complication after VTI, although it is more commonly associated with the underlying disease itself due to poor tube ventilation. Residual tympanic perforation, on the other hand, is associated with the duration of tube retention and the number of tube insertions. The prevalence of residual tympanic perforation varies, with rates ranging from 2-3% after the use of short-duration tubes and 14-24% after the use of long-duration tubes (Searight et al., 2023).

Myringosclerosis is a progressive and late event, primarily considered an aesthetic rather than functional sequel to VTI. It does not significantly impact hearing. The incidence of myringosclerosis reported in Ferlito's study (36.2%) is consistent with the percentages reported in the literature (23-70%). Myringosclerosis most frequently occurs (82.7%) in the quadrant of the tympanic membrane where the ventilation tube was inserted. Cholesteatoma is the most severe potential complication following VTI, but it occurs in a relatively small percentage of cases, approximately between 0.1% and 1% (Golz et al., 1999).

Reccurence of OME after ventilation tube extrusion may occure in some cases and it had been mentioned in different studies.

1. (Yaman et al., 2010). reported a recurrence rate of 20.7% in ears after the extrusion of Shepard tubes.
2. (Talmon et al., 2001) using T-tubes, reported a reinsertion rate of 4.9% of cases.
3. (Valtonen et al., 2005) found a higher recurrence rate of repeated ventilation tube insertion (VTI) in 53.2% of ears with OME. This study suggests that over half of the ears with OME required multiple VTIs.

Furthermore, the age of the child and the type of tube used can influence the recurrence rates:

1. (Iwaki et al., 1998) observed that the recurrence rate of OME was higher in the age range of 2 to 5 years compared to 7 to 8 years. This suggests that younger children may have a higher risk of OME recurrence after tube extrusion.
2. The study also found that Shepard tubes had a higher recurrence rate compared to T-tubes. This implies that the choice of tube type may impact the likelihood of OME recurrence.

Regarding patient demographics, (Ferlito et al., 2020) noted that male children were more likely to undergo VTI than female children, which is consistent with previous studies. Their analysis did not find a significant influence of patient age at surgery on sequelae and/or complications rates, although it's worth noting that there were no children younger than three years old included in the study, so this finding may differ in a larger population including younger cases (Ferlito et al., 2020). VT placement in children generally does not result in peri-

operative or immediate postoperative complications. However, the use of long-term tubes is associated with a higher incidence of complications such as otorrhea and residual perforation compared to short-term tubes. Cunningham MJ et al. reported a lower incidence of early and delayed otorrhea in their study compared to previous studies.

Swimming or bathing can potentially facilitate the entry of bacteria into the middle ear through the ventilation tube, increasing the risk of infection. Factors contributing to the low incidence of complications after VTI are difficult to establish conclusively. However, some potential factors that may contribute to a lower incidence include older patient age at the time of initial tube placement, the use of short-duration tubes, concurrent adenoidectomy, appropriate and regular follow-up by the same investigator, and strict water precautions after tympanostomy (Bassim & Drake, 2005).

Close and careful observation at each follow-up visit, including microscopic cleaning, especially at 2 months and 1 year after tube extrusion, can help minimize silent sequelae and prevent more severe pathologies of the eardrum and middle ear, which could lead to chronic otitis media and hearing problems later in life (Subramaniam et al., 2015).Similarly, determining the ideal type of ventilation tube can vary based on factors such as the patient's age, ear anatomy, and specific clinical indications. Different types of tubes, such as Shepard tubes and T-tubes, have been used with varying success rates and potential complications. Regarding the study by (Vahedian et al., 2020), they observed several surgical complications associated with tube placement, including otorrhea, eardrum atrophy, perforation, serous otitis, and tympanosclerosis. These findings are consistent with similar studies that have reported complications related to ventilation tube insertion.

RESULTS

A total number of 100 participants participated in this prospective study. The mean age was 4.9± 1.5 years among the participants. There were 36% males and 64% females. Among the participants; 5% had family history of the same illness while 95% had not. Upon assessing hearing level, 17% had slight hearing loss, 12% had mild hearing loss, 16% had moderate hearing loss, 16% had moderately severe hearing loss, 24% had severe hearing loss, and 15% had profound hearing loss as in fig.no.1.

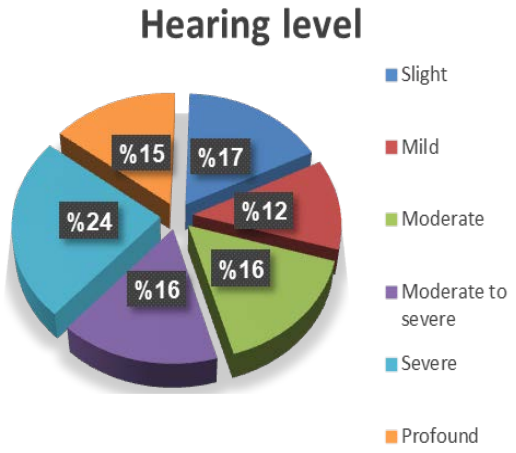

Figure (1). Hearing level among the included group.

Persistent otorrhea is the most common complication occurring in 14% and second in frequency is tympanosclerosis which is common among 9% of the participants. Persistent perforation is the third most common complication among 8% of the participants. Then serous otitis media occurred among 6% of the included participants while the least common was eardrum atrophy (5%). As seen in table No. 1

Table (1). Complications of the VTI among the included participants.

Persistent otorrhea	14	14%
Tympanosclerosis	9	9%
Persistent perforation	8	8%
Serous otitis media	6	6%
Tympanic membrane atrophy	5	5%
Total	42	42%

It is interesting to note. If table No.2 indicated that there was no statistically significant difference between males and females in terms of postoperative complications of VT insertion, including persistent otorrhea, eardrum atrophy, persistent perforation, serous otitis media, and tympanosclerosis, it suggests that the occurrence of these complications was similar in both males and females. In other words, the data did not show a significant association between gender and the risk of these complications following VT insertion.

Table (2). Comparison between both gender regarding complications.

Variable		Male n= 36	Female n= 64	P value
Persistent otorrhea	Yes	8 (22.2)	58 (90.6)	0.130
	No	28 (77.8)	6 (9.4)	
Eardrum atrophy	Yes	1 (2.8)	4 (6.3)	0.651
	No	35 (97.2)	60 (93.8)	
Persistent perforation	Yes	2 (5.6)	6 (9.4)	0.707
	No	34 (94.4)	58 (90.6)	
Serous otitis media	Yes	2 (5.6)	4 (6.3)	>0.999
	No	34 (94.4)	60 (93.8)	
Tympano-sclerosis	Yes	3 (8.3)	6 (9.4)	>0.999
	No	33 (91.7)	58 (90.6)	

Chi square test; Fisher Exact test; *p is significant at <0.05

DISCUSSION

According to (Restuti et al., 2022) untreated OME can lead to various consequences. These include hearing loss, eardrum rupture, adhesive otitis media, tympanosclerosis, and cholesteatoma. Hearing loss is a significant consequence of OME and can have a negative impact on various aspects of a person's life. In children, hearing loss due to OME can cause difficulties in language, speech, and cognitive development. It may lead to delays in acquiring language skills and affect communication abilities, which can subsequently impact academic performance. It's important to note that timely diagnosis and appropriate management of OME can help prevent or mitigate these consequences. Seeking medical attention, obtaining proper treatment, and addressing any underlying issues can significantly improve outcomes and reduce the potential impact on language, speech, and academic performance.

The key clinical-pathologic cause in otitis media is hypoventilation of the middle ear and serous fluid or mucoid collection. Eustachian tube dysfunction is commonly regarded as a significant element in the pathophysiology of this condition. Microorganisms enter the middle ear as a result of eustachian tube dysfunction, which results in air pressure and fluid buildup in the middle ear and precludes middle ear cleansing (MacKeith et al., 2022). The nasopharynx is the site of infection in 91% of patients, and the adenoid serves as a reservoir for harmful microorganisms. Otitis media and Eustachian dysfunction can both be brought on by enlarged adenoids (Searight et al., 2023).

(MacKeith et al., 2022). demonstrated that one of the greatest OME therapies is the ventilation tube (MacKeith et al., 2022). Furthermore, (Kancherla et al., 2022) cleared that reducing the incidence of future ear infections, treating hearing loss caused by middle ear fluid, improving speech and balance issues, are some advantages of ventilation tube insertion (Kancherla et al., 2022). The insertion of a ventilation tube has been shown to have a number of negative consequences on the tympanic membrane. Otorrhea, tympanosclerosis, chronic perforation, eardrum stretching, cholesteatoma, and ventilation tube granuloma are a few surgical problems associated with the installation of ventilation tubes. In many instances, it is challenging to discern between complications caused by the therapy and complications caused by the disease (Sogebi & Oyewole, 2022).

The most frequent ventilation tube problem, which can happen between 3% and 50% of the time, is otorrhea. Searight revealed that otorrhea affects two out of every three kids with VT every year. There isn't much proof

that taking measures when around water helps stop this sickness.(Searight et al., 2023). One of the key causes of tympanosclerosis, is frequently linked to myringotomy and VT insertion. Tympanosclerosis also has another etiology that involves bleeding into the eardrum (Mulvaney et al., 2022).

The study was aiming to evaluate the complications of VT insertion surgery in The speciality surgical center for Otolaryngology/ Benghazi/ Libya. This prospective study was carried out on 100 patients who underwent VT insertion surgery. The target samples were taken over a period of one year. Final sample size was compared to the found literature and the incidence of postoperative complications of myringotomy and ventilation tubes were approximately calculated. Regarding the demographic data in our study, the mean age was 4.9± 1.5 years among the participants. There were 36% males and 64% females. 49% participated and followed up in 2020 and 51% included in 2021. Among the participants, 5% had family history while 95% had no family history of same illness. Upon assessing hearing level;17% had slight hearing loss, 12% had mild hearing loss, 16% had moderate hearing loss, 16% had moderately severe hearing loss, 24% had severe hearing loss, and 15% had profound hearing loss.

Study by (Vahedian et al., 2020) on (VTI) in children. According to their study, out of the 205 children who underwent VT insertion surgery, approximately 57% were boys, and the remaining surgeries were performed on girls. It's interesting to note that no positive family history was recorded for any of the children in the study. The study by (Vahedian et al., 2020) did not find a higher incidence of complications, including hearing loss, in females compared to males. This finding contradicts some previous studies that have reported higher complications in females. The authors suggest that the non-normal distribution of gender variables in their study population may have contributed to this inconsistency. They specifically mention the study by (Khalili et al., 2007) which might have reported different results. It's important to consider that the relationship between gender and complications of (OME) and VT insertion can be complex and may vary across different populations and studies. Further research is needed to better understand the potential impact of gender on these complications.

Regarding complications of the VT insertion among our included participants, persistent otorrhea is the most common complication, occurring in 14% and second in frequency is tympanosclerosis which is common among 9% of the participants. Persistent perforation is the third most common complication among 8% of the participants. Then serous otitis media occurred among 6% of the included participants while the least common was eardrum atrophy (5%). There was no statistically significant difference between males and females regarding previously mentioned postoperative complications of VT insertion. studies by (Barati et al., 2012; van Dongen et al., 2015) that support the findings of many surgical complications associated with ventilation tube insertion (VTI) in children.

According to these studies, complications such as otorrhea, eardrum atrophy, perforation, serous otitis, and tympanosclerosis were observed. These complications highlight the importance of closely monitoring children who have undergone VTI to prevent and manage such complications effectively. The findings from these studies emphasize the need for proper care and follow-up after VTI to minimize the risk of complications. Regular monitoring and appropriate management can help detect and address any issues that arise, ensuring optimal outcomes for the children. It's worth noting that each study may have its own specific population and methodology, so the exact incidence and severity of complications may

vary. Nonetheless, the general consensus from multiple studies, including the ones mentioned, underscores the importance of postoperative care and vigilance to prevent and manage surgical complications associated with VTI in children. In the same line with us, (Barati et al., 2012; Klopp-Dutote et al., 2018) demonstrated that there was no statistically significant difference between males and females regarding postoperative complications of VT insertion (Barati et al., 2012; Klopp-Dutote et al., 2018).

These findings came to be inconsistent with the studies by (Hassmann-Poznańska et al., 2010; Yaman et al., 2010) who found a statistically significant difference between males and females regarding postoperative complications of VT insertion (Hassmann-Poznańska et al., 2010; Yaman et al., 2010). In addition, study by (Zielnik-Jurkiewicz et al., 2006) regarding eardrum atrophy after ventilation tube (VT) insertion surgery. According to Zielnik's findings, the incidence rate of eardrum atrophy was the same in both studied groups, indicating no difference in the occurrence of eardrum atrophy after VT insertion surgery. Furthermore, the study did not find a statistically significant difference between males and females concerning the postoperative complications of VT insertion that were previously mentioned. This suggests that gender may not play a significant role in the incidence of these complications.

Analysis conducted by (Vahedian et al., 2020) regarding the relationship between gender, elapsed time after surgery, and insertion of ventilation tubes (VT). According to their analysis, there was no significant relationship between the two groups of boys and girls in terms of elapsed time after the surgery and insertion of VT in different years. In other words, there was no significant difference in the timing of surgery and VT insertion between boys and girls. However, when studying the relationship between age and time of surgery, a significant difference was observed. The mean age of girls was significantly higher, indicating that girls tended to undergo surgery and VT insertion at a later age compared to boys. The authors suggest that the discrepancy in findings between the relationship of gender with elapsed time after surgery and age at surgery could be attributed to different study locations and different disease distribution. The distribution and prevalence of ear conditions requiring VT insertion may vary between different populations or regions, leading to differences in the timing of surgery and VT insertion among boys and girls.

In the same direction with our findings, Hassmann detected embedded ventilation tube in addition to cases of infections and recurrences with T-tube insertion among the participants of his study (Hassmann-Poznańska et al., 2010).

According to (Kancherla et al., 2022) there is a perspective that suggests a longer duration of time elapsed after surgery may increase the likelihood of developing serious otitis. In other words, the timing of post-surgical follow-up and ongoing monitoring is crucial in identifying and addressing potential complications, particularly serious otitis. This perspective highlights the importance of timely and regular postoperative care and follow-up visits to detect any complications that may arise and take appropriate measures to manage them effectively.

The study by (Vahedian et al., 2020) had several limitations, including:

1. Incomplete records: The presence of incomplete records may have resulted in missing data or reduced sample size, which could potentially affect the reliability and generalizability of the findings.
2. Refusal to cooperate: Patients or parents refusing to cooperate with the study may have introduced selection bias and lim-

ited the representativeness of the sample.

3. Coexisting infections and middle ear diseases: The presence of coexisting infections or other middle ear diseases among the study participants could have influenced the outcomes and introduced confounding factors that were not accounted for.

CONCLUSION

It is concluded that otitis media with effusion (OME) can lead to long-term changes in the middle ear and tympanic membrane, potentially resulting in hearing loss. Ventilation tubes are commonly used to prevent or mitigate complications associated with OME. However, certain complications can still arise, including persistent otorrhea, tympanosclerosis, serous otitis media), and eardrum atrophy.

To minimize the risk of postoperative complications related to ventilation tube insertion, close monitoring and follow-up are recommended. Regular check-ups and evaluations can help in the early detection and timely treatment of any complications that may arise. This allows for prompt intervention and can potentially prevent the development of more severe issues.

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Factors Influencing Postoperative Hospital Stay Following Colorectal Cancer Resection

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Abstract: Colorectal cancer, a prevalent ailment with a growing prevalence due to aging populations, presents surgical, chemotherapy, and radiation therapy as its chief treatment modalities. Our study sought to pinpoint the determinants of hospitalization duration (LOS) in colorectal cancer patients, encompassing both planned and emergency admissions, acknowledging the considerable impact on healthcare costs nationally and internationally. conducting a retrospective data analysis encompassing patients subjected to colorectal cancer surgery at Benghazi Medical Centre (BMC) from January 2016 to December 2018, we meticulously compiled and assessed demographics, clinical symptoms, surgical particulars, postoperative complications, comorbidities, and LOS. Statistical significance was ascertained at a threshold of $P < 0.05$. amongst the cohort of ninety-eight colorectal cancer surgery recipients, the exclusion of four due to missing data resulted in a study cohort of ninety-four cases. The median LOS stood at 11.7 days, with five notable factors significantly extending LOS: enter cutaneous fistula, wound infection, diabetes mellitus (DM), emergency procedures, and postoperative complications. This investigation conducted at BMC ascertained that diabetes mellitus, emergency admissions, postoperative wound infections, and fistula development correlated with prolonged LOS. A discernible increase in hospital stays was evident in elderly patients afflicted with comorbidities and diabetes. These revelations bear substantial financial implications.

العوامل المؤثرة على طول مدة الإيواء بعد إجراء جراحة لاستئصال ورم سرطاني من الأمعاء الغليظة والمستقيم

الكلمات المفتاحية :
سـ . رطان القولـ . ون والمستقيم؛ الأوضـ اع الاختيارية والطوارئ؛ مدة الإيواء؛ مضاعفات ما بعد الجراحة العلاج الإشعاعي؛ جراحة.

المستخلص: سرطان القولون والمستقيم، هو مرض شائع مع انتشار متزايد بسبب شيخوخة السـ كان. إذ يعتبـ ر الاستئصال بالتدخل الجراحي هو العلاج الأساسي إلى جانب العلاج الإشعاعي الكيميائي. يهـ دف هـ ذا البحـ ث للتعرف على العوامل المؤثرة في طول مدة الإيواء للمرضى بسرطان القولون والمستقيم ويشمل كلا من عمليات قبول المرضى المخطط لها والطارئة. حيث تعتبر طول مدة الإيواء من الدلالات على مستوى الخدمات وجـ ودة الرعاية الطبية. تم اجراء هذا البحث بدراسة استرجاعية، تضمنت مراجعة سجلات جميع المرضى الـ ذي تـ م إيوائهم وخضعوا لجراحة استئصال ورم سرطاني من القولون أو المستقيم في مركز بنغازي الطبي، في الفتـ رة من يناير 2016 إلى ديسمبر 2018. تم تجميع وتقييم التركيبة السـ كانية والأعـ راض السـ ريرية والتفاصـ يل الجراحية ومضاعفات ما بعد الجراحة والأمراض المصاحبة وخسارة الوزن بدقة. تـ م التأكـ دـ مـ ن الأهميـ ة الإحصائية عند عتبة $P < 0.05$. كان مجموع الحالات 98 حالة، استبعد منها 4 حالات وذلـ ك لعـ دم تـ وفر البيانات الكافية عليها. ومتوسط مدة الإيواء في المستشفى 11.7 يوم. ولوحظ خمسة عوامل تعمل على زيادة مدة الايواء بشكل كبير: الناسور المعوي الجلدي، والتهاب الجرح، ومرض السكري (DM)، وإجراءات الطـ وارئ، ومضاعفات ما بعد الجراحة. تم تقييم العلاقة بين المتغيرات المدخلة ومدة الإقامة في المستشـ فى تبـ ين وجـ ود علاقة بدلالة احصائية مع ثلاث متغيرات، مرض داء السكري، التدخل الجراحي الطارئ والمضاعفات ما بعـ د الجراحة خاصةً (التهاب الجرح والناسور المعوي الجلدي). حيث ادت إلى زيادة طول مدة الإيواء في المستشفى. وكانت الزيادة الملحوظة في الايواء بالمستشفى للمرضى المسنين المصابين بأمراض مصاحبة ومرض السكري. تحمل هذه الاكتشافات آثارًا مالية كبيرة.

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INTRODUCTION

Colorectal cancer (CRC) is a highly prevalent cancer and the third most common type worldwide. As the population ages, the number of cases is expected to increase. In 2018, it accounted for 10.2% of new cancer cases (1.8 million cases) and was the second leading cause of cancer-related deaths globally, with 862,000 fatalities (WHO, 2018). Colorectal cancer is the most common cancer among men in western Libya, with the highest regional incidence rate due to dietary and lifestyle habits. Improved accessibility and detection through endoscopic procedures may also contribute to the rapid increase in incidence rates (Bodalal et al., 2014). CRC is more complex and common in people over 50 (Brunicardi et al., 2018). Age is a significant risk factor; survival rates differ between genders (Yang et al., 2017). Behavioral and nutritional factors, such as inactivity, alcohol and cigarette use, high BMI, and poor diet, contribute to cancer and mortality. Family history is a risk factor for 20% of cases, emphasizing the need for genetic testing (Brunicardi et al., 2018)..

Colonoscopy is the most precise and sensitive diagnostic method for colorectal diseases, enabling polypectomy and biopsy when needed. CT scan with barium enema is occasionally useful, and metastasis can be detected via CT scan (Bailey et al., 2018). Screening programs significantly reduce morbidity and mortality from colorectal cancer. Starting at age 50, moderate-risk individuals should undergo colonoscopies every ten years, flexible sigmoidoscopies every five years, barium enemas every five years, and fecal occult blood testing (FOBT) annually (Lambert, 2013). Treatment for colorectal cancer varies depending on the cancer's stage, typically including a combination of chemotherapy, radiation, and surgery. Surgery is the only effective option for curing early and minimally metastatic disease (stage I-IV) (ASCO. 2018).

Our study on hospital LOS after colorectal resection was conducted at Benghazi Medical Centre, a public hospital without external budgetary constraints or fast-track treatment plans. We investigated how demographic factors, preoperative co-morbidities, clinical presentation, surgical methods, admission type, and postoperative complications affect hospital stay. Prolonged stay can have emotional, social, and financial consequences for patients, families, and society. Identifying risk factors associated with longer LOS can lead to more cost-effective care delivery. Preoperative preparation and postoperative treatment can be modified accordingly. These findings have significant cost implications for healthcare services and strategies to reduce emergency admissions and LOS.

This study aims to identify variables that prolong the stay after colorectal surgery. Hospital stay is a measure of treatment quality, and identifying its underlying causes can promote best practices, improve services, and reduce the stay period after colorectal cancer surgery.

MATERIALS AND METHODS

Study design and sitting: We conducted a retrospective cross-sectional study to identify all colorectal cancer patients admitted and operated on at BMC from January 2016 to December 2018. The study aims to determine how demographic factors, preoperative co-morbidities, surgical treatment, and postoperative complications impact hospital stay after colon cancer surgery.

Study population and data collection: Data was collected from medical records and operation notes of CRC patients admitted to the surgical department of BMC between Jan 2016 and Dec 2018 using a data sheet (Appendix). Demographics (Gender, Age), preoperative comorbidities (Diabetes mellitus (DM), hypertension (HTN), ischemic heart disease (IHD), cerebrovascular acci-

dent (CVA), bronchial asthma (BA), epilepsy, chronic obstructive pulmonary disease (COPD), and end-stage kidney disease (ESKD) are registered among the categories of variables used in the data collection sheet. The main clinical presentation (abdominal pain, constipation, per-rectal bleeding, anemia, acute obstruction, and perforation) is recorded. Operative procedures (low anterior resection (LAR), proctocolectomy, right colectomy, left colectomy, sigmoidectomy, complete colectomy, and abdominoperineal resection (APR) were detected. Mode of admission (emergency vs. elective), operative technique (laparoscopic vs. open), and postoperative complications (wound infection, wound dehiscence, seromas, hemorrhage, bowel leak, fistula, ileus, deep venous thrombosis [DVT], atelectasis, chest infection, and organ failure) are recorded.

Inpatient stay length was the primary outcome, and patients with incomplete data (<80%) were excluded.

This study defined prolonged hospital stay as a length exceeding the 75th percentile of the entire cohort. Accordingly, a prolonged hospital stay was defined as a length exceeding 20 days (about 3 weeks), representing the 75th percentile of the entire cohort.

Data statistical analysis: Data was analyzed using SPSS for Windows 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive analysis was used to characterize the study population's variables, while multivariable logistic regression was utilized to identify factors predicting prolonged hospital stay. Variables were included in the model if they were significant ($p < 0.1$) on likelihood ratio tests.

RESULTS

Over 36 months (about 3 years), 98 patients underwent significant colon surgical resections for colorectal cancer at BMC. After removing 4 cases with missing data, 94 patients were included in the analysis. Their mean age was 56.4 years (range, 18-80), with 55 males (58.5%) and 39 females (41.5%). Most patients (47%) were between 51 and 70 years old. Figure 1 displays the age distribution, and Table 1 shows gender frequencies.

Table: (1). Age groups frequency of study sample

	Frequency	Percent	Valid Percent	Cumulative Percent
<50	31	33.0	33.0	33.0
51-60	22	23.4	23.4	56.4
61-70	25	26.6	26.6	83.0
71-79	14	14.9	14.9	97.9
>80	2	2.1	2.1	100.0
Total	94	100.0	100.0	

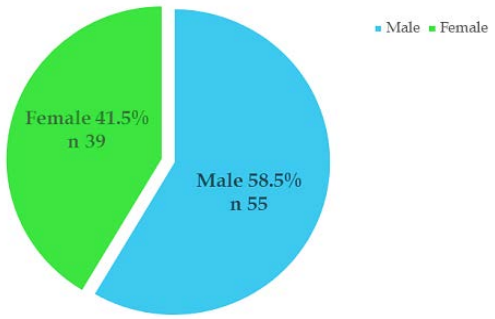

Figure: (1). Gender distribution of the study sample.

Comorbidities were present in 60 patients (63.8%). DM was the most prevalent in 29 cases (30.9%), followed by IHD (10.6%), HTN (9.5%), bronchial asthma (6.4%), anemia (5.3%), and COPD (3 cases) (3.1). ESKD, CVA, and epilepsy occurred in only one patient (1.06%) (Figure 2).

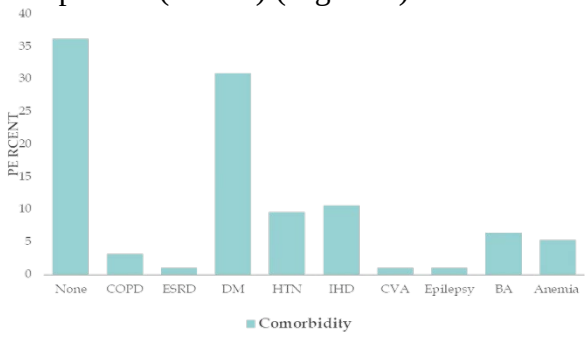

Figure: (2). Co-morbidity distribution of study sample.

The most frequent clinical manifestation was an acute intestinal blockage in 25.5%,

unexplained abdominal discomfort in 24.5%, constipation in 22.3%, rectal bleeding in 19.1%, anemia in 5.3%, and perforation in 3.2% (Figure 3). The tumor was commonly located at the sigmoid colon (43.6%), ascending colon (18.1%), splenic flexure (12.8%), rectum (8.5%), hepatic flexure (7.4%), descending colon (5.3%), and transverse colon (4.3%) (Figure 4).

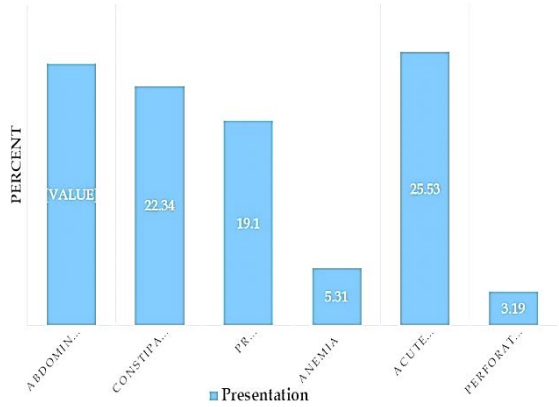

Figure: (3). Distribution of colorectal cancer presentation.

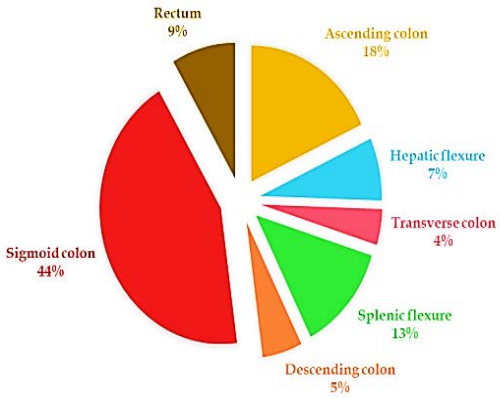

Figure: (4). Frequencies of tumor location.

Regarding surgical access, 71 cases (75.5%) were open, and 23 cases (24.5%) were laparoscopic (Figure 5). Sigmoid colectomies were performed in 42 cases (44.7%), right colectomies in 27, left colectomies in 16, APRs in 5, LARs in 3, and complete colectomies in one case (1.1%). Of the 94 patients, 45 (47.9%) were admitted as emergency cases, while 49 (52.1%) were elective admissions. The typical stay was 11.7 days (range 3-45 days), with 58 patients (61.7%) having a LOS between 6 and 20 days (Figure 6).

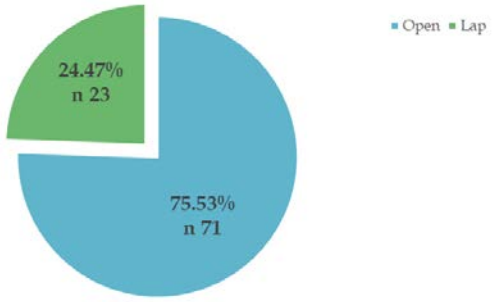

Figure: (5). Frequencies of surgical access.

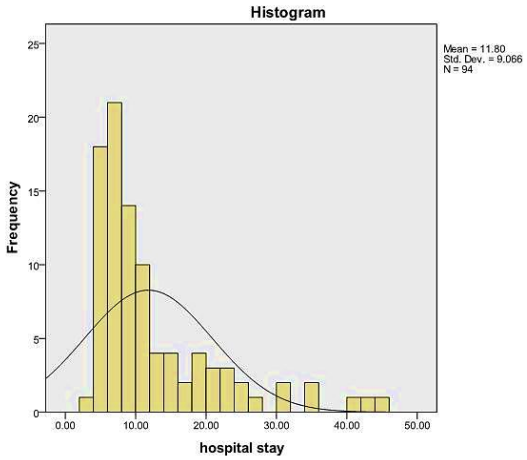

Figure: (6). Distribution of length of hospital stay (by days).

Compared to patients without DM (63.1%), those with DM (30.9%) had a longer median LOS (9 vs. 13.7 days (about 2 weeks), (P = 0.009) (Table 2). Emergency cases (47.9%) had a longer LOS (13 days) compared to elective cases (52.1%) with an LOS of 9.4 days (P = 0.002) (Table 3). Patients with postoperative problems (39.4%) had a longer median LOS (18 days) compared to those without (60.6%) with an LOS of 8 days (P = 0.006) (Table 4).

In regression analysis, wound infections and enterocutaneous fistula were identified as surgical complications that increased LOS. Patients with wound infections had a median LOS of 17 days (about 2 and a half

weeks), compared to 10.5 days for those without (P = 0.014). Patients with enterocutaneous fistula had a median LOS of 34 days, compared to 10 days for those without (P = 0.001). Age, gender, tumor location and type, surgical access, and other comorbidities did not predict hospital stay.

Table: (2). Hospital stay / Co-morbidity Cross tabulation

hospital stays	Co-morbidity									Total
	None	COPD	ESRD	DM	HTN	IHD	CVA	Epilepsy	BA	
<5	10	2	0	3	0	0	0	0	3	18
6-20	23	1	1	17	6	5	1	1	3	58
>21	1	0	0	9	3	5	0	0	0	18
Total	34	3	1	29	9	10	1	1	6	94

Table: (3). Hospital stay / Urgency Cross tabulation.

hospital stays	Urgency		Total
	Elective	Emergency	
<5	14	4	18
6-20	29	29	58
>21	6	12	18
Total	Total	45	94

Table: (4). Hospital stay / Complications Cross tabulation

Hospital Stay	Complications													Total
	wound infection	CVA	atelectasis	chest infection	organ failure	DVT	ileus	wound dehiscence	seroma	bleeding	collection	leaking	fistula	
<5	0	0	0	0	0	0	1	0	0	0	0	0	0	18
6-20	4	0	1	4	4	1	0	0	1	1	2	1	0	58
>21	5	1	0	1	2	1	0	1	0	0	1	1	4	18
Total	9	1	1	5	6	2	1	1	1	1	3	2	4	94

DISCUSSION

After colorectal resection, LOS patterns were examined, and factors contributing to "optimal" (5 days), or "prolonged" (21 days) hospital stays were analyzed via multivariable logistic regression.

Comparisons of LOS between countries: The healthcare system and LOS reduction measures should be considered when comparing median LOS among studies. Despite being conducted in a healthcare system without government initiatives, this study reported a longer median LOS than comparable studies in other countries. Surgery remains the primary treatment, and hospital stays are costly. Shortening stays raises concerns about patient safety and readmissions.

Reducing the hospital stay after major surgery indicates surgical success and lowers patient care costs (Nascimbeni et al., 2005). Hospital stay is the time between resection and discharge, typically lasting 10-12 days for colorectal surgeries (Aravani et al., 2016; King et al., 2006). A "fast track" reg-

imen aims to achieve a 2-day postoperative stay (Stephen & Berger, 2003).

Colorectal cancer is the most common digestive system cancer, with over 140,000 new cases and 50,000 deaths annually in the US (Deenadayalu & Rex, 2007). It ranks as the third most prevalent cancer in males worldwide and the most common cancer among men in eastern Libya (Bodalal et al., 2014). Although CRC can affect anyone, 90% of cases are diagnosed in those over 50, making age a significant risk factor (Brunicardi et al., 2018). Our investigation found that most cases fell between the ages of 51 and 70, which was consistent with prior research (Elzouki et al., 2014). Despite being common in Libya, few studies have investigated colorectal malignancies in this population. Our demographic analysis showed that men were more affected than women, consistent with a meta-analysis that found women have better survival rates (Yang et al., 2017). However, our study did not establish a statistically significant correlation between gender and survival due to the lack of long-term follow-up after discharge.

CRC symptoms are often nonspecific and present at advanced stages, with changes in bowel habits and rectal bleeding being the classic first signs (Brunicardi et al., 2018). However, our study found that abdominal discomfort and intestinal obstruction were the most common presenting symptoms, indicating larger tumors and advanced disease at diagnosis, consistent with another research (Aquina et al., 2017; Menegozzo et al., 2019; Xu et al., 2017). To improve early detection and optimal management, it is recommended that Libya establish colorectal cancer surveillance systems. Although colon cancer is commonly associated with the left side, there has been a rise in incidence on the right or proximal side in Europe, North America, and Asia (Kim et al., 2015; Vuik et al., 2019; Widmaier et al., 2007). In our study, over 60% of patients had tumors on the left side, with the sigmoid colon being the most frequently affected area.

The American Society of Clinical Oncology recommends combining surgery and radiochemotherapy for colorectal cancer treatment, based on the cancer's stage (Lambert, 2013). The primary treatment is oncological resection with curative intent, involving the removal of the tumor and lymphovascular supply. Surgical choice depends on the patient's condition, comorbidities, and clinical presentation, with en-block resection and sufficient lymphadenectomy being ideal. A diverting colostomy may be an option (Nelson et al., 2001). Although routine, colorectal resection may prolong hospital stay, causing a financial and psychological burden for patients.

Recent studies on hospital stay length after CRC procedures have focused on the impact of expedited pathways to reduce LOS (Sailhamer et al., 2007; Schwenk et al., 2008), while our study focuses on identifying risk factors with the most significant impact on hospital stay. Identifying these risk factors can inform pre- and postoperative treatment to deliver high-quality, cost-effective care. Median LOS varies between nations due to differences in practice styles and healthcare systems (Martin et al., 2016). For instance, Leung et al. found a median LOS of 8 days in the US (Leung et al., 2009), while Faiz et al. reported a median LOS of 11-14 days for colectomy and 13-15 days (about 2 weeks) for rectal surgeries in English NHS trusts (Faiz et al., 2011), which is more comparable to the findings in this study. In contrast to previous studies (Koperna et al., 1997; Tartter, 1988), advanced age was not associated with longer LOS in this study. However, given the elderly population studied (average age 56.4), the impact of age on LOS may be challenging to discern. Additionally, no significant difference in LOS was observed between genders, with men and

women having a LOS of 11.9 and 10.2 days, respectively.

DM was the only preoperative Co-morbidity found to be a risk factor for the increased stay period. Previous research has linked DM to post-operative complications, including slow wound healing, respiratory infections, cardiac issues, and ICU hospitalization (Chiu et al., 2017; Colibaseanu et al., 2018; Jin et al., 2019). After colorectal surgery, the body undergoes physiological and metabolic changes that can prolong hospital stays (Duggan et al., 2017). Hyperglycaemia can reduce tissue perfusion and oxygenation, leading to longer recovery times. Diabetes mellitus can also increase the risk of infections due to a deficiency in innate immunity. A systematic review and meta-analysis found that preoperative Hyperglycaemia harms short-term outcomes after colorectal surgery) (Reudink et al., 2021). This highlights the importance of preoperative treatment for improving recovery and reducing complications.

No significant association between LOS and co-morbidities such as HTN, IHD, COPD, ESKD, CVA, Epilepsy, and BA was found. Surgical complications significantly increased hospital stays, consistent with previous research (McNicol et al., 2007). In this study, enterocutaneous fistula and wound infections were the most common complications associated with longer LOS. In other studies, wound infections have been shown to prolong hospital stays by 7 to 10 days (Galie & Whitlow, 2006; Young & Khadaroo, 2014).

Preventative measures can reduce wound infections, such as maintaining cleanliness during surgery, using aseptic procedures, and limiting antibiotic use. Although rare, enterocutaneous fistulas can extend hospital stays by an average of 21-60 days (about 2 months) and increase mortality rates by up to 36%, with complications such as malnutrition and sepsis (Islam et al., 2018). Complications and prolonged LOS have a reciprocal relationship, leading to more problems like DVT and nosocomial infections. Early identification and management of postoperative complications are crucial (Nelson et al., 2001). We analyzed LOS and emergency readmissions in a population-based study. 63% of admissions were elective, and 37% were emergency cases. Median stay for elective and emergency admissions was 14 days (IQR = 11-20) and 21 days (about 3 weeks), respectively (McNicol et al., 2007; Xu et al., 2017). Emergency surgery patients had longer stays, due to additional testing needed for diagnosis before surgery. This highlights the importance of early screening programs for colorectal cancer (Schwenk et al., 2008).

Individuals who undergo emergency surgery for colorectal cancer often have advanced illness and may have distant metastases, which increases the risk of postoperative morbidity and longer stay (Kumar et al., 2014). Therefore, screening programs to detect colorectal cancer early are needed. Numerous studies have shown that laparoscopic surgery can reduce stay periods due to small incisions, less pain, and quicker recovery. However, this study did not find evidence of reduced hospital stays from laparoscopic surgery due to the small number of cases analyzed (Ueda et al., 2020). The study could not investigate the impact of increasing laparoscopic procedures on LOS due to the inability to distinguish between open and laparoscopic surgeries in the data.

Limitations of this study include its retrospective nature, with some patient file data missing. Factors influencing postoperative morbidity and hospital stay duration were not considered, such as operation duration, ASA grading, histology reports, illness staging, surgeon experience, and surgical volume. Additionally, the study's sample size was small, encompassing patients from only one institution, restricting the identification of further predictive variables. Furthermore,

failure to find matches may arise from typographical errors, missing data, or the absence of cancer mentions.

CONCLUSION

This retrospective cross-sectional study provides valuable insights into the factors associated with prolonged hospital stays in colorectal cancer patients undergoing surgery. The results emphasize the importance of addressing comorbidities, particularly diabetes mellitus, and implementing infection prevention measures to reduce wound infections and enterocutaneous fistulas. While the study has limitations, its findings have implications for optimizing patient care and resource utilization in colorectal cancer surgery. Further research is needed to validate these findings and explore additional factors affecting hospital stay duration.

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Abdominal Wall Endometriosis (AWE): A New Surgical Challenge (Case Report)

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Abstract: Endometriosis is defined as the appearance of functional uterine tissue that responds to hormonal stimuli in tissues other than the uterine cavity. Typically, the pelvic cavity is where endometriosis is found. The anterior abdominal wall, bone, adrenal glands, eyes, and heart are all occasionally involved in extrapelvic cases. The most typical symptom of extrapelvic endometriosis is abdominal wall involvement. There appears to be a strong correlation between pre-existing surgical scars and the presence of endometriomas within the abdominal wall. In cases when female patients exhibit a cyclically painful abdominal wall mass, it is crucial to maintain a heightened level of suspicion for endometrioma, particularly if there is a history of prior gynecologic surgery. The differential diagnosis may provide greater challenges when abdominal wall endometriomas are situated at a distance from the scar, as is commonly observed following Pfannenstiel's laparotomic incision. Many of these lesions can be distinguished by MRI, and intraoperative frozen section histological confirmation should be obtained to enable an oncological resection if necessary.

الانتباذ البطاني الرحمي في جدار البطن، تحدٍ جراحي جديد (تقرير حالة)

الكلمات المفتاحية : جدار البطن، ورم بطانة الرحم، الانتباذ البطاني الرحمي، الجراحة.

المستخلص : يتم تعريف الانتباذ البطاني الرحمي على أنه ظهور أنسجة الرحم الوظيفية التي تستجيب للمحفزات الهرمونية في الأنسجة بخلاف تجويف الرحم. عادة تجويف الحوض هو المكان الذي يوجد فيه الانتباذ البطاني الرحمي. من حين لآخر توجد أيضا تدخلات خارجية في جدار البطن الأمامي والعظام والقلب والغدد الكظرية والعينين والقلب. مشاركة جدار البطن هو أكثر مظاهر الانتباذ البطاني الرحمي نموذجية. يبدو أن الأورام البطانية الرحمية في جدار البطن، والندوب الجراحية القديمة مرتبطة ارتباطا وثيقا. يجب الحفاظ على مؤشر مرتفع للشك في ورم بطانة الرحم عند المريضات اللواتي يعانين من كتلة جدار بطن مؤلمة دوريا، خاصة في وجود جراحة أمراض النساء السابقة. قد يكون التشخيص التفاضلي أكثر صعوبة عندما توجد أورام بطانة الرحم في جدار البطن بعيدا عن الندبة، ويفترض أنه أكثر تواترا بعد شق بافانينستيل القطعي. يمكن أن يساعد التصوير بالرنين المغناطيسي في التمييز بين العديد من هذه الآفات، ويجب الحصول على التأكيد النسيجي أثناء الجراحة عن طريق القسم المجمد لتمكين استئصال الأورام إذا لزم الأمر.

INTRODUCTION

The implantation of endometrial cells during abdominal surgery, specifically a caesarean section, has the potential to lead to the development of endometriomas. These endometriomas are typically seen in close proximity to the surgical scar and exhibit a range of sizes. This case report discusses a unique manifestation of a sizable endometrioma located at a considerable distance from the site of a prior caesarean scar. The

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paper also outlines the diagnostic and treatment approach employed in this particular case. This report presents a case study involving the management of a 32-year-old female patient diagnosed with a 2 X 3 cm abdominal wall endometrioma. The therapeutic approach employed in this case was the utilization of a wide local excision technique.

CASE REPORT

A female patient, aged 32 and of Libyan nationality, was hospitalized at our medical facility presenting with a mass located in the lower region of the abdomen wall, accompanied by symptoms of discomfort and nausea. Throughout the previous year, there has been a gradual increase in the size of the bulk. The patient had previously endured four caesarean deliveries for each of her pregnancies. The incision resulting from the caesarean section exhibited satisfactory healing, while the protrusion located a few centimeters above the scar in the right paramedian suprapubic area was found to be unrelated to the Pfannenstiel incision. The observed mass had characteristics of tenderness, roundness, and a diameter of approximately 3 cm. Furthermore, it displayed a fixed nature and adherence to the underlying structures of the deep abdominal wall. Further gynecological and abdominal investigations did not yield any abnormal findings.

The blood tests indicate that the findings are within the usual range. The ultrasonic scan does not possess any diagnostic capabilities. The computer tomography (Figure 1) revealed two particularly interesting findings: a solitary increased nodule affecting the deep layers of the abdominal wall, and the measurement of its distance from the caesarean section scar.

The surgical procedure commenced with a comprehensive local excision of the lesion, followed by the removal of the mass. The mass exhibited numerous tracts that infiltrated the neighboring tissue and exhibited adherence to the fascia and rectus abdominis muscle, extending all the way to the peritoneal layer. Consequently, the removal of the mass proved challenging and resulted in a substantial wall defect, as depicted in Figure 2.

Figure: (1). CT-scan of abdominal wall shows AWE (arrowed).

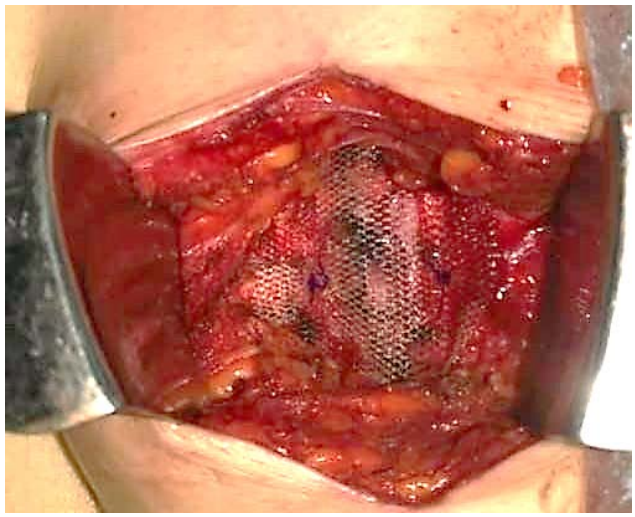

Figure: (2). AWE site after excision repaired by prolene mesh.

DISCUSSION

Endometriosis is a prevalent medical condition that impacts a substantial proportion, around 10%, of women within the reproductive age range (Viganò et al., 2004). The

phrase refers to the presence of glands and stroma that exhibit an unusual or heterotopic growth pattern, resembling those often found in the uterus lining. Endometrioma primarily manifests in the pelvic region; nevertheless, it has the potential to disseminate to several anatomical sites, giving rise to either a localized mass or multiple dispersed foci. Extrapelvic endometrioma is observed in approximately 0.1% to 0.2% of women following caesarean sections. Or less commonly after other surgical interventions such as amniocentesis, laparoscopy, hysterectomy, abdominoplasty, and similar treatments. The occurrence of extrapelvic endometrioma is commonly observed in conjunction with a preexisting abdominal scar. The authors (Blanco et al., 2003; Bumpers et al., 2002; Hughes et al., 1997; Khamechian et al., 2014) have conducted studies relevant to the topic.

In the majority of instances, women who have been impacted by endometriosis choose to refrain from disclosing their medical history pertaining to this condition.
Numerous explanations have been proposed with regard to the development of endometriosis nodules in locations outside of the uterus. The metaplasia and transport theories are the two theories that appear to receive the most support, as suggested by (Vellido-Cotelo et al., 2015).

The prevailing belief is that the origin of this condition can be attributed to the mechanical transplanting of endometrial tissue, particularly in surgical procedures that include the opening of the uterine cavity. The viability of shed endometrial tissue has been observed to be high, displaying significant angiogenic properties and the ability to generate endometriotic lesions across many model systems (Nap et al., 2004). Nevertheless, there have been documented cases of abdominal wall endometriomas located in the recti abdominis muscles that have spontaneously occurred, without any previous surgical intervention (Ideyi et al., 2003).

In a clinical context, symptoms of abdominal scar endometrioma may manifest at any point within a range of 6 months to 19 years following a previous surgical procedure. The study conducted by (Bumpers et al., 2002) is in contrast to the findings of (Patterson & Winburn, 1999).

The phenomenon known as AWE was initially documented by Meyer in the year 1903, as described by (Ideyi et al., 2003). The site of extrapelvic disease with the highest prevalence is reported to be between 0.03% and 1% according to studies conducted by (Bumpers et al., 2002; Ideyi et al., 2003).

Several explanations have been proposed, yet the exact etiology remains uncertain. The direct transplantation theory posits that endometrial cells have the potential to be transferred to the abdominal wall during surgical procedures involving the uterine cavity, such as hysterectomy or caesarean delivery. Based on the coelomic metaplasia idea, it is postulated that endometrial cells have the potential to originate from the mesothelial cells that border the peritoneum of the abdominal cavity.

The primary element of the symptomatology involves the presence of a mass growth and associated pain in the abdomen region, which is directly related to a scar. However, it is important to note that the severity of the pain and its exacerbation by menstruation may not always be consistent (Patterson & Winburn, 1999).

In the context of abdominal wall endometriosis, a significant proportion of patients exhibit symptoms such as cyclic abdominal discomfort, protrusion, and cutaneous irritation. The investigation of the pathophysiology of this entity was undertaken. Various explanations have been proposed regarding this phenomenon, with the retrograde reflux of endometrial tissue into the peritoneal cavity through the fallopian tubes being the

most widely recognized (El Muhtaseb et al., 2022).

The major symptom is characterized by the presence of a palpable mass lesion at the site of maximum soreness. The dimensions of this palpable lump undergo alterations in accordance with the menstrual cycle. According to (Kumar, 2021), findings indicate that the presence of endometriosis in a scar is attributed to concomitant pelvic endometriosis in approximately 26% of instances. Abdominal wall endometriosis, despite exhibiting characteristic symptoms, is often misdiagnosed by general surgeons due to its infrequent occurrence (Oh et al., 2014). The diagnosis of this condition often presents a significant challenge and is commonly established subsequent to the surgical removal of the lesion (Balleyguier et al., 2003).

In contemporary times, advancements in US technology have significantly enhanced the precision of diagnostic procedures, enabling skilled operators to precisely assess a patient's condition through first-level echography, hence informing surgical decisions. Moreover, the utilization of a high-resolution ultrasound presents itself as a rapid, cost- effective, and secure modality that possesses the capability to provide a suitable indication for surgical intervention. Furthermore, the utilization of intraoperative ultrasound facilitates the attainment of sufficient excision margins (Coccia et al., 2015).

According to (Balleyguier et al., 2003), magnetic resonance imaging (MRI) exhibits superior spatial resolution compared to computed tomography (CT) scans, hence enhancing its efficacy in delineating the borders between muscles and abdomen subcutaneous tissues.

In order to establish a definitive diagnosis, a biopsy is deemed necessary, as CT scans and MRIs are mostly employed to assess the disease's extent prior to surgical intervention. While FNAC is not considered a diagnostic tool, it can assist in the diagnostic process (Kumar, 2021).

Furthermore, it has the ability to discern the hemorrhagic nature of endometriotic lesions. When considering the diagnosis of endometrioma, it is crucial to consider the presence of incisional hernias as well as malignant or benign tumors located in the abdominal wall. Various imaging modalities, such as Doppler ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), should be employed for distinct diagnostic purposes. Histopathological investigation is the sole method by which a definitive diagnosis can be established as stated by (Çöl & Yilmaz, 2014). In the case study involving scar endometriosis subsequent to a caesarean section, when the condition was initially misdiagnosed as a stitch granuloma, the timely identification of the ailment might greatly influence the extent and aggressiveness of the therapeutic intervention.

The recommended therapeutic approach is performing a comprehensive local excision of the lesion with negative margins, although in certain cases, mesh implantation may be required. According to (Saliba et al., 2019), medical interventions such as non-steroidal anti-inflammatory drugs (NSAIDs), oral contraceptives, gonadotropin-releasing hormone (GnRH) analogues, and aromatase inhibitors have been employed in the treatment of this condition. These interventions have demonstrated efficacy in alleviating symptoms without affecting the size of the lesion.

The medical intervention yielded unsatisfactory results. The patient necessitated a comprehensive surgical excision of the lesion. The patient is currently undergoing continuous treatment due to the potential for recurrence (Saha et al., 2014).

According to (Khamechian et al., 2014), it is necessary to excise scar endometriomas with adequate margins following their dissection from the adjacent tissue. To prevent further instances, it is imperative to implement measures to prevent the intraoperative auto-inoculation of endometrial tissue (Çöl & Yilmaz, 2014). According to (Bachir & Bachir, 2002), wide excision is the recommended approach for managing endometriomas located on the abdominal wall as well as for recurrent lesions.

The necessity for abdominal wall reconstruction often arises following extensive mass removal, as a result of the mass's infiltration and attachment to the soft tissue. In such cases, the utilization of a polytetrafluoroethylene (Teflon) patch or polypropylene (Prolene) mesh graft is a common approach (Blanco et al., 2003). According to a study conducted by (Wasfie et al., 2002), there is a suggestion that performing meticulous surgical wound cleansing and irrigation using a high-jet saline solution before closure may be effective in preventing scar endometrioma in caesarean sections. While the likelihood of endometriosis progressing into cancer is low, it is crucial to consider this possibility due to the potential need for an oncological resection in cases where an abdominal endometrioma is detected (Matter et al., 2003).

According to (Lopes et al., 2019), the incidence of endometriosis in abdominal surgical scarring varies between 0.03% and 0.4%. Additionally, it has been shown that only 1% of these instances exhibit malignant change. Furthermore, since laparotomies are commonly conducted several years prior to the occurrence of endometriomas, postmenopausal women need to be aware of the uncommon potential for an endometrial implant to undergo malignant transformation.

In these instances, the utilization of frozen section intraoperative histological confirmation appears to be a justifiable precautionary measure. In order to exclude the possibility of an abdominal wall desmoid tumor that has manifested on the scar resulting from a caesarean section, it is imperative to do a differential diagnosis (Vellido-Cotelo et al., 2015). It is noteworthy to mention that the occurrence of cyclic pain during menstruation is specifically recorded in only 20% of cases involving abdominal wall endometrioma. From a clinical perspective, it is established that pain experienced in desmoid tumors is unrelated to the menstrual cycle (Chatziparadeisi et al., 2014).

CONCLUSION

In our case report study, the unusual occurrence of medium-sized deep endometriomas of the abdominal wall localized slightly above the caesarean scar rather than being in continuity with it, in a 32-year-old female, a year after a caesarean section, could be explained by taking into account the surgical method of the Pfannenstiel's laparotomic incision. The abdominal cavity can be longitudinally opened during dissection of the fascia and "recti abdominis" muscles, allowing the transport and implantation of endometrial tissue through surgical manipulation.

The differential diagnosis, which includes hernia, soft tissue tumor, abscess, and metastatic malignancy, can provide further difficulty when the endometrioma is located at a considerable distance from the abdominal scar. MRI can be utilized to distinguish between various lesions, and if an oncological resection is deemed required, intraoperative histological confirmation should be achieved through frozen section analysis.

The routine practice of cleaning and cleansing a laparotomy wound subsequent to a caesarean section is not implemented consistently throughout our hospital facilities, nor is it well incorporated into the training curriculum for medical trainees. In order to

mitigate the discomfort, anxiety, and morbidity associated with this delayed postoperative consequence, it is recommended that greater attention be given to this matter and that preventive measures be advocated.

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Development and Validation of HPLC-UV Method for Determination of Metformin Hydrochloride in Tablets Available in the Libyan Market

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Abstract: A rapid and simple HPLC-UV method has been developed and validated for the estimation of metformin HCl formulated in tablet dosage form, as well as to identify different commercial brands of metformin HCl using BP test Thin Layer Chromatography. The HPLC method was performed on a Reversed-Phase Brownlee Bio C18 column (250 x 4.6 mm, 5 µm) at a 1.0 mL min^{-1} flow rate with UV detection at 236 nm. The mobile phase was conducted in an isocratic manner and contained 52% acetonitrile and 48% aqueous phase (0.1M Potassium dihydrogen orthophosphate). The pH of the aqueous phase was adjusted to 5.5. The validation of the analytical method for the determination of metformin HCl in tablet formulation was performed following parameters, including system suitability, specificity, the limit of quantification, and limit of detection. Peak shapes asymmetries have resulted. The precision was calculated and showed excellent reproducibility (RSD = 0. 1-0.57 %, n=3). The linearity of the method has been tested in the range of 4.0–12.0 µg mL^{-1} for metformin HCl. The limits of detection and quantification have been also established to be lower than 2.7 µg mL^{-1} and 8.0 µg mL^{-1}, respectively. This method is suitable for estimating pharmaceutical formulations with no interference from excipients.

تطوير والتحقق من صحة طريقة HPLC-UV لتقدير الميتفورمين هيدروكلوريد في الأقراص المتوفرة في السوق الليبي

الكلمات المفتاحية:
الكروماتوغرافي؛
ميتفورمين هيدروكلوريد؛
اقراص؛
التحقق من صحة الطريقة.

المستخلص: تم تطوير طريقة HPLC-UV سريعة وبسيطة والتحقق من صحتها؛ لتقدير الميتفورمين هيدروكلوريد في شكل جرعة قرصية، وكذلك لتحديد العلامات التجارية المختلفة للميتفورمين هيدروكلوريد باستخدام اختبار BP كروماتوغرافيا الطبقة الرقيقة. تم إجراء طريقة الكروماتوغرافي السائل عالي الأداء على عمود C18 معكوس الطور (250x 4.6 مم ، 5 ميكرومتر) بمعدل تدفق 1.0 مل دقيقة $^{-1}$ مع الكشف بواسطة الأشعة فوق البنفسجية عند 236 نانومتر. تم إجراء الطور المتحرك بطريقة ثابتة وتحتوي على 52٪ أسيتونيتريل، و 48٪ طور مائي (0.1 مولار بوتاسيوم ثنائي هيدروجين أورثوفوسفات). تم تعديل الأس الهيدروجيني للطور المتحرك إلى 5.5. تم إجراء التحقق من صحة الطريقة التحليلية لتحديد ميتفورمين هيدروكلوريد في شكله الدوائي كأقراص بعد حدود مثل: ملاءمة النظام، والنوعية، وحد القياس الكمي، وحد الكشف، وأشكال الذروة وعدم التناسق تم إنتاجها. وكذلك تم حساب الدقة، وأظهرت استنساخًا ممتازًا (RSD = 0. 1-0.57)٪ وتم اختبار خطية الطريقة في نطاق 4.0–12.0 ميكروغرام مل $^{-1}$ للميتفورمين هيدروكلوريد. أيضا تحديد حدود الكشف، والقياس الكمي أيضًا لتكون أقل من 2.7 ميكروغرام مل $^{-1}$، و 8.0 ميكروغرام مل $^{-1}$ على التوالي.

INTRODUCTION

Diabetes mellitus is a category of metabolic disorders in which blood glucose levels are higher than normal due to insulin deficiency or inappropriate cell response to insulin (Nasri & Rafieian-Kopaei, 2014). Metformin mainly

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acts by reducing hepatic glucose production as well as increasing glucose uptake and utilization by muscles, which eventually leads to regulating blood glucose levels. (Foretz, Guigas, & Viollet, 2023). Metformin works by assisting in the restoration of the body's insulin response. It has been used in addition to dietary control and exercise to prevent diabetes in those who are at high risk of developing the disease. It is also used to treat polycystic ovarian syndrome in women. It may help regulate menstrual periods and enhance fertility (Hundal & Inzucchi, 2003). Metformin is a biguanide derivative that contains a lot of guanidine, a hypoglycemic chemical (C. J. Bailey & Day, 1989; Hill, 1771). It is also known as 1, 1 dimethylbiguanide hydrochloride, and comes as a white crystalline powder that is hygroscopic and freely soluble in water, slightly soluble in alcohol, and practically insoluble in acetone and methylene chloride. The melting point ranges from 223 to 226 ºC (da Trindade, Kogawa, & Salgado, 2018).Only a few methods, such as HPLC and GC, have been published for estimating metformin hydrochloride in pharmaceutical formulations and biological fluids (Zounr, Khuhawar, Khuhawar, Lanjwani, & Khuhawar, 2023) (Arayne, Sultana, & Zuberi, 2006).

HPLC methods were the most widely used for the analysis of metformin. The sensitive ion-pair HPLC methods were used for the quantification of metformin in plasma and the simultaneous determination of it with gliclazide and glipizide present in multicomponent dosage forms (Arayne et al., 2006). Liquid chromatography with electrospray ionization tandem mass (LC-ESI-MS/MS) spectrometric detection was used to analyze metformin in combination with glipizide in human plasma (Ding, Zhou, Ge, Zhi, & Ma, 2007). The official method for estimating the active ingredient in tablets is the UV spectrophotometric approach (Pharmacopoeia, 1996). Metformin was first synthesized in the 1920s (Thomas & Gregg, 2017; Werner & Bell, 1922), it was found to be beneficial in the 1940s and 1950s when used to treat an influenza infection, and it was found to drop glucose but not below physiologic values. Because other biguanide drugs have safety concerns, metformin fell out of favour until the 1990s. It was currently approved by the Food and Drug Administration (FDA) as a first-line treatment for type 2 diabetes, as well as on and off-label indications for diabetes prevention in prediabetes, polycystic ovarian syndrome, antipsychotic-associated weight gain, weight loss, gestational diabetes, and fertility enhancement (Control & Prevention, 2014; Finkelstein, Trogdon, Cohen, & Dietz, 2009).

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) have recommended metformin as the first-line oral medication for the treatment of type 2 diabetes since 2009 (Nathan et al., 2009) after phenformin and buformin were withdrawn from the market in most countries due to their high risks of lactic acidosis (Nattrass & Alberti, 1978). Metformin has been shown to have long-term metabolic effects as well as lower cardiovascular risk. Metformin is becoming more widely recognized as a potential anticancer agent due to a lower cancer rate in diabetes people who take the medicine (Evans, Donnelly, Emslie-Smith, Alessi, & Morris, 2005). Recently, patients taking metformin were associated with a reduced risk of COVID-19-related mortality (Bramante et al., 2021; Crouse et al., 2021). In humans, oral absorption of metformin from immediate-release dose formulations is incomplete, with a population mean bioavailability of 55% (Graham, Punt, Arora, Day, & Doogue, 2011). Metformin's blood-glucose-lowering impact is attributed to numerous tissues, although its mechanism of action remains unknown after long-term therapeutic use. Reduced hepatic gluconeogenesis is the main mechanism of action mediated through the control of mitochondrial enzymes and antagonistic regulation of the glucagon signaling pathway (Madiraju et al., 2014). Metformin was found to improve the insulin sensitivity of muscle in rodents in vitro by boosting insulin receptor expression and activity, resulting in

higher insulin-dependent glucose uptake in cells (C. Bailey & Puah, 1986; Rossetti et al., 1990). The current method reported a new, simple, sensitive, precise, accurate, linear, and isocratic RP-HPLC method for the quantitative estimation of Metformin HCl.

MATERIALS AND METHODS

Chemicals and Reagents: Metformin HCl reference standard with a certified purity of 99% was purchased from Glentham Life Sciences (UK). HPLC grade acetonitrile was obtained from Carlo Erba (France). Potassium dihydrogen orthophosphate was obtained from El Nasr Pharma (Egypt), and water was obtained in-house by distillation. All other reagents and solvents used were of analytical grade.

Brand Selection and Sample Collection: This study is based on the comparison of different doses of metformin 500, 850, and 1000 mg tablet brands in the Libyan market that are available for consumer use. There are approximately twenty brands of metformin tablets in the Libyan pharma market. Among them, eight available brands were selected for the study of some physical and chemical parameters, and all brands were labelled by their trade names. Study samples were coded as shown in Table 1.

Table: (1). Metformin hydrochloride (MTF HCL) tablets available in the Libyan market

MTF HCl Brand Names	Sample Codes		
	500 mg	850 mg	1000 mg
Mylan France	MTF 1-1	MTF 1-2	MTF 1-3
Bristol	MTF 2-1	MTF 2-2	MTF 2-3
Dialon XR UAE	MTF 3-1	MTF 3-2	MTF 3-3
Glyformin Cyprus	MTF 4-1	-	-
Metforal Italy	-	MTF5-2	-
Glucophage Turkey	-	MTF 6-2	-
Diabitos Tunisia	-	-	MTF 7-3
Glucophage France	-	-	MTF 8-3

Analytical Methods

Melting Point Determination: The melting point of Metformin was determined by using Stuart Equipment SMP 10 capillary melting point apparatus.

Thin Layer Chromatography Materials: Thin-Layer Chromatography was carried out on aluminum-backed (Silica gel 60 F 254, Merck, Germany) by using TLC Nanomate 4 device, and spots were visualized using ultraviolet light (254 nm). The eluting solvent was according to BP. Its solvent system was prepared to be used in chromatographic controls of compounds which were Glacial Acetic Acid: Butanol: Water (10: 40: 50).

Development of TLC Plate: Dragging conditions: The solvent system was poured into the TLC chamber and remained for 24 hours to reach saturation, then filled with the development solvent to a depth of no greater than 0.5 cm.

Different brands of metformin HCl and the standard material were dissolved in water and then applied to thin-layer chromatography (TLC) plates which were dragged for 15 cm at room temperature.

HPLC Instrumentation: HPLC operating conditions used a Mobile phase consisting of 0.1M Potassium dihydrogen orthophosphate (pH 5.5), 52% acetonitrile, and 48% aqueous phase. The flow rate was 1.0 mL min^{-1} with an injection volume of 20 µL. Three replicate injections of each calibration standard were performed. Data analysis was carried out using Thermo Electron Corporation software (ChromQuest).

Validation of HPLC method: Validation was acceptable for its intended purpose, as defined in the International Conference on Harmonization (ICH) guidelines (Harmonization 1994). Analytical validation was assessed the accuracy, linearity, precision, LOD, and LOQ.

The precision (RSD) was expressed with respect to variation in the expected drug concentrations. The accuracy was determined by measuring a known amount of standard material under different conditions. After validation, the developed method was applied to a pharmaceutical dosage form containing Metformin HCl.

Preparation of Standard Solution: Calibration standard was prepared as 10.0 mg of the metformin HCl weighed accurately into a 100 ml volumetric flask and diluted to 100 µg mL-1 with the mobile phase. Concentrations of 4.0, 6.0, 8.0, 10.0, and 12.0 µg mL^{-1} were used for the construction of the standard r curve.

Preparation of Sample Solution: Twenty tablets were randomly selected from each brand, weighted and crushed. An accurately weighed amount of powder equivalent to 10.0 mg of metformin HCL was transferred into a 100 mL volumetric flask, shaken with 70 mL of mobile phase for 10 min, and then diluted to 100 mL with the same solution, then filtered and diluted to 8.0 µg mL^{-1}. The sample was analysed using HPLC, three injections were run on each brand, and the area of metformin-HCl peaks was quantified with the equation of calibration series of metformin standard to get the amount of metformin in the percentage present in each brand.

Statistical Analysis: Data were analyzed using Minitab version 20 software. Statistical differences were tested using one-way ANOVA. Differences were considered significant at p values ≤ 0.05, and LD analysis was then performed to determine whether there were statistically significant differences at p values ≤ 0.05 between the concentrations of the different samples.

RESULTS AND DISCUSSION

Identification of Metformin HCl in Formulated Tablet by TLC: Because the detected structure of the active ingredient has a chromophore, all the spots of Metformin HCl in commercial brands and standards appeared clearly under UV lights. Thus, the distance traveled by the Metformin HCl substance from the origin (where the compound was applied onto the TLC plate) was divided by the distance traveled by the solvent from the origin to obtain the Rf value shown in Table 2.

Table: (2). Rf values of standard and tested brands

No.	Brand code of MTF tables	Rf Values
0	Standard	0.88
1	MTF1-1	0.81
2	MTF-2-1	0.85
3	MTF-3-1	0.88
4	MTF4-1	0.85
5	MTF-1-2	0.85
6	MTF2-2	0.85
7	MTF3-2	0.84
8	MTF5-2	0.85
9	MTF6-2	0.88
11	MTF1-3	0.88
12	MTF2-3	0.85
13	MTF3-3	0.88
14	MTF7-3	0.84
15	MTF8-3	0.88

Development and Validation HPLC Method: A suitable analytical method was developed after evaluating the major and critical separation parameters of chromatography. Isocratic elution was carried out using the HPLC method, and the first parameter to be changed was the pH of the mobile phase buffer which increased from 3.5 to 5.5. The organic modifier was also increased to 52% using pH 5.5 with the aqueous component. The result is shown in Figure 1. The retention time was reduced to 2.9 min. Thus, this eluting time was satisfactory and showed that there was no interference of excipients. Therefore, this showed that the method used was selective for metformin HCl analysis. Utilizing a UV–vis spectrophotometer, the absorbance maxima of metformin HCl were determined to be 236 nm.

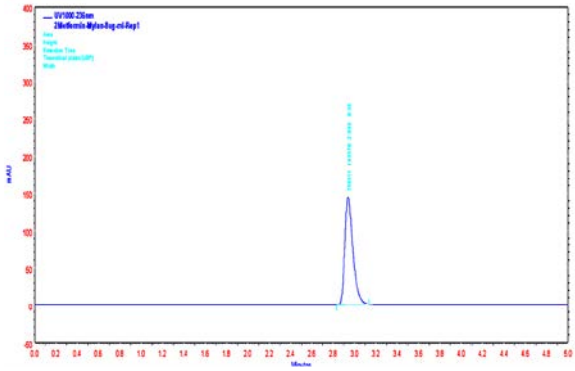

Figure: (1). Representative chromatogram of the solution containing Metformin HCl standard (15 µgmL-1) obtained using the Isocratic HPLC method (mobile Phase: Potassium dihydrogen orthophosphate buffer (0.1M, pH 5.5): Acetonitrile)

The optimized method for analyzing metformin HCl was validated and the reproducibility was tested. This involved making up a fresh solution of calibration standards that contained metformin HCl in the mobile phase and then running it three times on the HPLC system(Khreit, Elfowiris, Aljali, & Abduljalil, 2021). The linearity of metformin HCl was established by preparing a series of concentrations with metformin HCl standard solutions ranging from 4.0 µg mL^{-1} to 12.0 mg mL^{-1}. The calibration standards demonstrated a linear response (r 2 = 0.999 -1.0). The linear equation was extrapolated, and the correlation coefficient, slope, and intercept were for the calibration curve.

Repeatability of the system (interday precision) was checked by injecting the different concentrations of standard solution on the same day, and the relative standard deviation (RSD) of the chromatographic method was less than 2 % within a day, which complies with the BP requirements (RSD = 0. 1-0.57 %, n=3). The limits of detection and the limit of quantification for metformin HCl were determined to be 2.70 and 8.20 µg mL^{-1}, respectively.

Table 3 shows also some other validation parameters that were been calculated from the calibration series chromatograms, including retention time, capacity factor, as well as symmetry factor, which showed metformin HCl standard solution eluted with a slight peak tailing (A_s =1.21).

Table (3): Summary of validation data for the quantification of Metformin HCl using Brownlee Bio C18 column (250x4.6 mm and 5 µm particle size); mobile phase: 0.1M Buffer: Acetonitrile;
Detector wavelength: 236 nm.

	Metformin HCl
t_R (min) (t0 = 2.2 min)	2.9 min.
Capacity factor (k′)	0.32
Symmetry factor (A_s)	1.21
LOD (µg mL^{-1})	2.70 µg mL^{-1}
LOQ (µg mL^{-1})	8.20 µg mL^{-1}
Co-efficient of regression (r2)	0.999 (101079x+3779.4)
Precision (% RSD) (n= 3)	
4.0 µg mL^{-1}	0.37
6.0 µg mL^{-1}	0.38
8.0 µg mL^{-1}	0.57
10.0 µg mL^{-1}	0.39
12.0 µg mL^{-1}	0.10

The results obtained in the evaluation of robustness showed that a small variation in the composition of the mobile phase, its pH variations, flow rate during chromatography, and column temperature, has an insignificant impact on metformin HCl chromatogram.

Application of HPLC Method for Determination of Metformin HCl in Commercial Products : Once the final method had been developed and validated, the fourteen metformin HCl samples of eight brands were tested using this method to identify the metformin HCl compound in tablet samples. The samples were powdered and then dissolved in the mobile phase as previously described and analyzed by HPLC based on the HPLC method described above. The injection of each sample was repeated three times, and chromatograms were recorded. Representative chromatograms for the fourteen samples are shown in Figure 2. This result indicates that the peak of the analyte was pure, and this confirmed the specificity of the method.

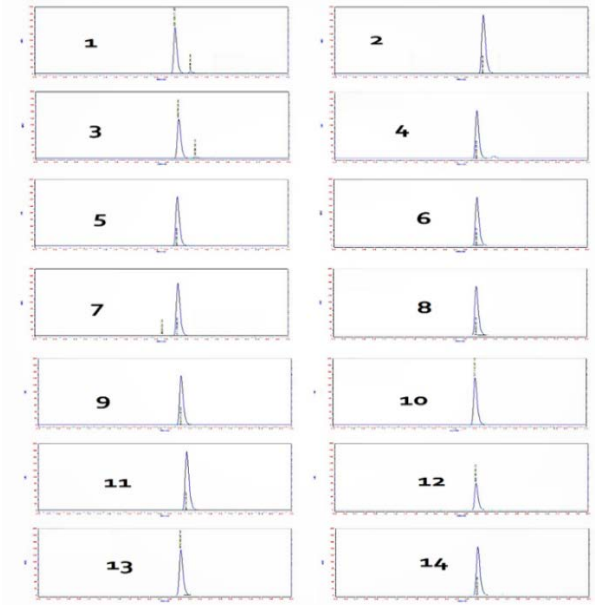

Figure: (2). Representative chromatogram for tested tablets of different brands of metformin (1) Mylan 500mg (2) Bristol500mg (3) Dialon xr 500 mg (4) Glyformin 500mg (5) Metforal 850mg (6) Dialon 850mg (7) Glucophage 850 (8) Bristol 850 (9) Mylan 850mg (10) Mylan 1000mg (11) Bristol 1000mg (12) Dialon 1000mg (13) Diabitos 1000m **(**14) Glucophage 1000mg

The regression equation of linearity was used to calculate the concentration of extracted tablet samples. The state of the content of metformin HCl in powder assayed from the different brand samples is shown in Table 4.

Table: (4). Assay result of the amount of metformin HCl obtained from HPLC analysis.

Sample Code	State of Content (±SD)		
	500 mg	850 mg	1000 mg
MTF 1	95.56 ± 0.019	95.5 ±0.32	87.60± 0.081
MTF 2	91.14 ±0.29	97.31 ± 1.7	95.49± 0.074
MTF 3	88.97 ±2.5	92.72± 0.27	83.55± 0.24
MTF 4	73.51 ±0.12		
MTF 5		1.75±96.69	
MTF 6		105.45±0.79	
MTF 7			87.60±0.86
MTF 8			83.55±0.29

Metformin HCl was studied for its active pharmaceutical ingredient percentage at three concentrations (500 mg, 850 mg, and 1000 mg) manufactured by different commercial brand companies (State of Content). These samples were collected from various pharmacies in El-Beida, and an HPLC instrument was utilized to analyze them.

Table 3 shows that some formulations containing 500, 850, and 1000 mg metformin HCl per tablet complied with BP specifications for metformin HCl content (95% - 105% of the labelled content(, with the majority of them lying outside of the BP 2012 specification range. In the first series of different brands of metformin tablets 500 mg, MTF 1-1 only compiled with the BP specifications and showed a significant difference from the other brands tested. However, MTF 2-1, MTF 3-1, and MTF 4-1 did not comply with BP specifications. In the second metformin 850 mg series with different brand companies. The results showed a significant difference between the MTF 6-2 and the other brands tested. However, MTF 1-2, MTF 2-2, and MTF1 complied with BP specifications. In the last group of metformin series with 1000 mg analysis of different companies, MTF 1-3, MTF3-3, MTF 7-3, and MTF 8-3 did not comply with BP specification except for MTF 2-3, which was within the BP specification range.

CONCLUSION

This study investigated different commercial brands of metformin HCl collected from different pharmaceutical markets in El-Beida under wild environmental conditions, and their state of content was evaluated using the HPLC method. The presented, developed, and validated method was rapid, economic, simple, accurate, sensitive, robust, specific, and linear. This optimized isocratic method can be employed as an absolute qualification and quantification method for routine analysis of metformin tables, either alone or in combination products.

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Expression and Significance of Vascular Endothelial Growth Factor Receptors 2 and 3 in Endometrial Carcinoma

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Endometrial carcinoma, VEGF receptor, VEGF receptors2-3.

Abstract: The aim of this work is to evaluate the expression and significance of Vascular Endothelial (VEGFR2,3) in endometrim. The study was applied to 70 females selected from El-Shatby University Hospital. Group I: 35 patients with dysfunctional uterine bleeding. Group II: 35 patients diagnosed with endometrial carcinoma. In the present work, VEGFRs 2 and 3 expression was detected by quantitative real-time PCR. The results revealed that: regarding relative VEGFR gene quantitation, there were no significant differences in the rates of VEGFR 2 and 3 expression between controls and cases. Regarding VEGFR 2 and 3 with type I & II, low and high histological grade, early and late stage, and lymphovascular invasion of endometrial cancer cases, there was no statistically significant relation. In this particular study, it was concluded that the expression levels of VEGFR2 and VEGFR3 do not exhibit any significant increase in endometrial cancers compared to dysfunctional uterine bleeding. Furthermore, we have observed that there exists no discernible correlation between VEGFR2 and VEGFR3 with regard to the histological type, grade, stage, or lymphovascular invasion of the endometrial cancer case.

دراسة مستقبلات عامل نمو بطانة الأوعية الدموية 2 و3 و دلالتها في سرطان بطانة الرحم

الكلمات المفتاحية :
سرطان الرحم، مستقبلات عامل نمو بطانة الأوعية الدموية 2و3.

المستخلص : الهدف من هذا العمل هو تقييم تعبير مستقبلات عامل النمو البطاني الوعائي من النوع 2 و3 (VEGFR2،3) في بطانة الرحم، وأهميته. طبقت الدراسة على 70 أنثى تم اختيارهن من مستشفى الشاطبي الجامعي. المجموعة الأولى: 35 مريضة تعاني من نزيف رحمي مختل. المجموعة الثانية: تم تشخيص 35 مريضة على أنه سرطان بطانة الرحم. في العمل الحالي ، تم الكشف عن تعبير VEGFRs 2 و3 بواسطة تفاعل البوليميراز المتسلسل في الوقت الحقيقي الكمي. أظهرت النتائج ما يلي: فيما يتعلق بالكمية النسبية للجينات VEGFRs، لا توجد فروق ذات دلالة إحصائية في معدلات التعبير VEGFR2 & 3 بين الضوابط والحالات. بخصوص VEGFR 2 & 3 مع النوع الأول والثاني، الدرجة النسيجية المنخفضة والعالية، المرحلة المبكرة والمتأخرة، الغزو اللمفاوي لحالة سرطان بطانة الرحم، لم تكن هناك علاقة ذات دلالة إحصائية. في هذه الدراسة، خلصنا إلى: لا يزيد التعبير عن VEGFR2 و VEGFR3 في سرطانات بطانة الرحم بالمقارنة مع نزيف الرحم المختل. لم يلاحظ أي ارتباط بين VEGFR2 و VEGFR3 مع النوع النسيجي، أو الدرجة، أو المرحلة، أو الغزو اللمفاوي لحالة سرطان بطانة الرحم.

INTRODUCTION

Endometrial cancer represents the most prevalent invasive gynaecological malignancy in Europe and North America. This particular malignancy's occurrence is on the rise, with a yearly diagnosis rate of 150,000 cases across the globe. It stands as the fifth most prevalent type of cancer and the seventh most frequent cause of mortality among women (Amant et al., 2005; Plataniotis & Castiglione, 2010).

The racial difference in the occurrence of uterine corpus cancer can be related to the distribution of identified risk factors such as socioeconomic status (Elwood et al., 1977),

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reproductive history (Brinton, 1992), and use of exogenous estrogens (McDonald et al., 1977). The bases for racial variances in cancer survival are not as clearly described (Hill et al., 1996).

Angiogenesis is a pivotal process in the advancement of tumors. Folkman in 1971, studied the significance of angiogenesis in cancer biology. In 1990, (Folkman, 1990) collated supporting data on this matter. It is currently recognized by a vast majority of scholars and researchers that the process of angiogenesis, which refers to the formation of new blood vessels, plays a critical role not only in facilitating the growth and development of tumors, but also in the initial advancement from a pre-cancerous tumor state to a full-blown cancerous condition. (Hanahan & Folkman, 1996).

In 1995, (Folkman, 1995) conducted a review of emerging clinical applications of angiogenesis research, which have since focused primarily on two aspects. The quantification of angiogenesis in cancer patients is employed for the purpose of diagnosis and prognosis, as well as for impeding tumor growth by means of angiogenesis inhibition. Notably, significant advancements have been made in both directions in recent years (McNamara et al., 1998; Thompson et al., 1999).

Angiogenic factors are produced by both malignant and infiltrating cells. The angiogenic switch is associated with the activation, manifestation, and release of angiogenic factors by malignant cells during the development of tumors. Moreover, tumors possess the ability to generate inhibitors of angiogenesis. (Takahashi et al., 1996).

Vascular endothelial growth factor (VEGF) is categorized among the most influential angiogenic factors. It exhibits a discernible mitogenic effect on endothelial cells and seems to be devoid of mitogenic effect on other cell types.(Ferrara et al., 1992). This peptide that binds to heparin generates five distinct molecular isoforms, which arise through the process of alternative splicing of mRNA (Neufeld et al., 1999).

Most types of tumor cells generate multiple isoforms of VEGF concurrently, although the prevailing variants are typically VEGF121 and VEGF (Neufeld et al., 1999). VEGF appears to have a pivotal function in the regulation of tumor angiogenesis. The induction of VEGF release by tumor cells is prompted by hypoxia (Shweiki et al., 1992). As solid neoplasms enlarge, the cells within the enlarging conglomeration often experience hypoxia due to the progressive separation from adjacent blood vessels. The upregulation of vascular endothelial growth factor (VEGF) by neoplastic cells is also enhanced by the activation of oncogenes, such as ras. (Rak et al., 1995) or inactivation of tumor suppressor genes, such as p53, (Kieser et al., 1994), and by other cytokines, such as transforming growth factor beta (TGF-b) (Pertovaara et al., 1994) and nitric oxide (Chin et al., 1997). Suppression of tumor growth has been demonstrated in vivo through the inhibition of Vascular Endothelial Growth Factor (VEGF). The VEGF family, comprising VEGF – A to D, is a multifunctional cytokine that is an important regulator of tumor angiogenesis (Berchuck et al., 1989; Boocock et al., 1995).

VEGF-A elicits angiogenic consequences through its interactions with the unique receptors VEGFR-1 and VEGFR-2, predominantly eliciting effects within vascular endothelial cells (Brown et al., 1993; Jeltsch et al., 1997). The localization of Flt-4 is significantly confined to the cells of the lymphatic endothelium, thereby implying that Flt-4 serves as a distinctive identifier for the cells of the lymphatic endothelium (Guidi et al., 1995; Smith, 1998). The investigation was executed to evaluate the manifestation and importance of Vascular Endothelial Growth Factor Receptors type 2 and 3 in the context of endometrial cancer.

MATERIALS AND METHODS

This investigation was conducted on a cohort of seventy individuals, 35 diagnosed with endometrial carcinoma and 35 with endometrial hyperplasia, presented to the Shatby Maternity University Hospital. Laboratory work was done in the Clinical Pathology Department, University of Alexandria, in the period from 1/2015 to 8/2015.

Group I: Thirty-five patients presented with endometrial carcinoma attended the gyne-oncology clinic of Shatby Maternity University Hospital, Alexandria University.

Group II: Thirty-five subjects matched for age with the study group presented with abnormal uterine bleeding served as a control group.

All patients in group I were diagnosed according to: pathologically proven endometrial carcinoma by endometrial biopsy.

The study was subjected to the following: medical examination and routine investigations: D&C biopsy for preliminary histopathological examination and imaging investigations.

Total abdominal hysterectomy with bilateral salpingo-oophorectomy: The biopsies were histopathologically tested, diagnosed, and graded using the criteria of the modified (FIGO) surgical staging and grading system for uterine corpus carcinoma (Beddy et al., 2012; Holland, 2010; McCluggage et al., 2010).

(VEGFRs) 2 and 3 mRNA expression analysis by quantitative real-time PCR**:**

Quantitation of VEGFR2 and VEGFR 3 mRNA expression by quantitative real-time polymerase chain reaction (qRT- PCR), which was done for all patients and controls included in this study (van't Veer et al., 2006).

RNA isolation and cDNA preparation followed by quantitative real-time qRT-PCR were done to assess VEGFRs 2 and 3 mRNA expression in all cases and controls.

Reverse transcription, commonly referred to as RT, followed by polymerase chain reaction or PCR, represents the most preferred technique for analyzing mRNA expression originating from diverse sources. Real-time PCR, renowned for its high sensitivity, enables researchers to quantify even the slightest changes in gene expression (van't Veer et al., 2006).

a) Sample collection two milligrams (mg) of fresh endometrial tissue were collected in tubes containing RNA latter QIAGEN Inc. 2006

b) RNA isolation purification of mRNA from human tissues was done using QIAamp® RNA blood mini kit (Qiagen, Germany, catalog # 52304

c) Quantification and storage of total RNA. The concentration of RNA was determined by measuring the absorbance at 260 nm (A 260) using Nanodrop® 2000 spectrophotometer

d) One-step reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) and QuantiTect Probe RT-PCR assays were used for quantitative real-time one-step RT-PCR using sequence-specific probes.

Data Analysis

Data processing was performed using Rotor Gene Q software.

Relative quantitation of VEGFR 2 and 3 mRNA expression: relative quantitation was expressed by a comparative Ct method where the amount of target, normalized to an endogenous reference; GAPDH and relative to the average ΔCt of normal controls, Livak

KJ and Schmittgen TD 2001 was given by: $2^{-\Delta\Delta Ct}$

Statistical analysis: Data were analyzed by using SPSS software version 20.0. Significance of the obtained results was judged at the 5% level.

RESULTS

Characteristics of Patients Included in the Study

A. Age.
B. Obstetrics and menstrual histories.

Clinical Characteristics of Tumors According to:

- Pathological types.
- Histopathological grade (Low and High).
- Stage: early (I and II) and late (III and IV).
- Lymphovascular invasion.

Relative VEGFRs Gene Expression

- Analysis of studied cases according to relative gene quantitation.
- Correlation between relative VEGFR2 and VEGFR3 genes quantitation.
- Correlation between VEGFR2 and age.
- Correlation between VEGFR3 and age.
- Relation between relative VEGFR2 quantitation and clinical characteristics of tumors (pathological type, histopathological grade, FIGO stage, lymphovascular-invasion and uterine enlargement).
- Relation between relative VEGFR3 quantitation and clinical characteristics of tumors (pathological type, histopathological grade, FIGO stage, lymphovascular invasion, and uterine enlargement).

Characteristics of Patients Included in the Study

Age: Table (1) shows a comparison between the two studied groups according to age. There were no statistically significant differences between the two studied cases regarding age (P=0.178).

Table: (1). Comparison between the two studied groups according to age

Age (years)	Cases (n= 35)		Controls (n= 35)		Test of sig.	p
	n	%	n	%		
<50	5	14.3	8	22.9	□□□ □□□ □□	0.459
50 - <60	6	17.1	8	22.9		
≥60	24	68.6	19	54.3		
Min. – Max.	43.0 – 74.0		44.0 – 77.0			
Mean ± SD.	61.46 ± 9.28		58.49 ± 8.98		t= 1.361	0.178
Median	65.0		60.0			

χ^2: Chi square test
t: Student t-test

Obstetrics and Menopausal histories: Table (2) shows a comparison between cases and controls according to obstetric and menstrual histories. There were no statistically significant differences between cases and controls regarding parity (p=0.380), abortion (0.809), menopausal history (p=0.673), and duration since menopause (p=0.704).

Table: (2). Shows a comparison between cases & controls according to obstetric and menstrual histories

Gravidity	Cases (n= 35)	Controls (n= 35)	Test of sig.	p
Min. – Max.	0.0 – 14.0	0.0 – 9.0		
Mean ± SD.	5.31 ± 3.66	5.54 ± 2.39	Z=0.668	0.504
Median	5.0	5.0		
Parity				
Min. – Max	0.0 – 11.0	0.0 – 9.0		
Mean ± SD.	4.31 ± 3.22	4.69 ± 2.29	Z=0.877	0.380
Median	4.0	5.0		
Abortion				
Min. – Max.	0.0 – 10.0	0.0 – 5.0		
Mean ± SD.	± 1.94	0.83 ± 1.42	Z=0.242	0.809
Median	0.0	0.0		
Menopausal history	N %	n %		
Premenopausal	2 5.7	4 11.4	χ^2= 0.729	p= 0.673
Postmenopausal	33 94.3	31 88.6		
Duration since menopause	(n= 33)	(n= 31)		
Min. – Max.	1.0 – 27.0	2.0 – 25.0		
Mean ± SD.	13.61 ± 7.24	14.39 ± 8.12	Z= 0.379	0.704
Median	12.0	15.0		

Z: Z for Mann Whitney test
χ^2: Chi square test
FE: Fisher Exact test

Clinical Characteristics of Tumors According to:

Pathological Types: As shown in Table (3), 32 patients (91.4%) had endometriod adenocarcinoma (type I), while 2 patients (5.7%) with papillary and 1 patients (2.9%) with clear cell carcinoma (type II).

Histopathological Grade: Grade I (well differentiated): included 13 patients (37.1%).

Grade II (Moderately differentiated): included 15 patients (42.9%).

Grade III (Poorly differentiated): included 7 patients (20%).

FIGO Stage

Stage I: was present in 20 patients (57.1%)

Stage II: 8 patients (22.9%)

Stage III: 7 patients (20%)

Stage IV: no patients

Table: (3). Distribution of the studied cases (n=35) according to pathological type, histological grade, and FIGO stage

Type	N	%
Type I (Endometriod)	32	91.4
Type II (papillary & clear cell)	3	8.6
Histological Grade		
Low	28	80.0
Grade I (well differentiated)	13	37.1
Grade II (moderately differentiated)	15	42.9
High	7	20.0
Grade III (poorly differentiated)	7	20.0
Stage		
Early	28	80.0
I	20	57.1
II	8	22.9
Late	7	20.0
III	7	20.0
IV	0	0.0

Lymphovascular Invasion: Table (4) shows the distribution of the studied cases according to lymphovascular infiltration. Lymphovascular invasion was present in 7 patients (20%).

Table: (4). Distribution of the studied cases (n=35) according to lymphovascular infiltration

	n	%
Lymphovascular infiltration		
Negative	28	80.0
Positive	7	20.0

Relative VEGFRS Gene expression

Analysis of Studied Cases according to Relative Gene Quantitation: A descriptive analysis of the studied cases according to relative gene quantitation is shown in Table (5) In endometrial adenocarcinomas, gene expression was scored as normal expression (1), under expression (<1), over expression (>1).

VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%).

VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%).

Table: (5). Descriptive analysis of the studied cases (n = 35) according to relative gene quantitation

Relative VEGFR2 Gene Quantitation	N	%
Normal "1"	1	2.9
Under expression "<1"	31	88.6
Over expression ">1"	3	8.6
Min. – Max.	0.01 – 2.48	
Mean ± SD.	0.40 ± 0.57	
Median	0.12	
Relative VEGFR3 Gene Quantitation	n	%
Normal "1"	0	0.0
Under expression "<1"	32	91.4
Over expression ">1"	3	8.6
Min. – Max.	0.01 – 3.39	
Mean ± SD.	0.30 ± 0.69	
Median	0.06	

Correlation between Relative VEGFR2 and VEGFR3 Genes Quantitation: Correlation between the relative VEGFR 2 and 3 genes quantitation is shown in Figure (1). A statistically significant positive correlation was evident between the relative VEGFR2 and VEGFR3 genes.

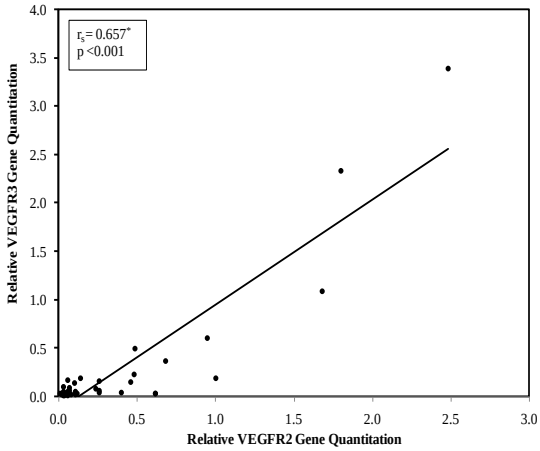

Figure: (1). Correlation between relative VEGFR2 gene quantitation and relative VEGFR3 gene quantitation

Correlation between VEGFR2 and Age: Figure (2) shows a correlation between relative VEGFR2 gene quantitation and age. It illustrated that there was no significant correlation between VEGFR2 and age (p=0.920).

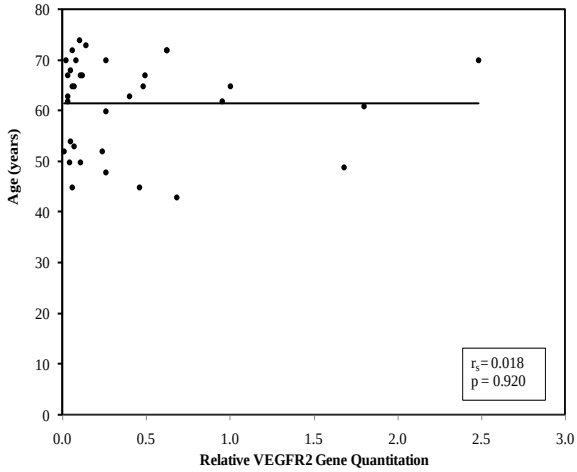

Figure: (2). Correlation between relative VEGFR2 gene quantitation and age

Correlation between VEGFR3 and Age: Correlation between relative VEGFR3 gene quantitation and age. It illustrated that there

was no significant relation between VEGFR3 and age (p=0.182).

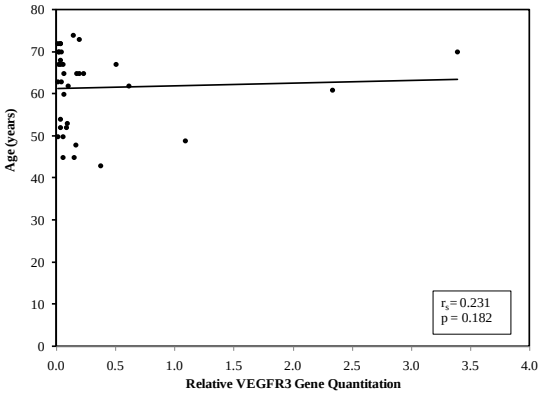

Figure: (3). Correlation between relative VEGFR2 gene quantitation and age

Relation between Relative VEGFR2 Quantitation and Clinical Characteristics of Tumors (Pathological Type, Histopathological Grade, FIGO Stage, and Lymphovascular-Invasion: There was no statistically significant relationship between the VEGFR2 gene and pathological type (p=0.443), histopathological grade (p=0.741), stage (p=0.132), and lymphovascular infiltration (p=0.079).

Relation between relative VEGFR3 gene quantitation and clinical characteristic of the tumor (pathological type, histological grade, FIGO stage, and lymphovascular infiltration

There was no statistically significant relationship between the VEGFR3 gene and pathological type (p=0.193), histopathological grade (p=0.535), stage (p=0.172), and lymphovascular infiltration (p=0.222).

Table: (6). Relation between Relative VEGFR2 Quantitation and Clinical Characteristics of Tumors

	n	Relative VEGFR2 Gene Quantitation			Z	p
		Min. – Max.	Mean ± SD.	Median		
Type I (Endometriod)	32	0.01 – 1.80	0.35 ± 0.46	0.12	0.767	0.443
Type II	3	0.06 – 0.26	0.16 ± 0.14	0.16		
Histological Grade						
Low	28	0.01 – 1.80	0.38 ± 0.48	0.12	0.330	0.741
High	7	0.03 – 2.48	0.47 ± 0.89	0.14		
r_s(p)			0.039(0.823)			
Stage						
Early	28	0.01 – 2.48	0.47 ± 0.62	0.25	1.507	0.132
Late	7	0.03 – 0.46	0.13 ± 0.15	0.07		
r_s(p)			0.306(0.073)			
Lymphovascular Infiltration						
Negative	28	0.01 – 2.48	0.48 ± 0.61	0.26	1.755	0.079
Positive	7	0.03 – 0.14	0.08 ± 0.04	0.07		

Z: Z for Mann Whitney test
r_s: Spearman coefficient

Table: (7). Relation between relative VEGFR3 the tumor gene quantitation and clinical characteristics

	n	Relative VEGFR3 Gene Quantitation			Z	p
		Min. – Max.	Mean ± SD.	Median		
Type I (Endometriod)	32	0.01 – 2.33	0.21 ± 0.45	0.06	1.301	0.193
TypeII	3	0.05 – 0.16	0.11 ± 0.08	0.11		
Histological Grade						
Low	28	0.01 – 2.33	0.23 ± 0.47	0.06	0.621	0.535
High	7	0.01 – 3.39	0.56 ± 1.25	0.10		
r_s(p)			0.061(0.730)			
Stage						
Early	28	0.01 – 3.39	0.36 ± 0.76	0.06	1.366	0.172
Late	7	0.01 – 0.15	0.06 ± 0.05	0.03		
r_s(p)			0.162(0.353)			
Lymphovascular Infiltration						
Negative	28	0.01 – 3.39	0.36 ± 0.76	0.06	1.221	0.222
Positive	7	0.01 – 0.19	0.07 ± 0.06	0.03		

DISCUSSION

Endometrial carcinoma ranks as the most prevalent intrusive gynecological neoplasm in Europe and North America (Chan et al., 2007; Papanikolaou et al., 2006).

The phenomenon of angiogenesis exhibits a paramount significance in the advancement of diverse tumors, such as endometrial carcinomas. Several cytokines, along with their corresponding receptors, have been demonstrated to be implicated, specifically in relation to VEGFR1, -2, and -3 (Guidi et al., 1995).

The scrutiny of the expression of various factors that promote the growth of new blood vessels and the receptors that bind to them has been extensively studied and confirmed to be apparent in a wide range of cancerous conditions, including breast, pancreatic, and colorectal tumors (Kim et al., 2011; Lozano-Leon et al., 2011).

The examination of their expressions in cases of endometrial cancers has been the subject of prior investigation, as evidenced by the works of (Brys et al., 2007; Donoghue et al., 2007). However, several of these studies have produced conflicting data, with certain instances indicating an increase in expression relative to normal endometrium, while others fail to demonstrate such an effect. Similarly, some studies have established a correlation between the expression of VEGFs or their receptors and prognostic factors, while others have not observed this association. (Guidi et al., 1995).

In the present work, VEGFRs 2 and 3 expression, with the occurrence of a particular event, specifically the detection of a specific element, was identified and established through the utilization of a highly precise and quantitative technique known as quantitative real-time polymerase chain reaction (PCR) in all patients with a wide range of expression between the highest and the lowest values.

In the current study, relative VEGFR2 gene quantitation ranged from 0.01 to 2.48 with a mean of 0.40 ± 0.57, and between 0.01 and 3.39 with a mean of 0.30 ± 0.69 in the VEGFR3 gene. VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%). VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%). There were no notable disparities observed in the frequencies of VEGFR 2 and 3 manifestation among the control group and the affected individuals.

Our study revealed that the relative VEGFR2 gene ranged from 0.01 to 2.48 with a mean of 0.40±0.57, and between 0.01 and 3.39 with a

mean of 0.30±0.69 in the VEGFR3 gene. VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%). VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%). There were no notable variations found in the rates of expression of Vascular Endothelial Growth Factor Receptor 2 and 3 (VEGFR2&3) between the control group and the group of individuals with the condition under investigation.

In agreement with our study, Erdem et al. (2007) conducted a comparative analysis of various markers of angiogenesis, including vascular endothelial growth factor (VEGFRs), CD34, and endoglin, in proliferative endometrium (PE), endometrial hyperplasia (EH), and endometrial carcinoma (EC). The aim was to assess the potential impact of angiogenesis on the process of malignant transformation.

The present study comprised a cohort of 66 individuals, out of which 12 exhibited proliferative endometrium, 23 showed endometrial hyperplasia (11 with simple hyperplasia and 12 with complex hyperplasia exhibiting atypia), and 31 manifested endometrial carcinoma, and were all incorporated in this investigation.

Histological specimens of both proliferative endometrium (PE) and endometrial hyperplasia (EH) were extracted via (D&C) and (TAH) procedures. Meanwhile, histological specimens of endometrial adenocarcinoma (endometrioid type) were procured from surgically treated patients. In cases of endometrial cancer (EC), tumors were categorized according to the (FIGO) staging. The cohort consisted of 16 patients with stage I disease, 7 with stage II disease, and 8 with stage III disease. Histologically, 15 patients were diagnosed with (grade [G1]), while 16 patients had (G2) and (G3) adenocarcinomas. Furthermore, five out of eight patients with stage III disease were found to have metastases to the pelvic lymph nodes. It was observed that Vascular Endothelial Growth Factor Receptors (VEGFRs) expression was significantly higher in EC and EH specimens than in PE specimens, but no difference in expression was detected between EC and EH samples (Erdem et al., 2007).

(Wang et al., 2014) the investigation delved into the examination of VEGF-A, VEGFR2, and VEGFR3 expression in endometrial tumors in comparison to the normative endometrium. The investigation consisted of a collective of 76 individuals who had received a medical diagnosis of endometrial adenocarcinomas. The average age of these individuals was determined to be 64 years, with a minimum age of 39 years and a maximum of 88 years. Among the 76 cases, there were 43 endometrioid adenocarcinomas, 22 serous carcinomas, 7 clear cell carcinomas, and 4 carcinosarcomas. It was observed that a total of 10 tumors were categorized as grade I, while 25 tumors were classified as grade II, and 41 tumors were designated as grade III. These classifications encompassed all types of tumors including serous, clear cell, and carcinosarcomas, which are specifically labeled as high-grade.

The reasons that have led to the different results between the current and all other existing studies, such as that of (Wang et al., 2014), is that the majority of studies about VEGFRs expression in endometrial cancer have compared normal endometrium in controls group with endometrial cancer, whereas in our study, the control group had dysfunctional uterine bleeding. This is due to the fact that the ethics committee refused to collect samples from normal cases. In addition, the number of cases in our study is small in comparison with other studies.

In contrast to our study, (Yokoyama et al., 2000) in the course of their research, the researcher acquired newly obtained surgical

samples of endometrial carcinoma from a total of 86 patients. The (FIGO) criteria was followed for the surgical staging of all patients. The surgical procedure included radical or modified radical (TAHSOP), pelvic and para aortic lymphadenectomy. The analysis of endometrial carcinoma staging demonstrated that there were 9 patients classified as being at stage Ia, 34 patients classified as being at stage Ib, 7 patients classified as being at stage Ic, 1 patient classified as being at stage IIa, 4 patients classified as being at stage IIb, 9 patients classified as being at stage IIIa, and 22 patients classified as being at stage IIIc.

The histological types were categorized into 80 instances, along with an additional occurrence of endometrioid adenocarcinoma, three instances of adenosquamous carcinoma, one instance of adenoacanthoma, and two instances of clear cell adenocarcinoma. Furthermore, surgical specimens were procured from 14 women who had complex atypical endometrial hyperplasia (AEH) and from 15 women who underwent surgical treatment for uterine cervical neoplasia or ovarian tumor and had histologically confirmed normal endometrium. There was a noteworthy dissimilarity in the occurrence of VEGFR-3 identification between the conventional endometrium and CAH. The frequency of VEGFR-3 identification in stage I/II carcinoma was notably more elevated than that in the conventional endometrium, even though there was no substantial contrast in the frequency of VEGFR-3 detection between CAH and stage I/II carcinoma. The frequency of VEGFR-3 detection in stage III/IV carcinoma was notably higher than in the conventional endometrium, CAH, and stage I/II carcinoma.

(Guidi et al., 1995) the focal strong expression of VEGFr mRNA by endometrial tumor cells was initially described, accompanied by an observation that flt-1 and KDR mRNAs were strongly expressed by the endothelial cells surrounding microvessel density MVs.

(Holland, 2010) the mRNA of VEGF-A was verified to be present in the epithelial cells of the EC, yet not within the normal endometrium and atypical complex hyperplasia.

The observed discrepancies in results may be attributed to the varying methodologies employed, as our investigation utilized qRT-PCR, a modality that demonstrates enhanced sensitivity towards variations in gene expression, in contrast to other studies that made use of immune-histochemical techniques.

In our investigation, no discernible association was observed between Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) and Vascular Endothelial Growth Factor Receptor 3 (VEGFR3) in relation to the histological type grade stage or lymphovascular invasion. Relative VEGFR2 gene expression ranged from 0.01 to 1.80 with a mean of 0.35±0.46 in type I (endometriod type) and ranged between 0.06 to 0.26 with a mean of 0.16±0.14 in type II. There was no statistically significant relationship (p=0.443). The expression of the VEGFR2 gene with low histological grade ranged from 0.01 to 1.80 with a mean value of 0.38±0.48, and from 0.03 to 2.48 with a mean value of 0.47± 0.89 in high grade. There was no statistically significant relationship. (p=0.741), VEGFR2 expression ranged from 0.01 to 2.48 with a mean of 0.47 ± 0.62 in early-stage patients and ranged from 0.03 to 0.46 with a mean of 0.13 ± 0.15 in late stage. There was no statistically significant relationship (p=0.132), and VEGFR2 expression ranged from 0.03 to 0.14 with a mean value of 0.08±0.04. There was no statistically significant relation between relative VEGFR2 gene quantitation and lymphovascular infiltration (p=0.079).

Relative VEGFR3 gene expression ranged from 0.01 to 2.33 with a mean of 0.21±0.45 in

endo metriod type and ranged between 0.05 and 0.16 with a mean of 0.11± 0.08 in type II. There was no statistically significant relation between relative VEGFR3 gene quantitation and type I (p=0.193) or with type II (p=721). The expression of the VEGFR3 gene with low histological grade ranged from 0.01 to 2.33 with a mean value of 0.23±0.47, and from 0.01 to 3.39 with a mean value of 0.56±1.25 in high grade. There was no statistically significant relation between relative VEGFR3 gene quantitation and histological grade (p=0.535). VEGFR3 expression ranged from 0.01 to 3.39 with a mean of 0.36 ± 0.76 in early-stage patients and ranged from 0.01 to 0.15 with a mean of 0.06 ± 0.05 in late stage. There was no statistically significant relation between relative VEGFR3 gene quantitation and stage (p=0.172), and VEGFR3 expression ranged from 0.01 to 0.19 with mean value of 0.07±0.06 in positive lymphovascular invasion patients. There was no statistically significant relation between relative VEGFR3 gene quantitation and lymphovascular infiltration (p=0.222). In agreement with our study, Wang et al. (2014) conducted analysis which also discovered a lack of any connection between VEGFR2 and the histological type, grade, stage, or lymphovascular invasion. However, it was ascertained that the manifestation of VEGFR3 exhibited a significant correlation with the tumor stage, although it did not exhibit a significant association with the histological type, grade, or lymphovascular invasion.

(Giatromanolaki et al., 2001) found that no significant correlation was found between the expression of (VEGFRs) and the histologic type, histologic grade, depth of myometrial invasion, or lymph vascular space invasion. However, it is worth noting a slight connection between the increased expression of VEGF and the advanced International Federation of Gynecology and Obstetrics (FIGO) stage. The diversity of the angiogenic function in distinct areas of a neoplasm poses a difficulty in the precise evaluation of neovascularization in tumor tissue. Specifically, the expression of Vascular Endothelial Growth Factor (VEGF) is known to be highest in hypoxic areas of the tumor near necrotic regions. As such, the specific location within the tumor that is examined may significantly impact the results of the evaluation of VEGF expression in the tumor. These variables are likely to contribute to the variability observed in studies examining the expression of angiogenic factors in tumors (Poon et al., 2001).

CONCLUSION

In this particular investigation, we have reached a definitive conclusion.The upregulation of VEGFR2 and VEGFR3 is not observed in endometrial malignancies when compared to dysfunctional uterine bleeding. None correlation was observed between the expression levels of vascular endothelial growth factor receptor 2 (VEGFR2) and vascular endothelial growth factor receptor 3 (VEGFR3) and the histological type, grade, stage, or lymphovascular invasion of endometrial cancer cases. More researches and studies on a larger number of cases, The period should be the longest follow-up period to prove the impact of VEGFR2&3 on endometrial cancer and its prognosis**.**

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ETHICS

All the patients were counseled about the procedure and an informed consent was taken before the beginning of the study.

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The Relationship of Betatrophin and Kisspeptin with Related Hormones in Women Newly Diagnosed with Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is one of the most widespread endocrine hormone disorders that affects women of reproductive age. It's a major public health concern that leads to dysregulations in the reproductive, metabolic, and psychological systems. Anovulation, which has a significant impact on female fertility, and hyperandrogenism are the most characteristic of PCOS. Because of its endocrine role, adipose tissue secretes several proteins called adipokines that work as hormones controlling bodily functions, including resistin, betatrophin, etc. The cases involved in this study were 90 women. For comparison, 30 healthy women with regular periods suitable with age were used as a control group. 30 Obese PCOS women were diagnosed depending on the Rotterdam criteria as $group_2$, and 30 non-obese PCOS women as $group_3$. The results showed there was a significant elevate in insulin and insulin resistance and related hormones between groups compared to the control, and a significant increase at ($P \leq 0.01$) in levels of betatrophin in $group_2$ compared to the control, with a significant difference between the $group_2$ and $group_3$, and a significant increase at ($P \leq 0.01$) in levels of kisspeptin in $group_2$ compared to the control. No significant difference between $group_2$ and $group_3$ and a significant difference between $group_3$ and the control group.

العلاقة بين البيتاتروفين والكيسبيتين مع الهرمونات ذات الصلة في النساء المشخصات حديثا بمتلازمة المبيض متعدد الأكياس

الكلمات المفتاحية :
البيتاتروفين . الكيسبيتين؛ متلازمة المبيض متعدد الاكياس مقاومة الانسولين.

المستخلص : متلازمة المبيض متعدد الأكياس واحدة من أكثر اضطرابات هرمونات الغدد الصماء انتشارا التي تصيب النساء في سن الإنجاب، وهي مصدر أساسي لها علاقة بالصحة العامة التي تؤدي إلى خلل في الأنظمة التناسلية والأيضية والنفسية، يعد انقطاع الإباضة الذي يلعب دورا أساسيا في خصوبة المرأة ، وفرط الأندروجين من أكثر خصائص المتلازمة بسبب دوره في الغدد الصماء ، يفرز النسيج الدهني عددا من البروتينات التي تسمى بالاديبوكاينز والتي تعمل بوصفها هرمونات تتحكم بوظائف الجسم ، مثل الريزستين والبيتاتروفين. هذه الدراسة شملت 90 امرأة. للمقارنة: 30 امرأة تتمتع بصحة جيدة مع فترات حيضية منتظمة ومناسبة للعمر، والوزن عرفت بمجموعة السيطرة ، 30 امرأة بدينة مصابة بمتلازمة المبيض متعدد الأكياس تم تشخيصهن بالاعتماد على معايير روتردام عرفت بالمجموعة الثانية، 30 امرأة ليست بدينة مصابة بمتلازمة المبيض متعدد الأكياس عرفت بالمجموعة الثالثة. النتائج أظهرت وجود ارتفاع كبير في الإنسولين، ومقاومة الإنسولين مع الهرمونات ذات الصلة بين المجموعات مقارنة بمجموعة السيطرة ، مع وجود زيادة معنوية في مستويات البيتاتروفين في المجموعة الثانية مقارنة بمجموعة السيطرة ، مع اختلاف معنوي بين المجموعتين الثانية والثالثة، إضافة إلى وجود زيادة معنوية في مستويات الكيسبيتين في المجموعة الثانية مقارنة بمجموعة السيطرة، مع عدم وجود اختلافات بين المجموعتين الثانية والثالثة ، مع وجود اختلافات في المجموعة الثالثة مع مجموعة السيطرة.

INTRODUCTION

Adipose tissue secretes a variety of chemicals known as adipokines and functions as both an active endocrine organ and a passive energy store. Adipokines are a class of cytokines that control several physiological processes, including immunity, metabolism, hunger, inflammation, and cardiovascular health. Among the numerous additional adi-

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pokines are resistin, adiponectin, and leptin. Adipose tissue's production of adipokines is contingent upon the nature of the adipocytes (brown or white), as well as its size, quantity, arrangement, and interactions with other cells. White adipocytes and brown adipocytes are the two primary subtypes of adipocytes (Mancuso, 2016).

Produced by the fat cells are physiologically active compounds with properties similar to those of traditional hormones. These are signaling proteins found in cells that control or modify a range of biological functions in the target organs, such as the immune system, blood vessels, brain, liver, muscles, and heart (Fasshauer & Blüher, 2015). They have a variety of roles and can affect a wide range of processes, such as the regulation of hunger and energy, the metabolism of lipids and glucose, insulin sensitivity, endothelial cell function, inflammation, angiogenesis, blood pressure, hemostasis, the development of atherosclerosis, and metabolic syndrome. (Farkhondeh et al., 2020; Metz et al., 2020). Also, they may help to explain some pathophysiologic infertilities like polycystic ovary syndrome (PCOS) (Bongrani et al., 2019).

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine system conditions affecting women of reproductive age, also known as hyperandrogenic anovulation (HA) or Stein –Leventhal syndrome (El Hayek et al., 2016). This chronic and heterogeneous disorder manifests as menstrual dysfunction, infertility, hirsutism, acne, and obesity (Motlagh Asghari et al., 2022). It depicts a syndrome in which at least one ovary develops an estimated ten tiny cysts, with diameters ranging from 2 to 9 mm, and at least one ovary has an ovarian capacity larger than 10 ml (Balen et al., 1993). It is usually only diagnosed when complications develop that significantly reduce a patient's quality of life (e.g., hair loss, alopecia, acne, and infertility–related problems) (Azziz, Sanchez, et al., 2004). According to a systematic screening of women using the National Institutes of Health (NIH) diagnostic standards, 4–10% of reproductive-age women are predicted to have PCOS worldwide (El Hayek et al., 2016). A changed LH/FSH ratio is caused primarily by hormonal abnormalities, specifically elevated luteinizing hormone (LH), and normal or suppressed follicle-stimulating hormone (FSH). Additionally, hyperinsulinemia and insulin resistance are linked to the clinical signs of hyperandrogenism. It is unclear what factors may predispose a woman to develop PCOS, but it has been observed in some instances that the condition is genetic in nature and that obesity contributes to hyperinsulinemia, which predisposes people to the condition (Asunción et al., 2000; Barth et al., 2007).

Betatrophin (angiopoietin-like protein 8 (ANGPTL8)) is a hormone that was only recently found in the liver of humans. White adipose, brown adipose, and mammalian liver tissues have all been found to contain numerous homologous sequences. Insulin resistance, lipid metabolism, and the onset of type 2 diabetes (T2D) are all dependent on betatrophin (Guo et al., 2022). Betatrophin was identified in 2004 as a serum antigen unique to tumors (Dong et al., 2004). Cumulating evidence suggests that Betatrophin is significantly associated with adiposity, type 2 diabetes, and metabolic syndrome (Abu-Farha et al., 2016; Ghasemi et al., 2015; Yamada et al., 2015). Most women with PCOS display impaired glucose tolerance and are at higher risk for developing T2DM. Moreover, Betatrophin has a close relationship with insulin resistance and T2DM (Chen et al., 2015; Stepto et al., 2013; Yamada et al., 2015).

Kisspeptin was first discovered in 1996 as a metastasis inhibitor in melanoma cell lines (Lee et al., 1996). It is a peptide that is mostly expressed in the hypothalamic infundibular nucleus. Kisspeptin is essential for controlling reproductive processes. It is thought to be the primary factor in the regulation of the menstrual cycle and fertility, the

start of puberty, and the hypothalamic-pituitary-gonadal axis. Many processes, including follicular maturation, ovulation, steroidogenesis, and ovarian senescence, are influenced by kisspeptin activity. Studies on the function of kisspeptin in puberty have been sparked by the discovery of kisspeptin receptor mutations that result in hypogonadotropic hypogonadism. A significant factor in the development of PCOS, functional hypothalamic amenorrhea, and perimenopausal vasomotor symptoms is pathologies affecting the neurons secreting kisspeptin (Szeliga & Meczekalski, 2022).

MATERIALS AND METHODS

A total of 90 women between 18– 40 years old were collected from Iraqi hospitals and private laboratories in Mosul and Erbil governorate from the period 15/8/2022 to 22/12/2022.

A gynecologist made a diagnosis based on the Rotterdam criteria, which includes: oligo/anovulation, hyperandrogenism, quantifying it biochemically, noting its signs such as hirsutism, acne, baldness, and examining the ovary's morphology using pelvic ultrasound (ESHRE & Group, 2004). Age, BMI, blood pressure, smoking history, T2DM, primary and secondary infertility, and symptoms of hyperandrogenism such as hirsutism, acne, and male pattern baldness were among the questions on a questionnaire created for the women participating in this study (Halawa et al., 2020), All women were divided into three groups:

Group1: Control, included 30 healthy women who have normal serum parameters level with no PCOS symptoms.

Group2: This group included 30 obese women with PCOS, and the diagnosis was relied upon by a specialized gynecologist relying on the Rotterdam criteria with BMI (31.72) Kg/m2.

Group3: This group included 30 normal-weight women with PCOS and BMI (21.09) Kg/m2. None of the women had used oral contraceptives, glucocorticoids, ovulation induction agents, anti-obesity drugs, or any other steroid-containing drug, but they were diagnosed for the first time with PCOS.

Collecting Samples: After 16 h of overnight fasting, 5 ml of venous blood was collected from the women and placed in gel tubes. The serum was separated by a centrifuge and the separated serum was placed in a sterile plastic Eppendorf tube and frozen at (– 20) C until assayed.

Hormonal and Biochemical Tests: Laboratory investigations included the following: BMI, (calculated as Kg/m^2). Betatrophin, KISSPEPTIN, Insulin, LH, and Testosterone, determined by ELISA methods. The index for insulin resistance; HOMA IR was calculated according to the equation: Fasting plasma glucose (mg/dl) × Fasting serum insulin μIU/ml/405 (Matthews et al., 1985).

Statistical Analysis: Data were gathered, updated, coded, and put into the IBM SPSS statistical software program. When the distribution of the quantitative data was determined to be parametric, the mean, standard deviations, and ranges were reported. The Duncun's multiple range test revealed that the different coefficients were substantially separated by various letters of the alphabet under the probability threshold of ($P \leq 0.01$). Therefore, the data were investigated using a system of simple experiments and a full random design. The variables were also correlated with one another (Antar & Al-Wakaa, 2017).

RESULTS

Depending on the table below, the results showed a significant increase in BMI in obese women with PCOS ($Group_2$), while there was no significant difference between non-obese women with polycystic ovary

syndrome ($Group_3$). In addition, there was a significant increase in fasting glucose, insulin, and HOMA–IR in $group_2$ and $group_3$ compared to the control group, noting a significant difference between $group_2$ and $group_3$ with these parameters. A significant increase was observed in both LH and testosterone in the $group_2$ compared to the control group, with no significant difference observed between $group_2$ and $group_3$. Finally, the results showed a significant increase in Betatrophin in obese women with PCOS ($Group_2$), while there was no significant difference between non-obese women with polycystic ovary syndrome ($Group_3$). Also, the results showed of Kisspeptin that there was no significant difference between $group_2$ and $group_3$ with a significant difference between $group_2$ and $group_3$ with the control group.

Table: (1). The value of all study parameters in the different groups

Group Parameters	$Group_1$ (no.=30)	$Group_2$ (no.=30)	$Group_3$ (no.=30)
BMI (Kg/m^2)	21.10 ± 1.91b	31.72 ± 2.81 a	21.07 ±1.68 b
Fasting glucose(mg/dl)	88.07 ± 6.64 c	155.8 ± 12.1 a	102.7 ±13.8 b
Insulin (µIU/ ml)	7.42 ± 0.86 c	8.29 ± 0.81 a	7.91± 0.53 b
HOMA–IR	1.62 ± 0.26 c	3.18 ± 0.49 a	2.01± 0.38 b
LH (µIU/ ml)	2.8 4± 0.91 b	13.04 ± 1.74 a	13.28± 1.24 a
Testosterone(ng/ml)	1.12 ± 0.34 b	3.13± 0.61 a	3.15± 0.69 a
Betatrophin (pg/ml)	202.94± 23.79 b	507.99 ± 102.45a	193.44± 25.31 b
Kisspeptin (ng/ml)	150.15±6 .97 b	193.97±5 .75 a	195.85±6 .51 a

The no. followed by different letters means there is significant difference.
The values is means ± standard deviation SD

DISCUSSION

Adipose tissue releases a variety of adipokines that control lipid metabolism, glucose metabolism, and insulin resistance (Coelho et al., 2013). The results showed a significant increase in BMI in $group_2$ compared with the control group. Numerous processes regulate how obesity and excess weight affect the emergence of PCOS. The metabolic impacts of insulin resistance, as well as the steroidogenic and reproductive implications of hyperinsulinemia, are significant processes. It appears that adipokines involved in metabolism, are produced by visceral and subcutaneous fat. It's important to consider any possible effects of PCOS on future weight gain, or at the very least, on attempts to reduce weight and maintain it through dietary and lifestyle changes, given the complex nature of PCOS etiology (Barber et al., 2019).

Research results indicated that in PCOS–affected women, Betatrophin concentrations had a strong association with BMI and insulin resistance (Calan et al., 2016). Insulin resistance promotes Betatrophin to be produced. In insulin-resistant mice, Betatrophin has been observed to increase the rate of beta cell division, stimulate pancreatic beta cell proliferation, and improve metabolic regulation (Yi et al., 2013).

(Sun et al., 2017) displayed that in mice who had diabetes induced by streptozotocin, the transplantation of Betatrophin –expressing adipose-derived mesenchymal stem cells caused β–cell proliferation (Sun et al., 2017). They found that Betatrophin overexpression enabled islet cell proliferation, expression of transcription factors unique to beta cells, and insulin production when glucose was stimulated (Bulmuş et al., 2020). IR and associated compensatory hyperinsulinemia are one of PCOS's causes, and it has been estimated that 70% of PCOS patients reflect IR symptoms. However, the pathogenesis of PCOS is still not fully understood (Hillman & Dale, 2018). Furthermore, HOMA-IR and fasting insulin levels were both substantially positively correlated with serum Betatrophin levels according to a Spearman rank analysis.

These results support those of older populatin-based studies that showed that insulin

resistance and higher circulating Betatrophin levels were present in T2DM patients (Chen et al., 2015). In another research, we observed that insulin stimulation more prominent Betatrophin expression in hepatocytes. Treatments with metformin and rosiglitazone reduced the expression of Betatrophin in insulin-stimulated hepatocellular (Wang et al., 2017). This study's results are in agreement with the majority of other examinations that found higher Betatrophin levels in IR individuals or PCOS women (Erol et al., 2017). In a previous study, (Adamska et al., 2017) demonstrated a connection between Betatrophin and island β-cell release and IR (Adamska et al., 2017).

As a result, IR and PCOS may be determined by excessive Betatrophin levels. We think that circulating Betatrophin has a significant clinical effect on adipose IR reflection. The mechanism through which adipose IR raises circulating Betatrophin levels is unknown. We suggest that IR may be the cause of the enhanced production and release of Betatrophin in adipose tissue as a compensatory mechanism. Additionally, in IR circumstances, macrophage-secreted inflammatory cytokines may encourage Betatrophin expression and production (Wang et al., 2017). A peptide hormone termed Betatrophin is synthesized from adipose and liver tissue (Erol et al., 2017). This protein influences lipid metabolism and glucose to a healthy equilibrium (Eksi Haydardedeoglu et al., 2019). Circulating Betatrophin levels have been observed in T1DM and PCOS (Ersahin et al., 2017). However, the pathogenic effects of Betatrophin on insulin secretion and glucose homeostasis are not fully recognized (Erbag et al., 2016).

The reproductive phenotype of PCOS and its pathogenesis. LH secretion is specifically increased by increased pulsatile GnRH release. LH facilitates the generation of testosterone in ovarian theca cells. Due to a relative FSH shortage, the surrounding granulosa cells are unable to appropriately aromatize testosterone. Additionally, the activity of several steroidogenic enzymes is constitutively elevated in polycystic ovaries, which boosts androgen synthesis. Additionally, PCOS may produce more adrenal androgen. Alopecia, hirsutism, and acne are all signs of androgen excess that are indirectly caused by testosterone. As a result of unopposed estrogen activity on the endometrium, androstenedione, and testosterone may be capable of aromatizing extragonadally to produce estradiol and estrone. Testosterone feeds back on the hypothalamus to lessen sensitivity to the effects of estradiol's typical feedback (Diamanti-Kandarakis & Dunaif, 2012). According to certain studies, the LH reacts strongly to the GnRH released by the hypothalamus in PCOS. Additionally, one of the primary features of PCOS is hyperandrogenism, particularly with regard to testosterone. This condition results in an increase in LH production without a negative feedback loop, which in turn causes an increase in androgens with a reduction in SHBG (Hřebíček et al., 2002).

According to several research, serum Betatrophin levels are strongly correlated with PCOS progression and are important to the IR process. Since Betatrophin may be a sensitive biomarker of PCOS development, it may also be a useful target for PCOS therapy (Gong et al., 2021). In addition, increasing androgen levels in PCOS patients also cause ovarian cells to release more Betatrophin (Eksi Haydardedeoglu et al., 2019). This might be the cause of its rise in PCOS patients and its link to LH and Testosterone, as one of the most significant symptoms of this medical condition is a rise in LH (Azziz, Woods, et al., 2004), which then causes a surge in testosterone and, subsequently, a rise in Betatrophin (Eksi Haydardedeoglu et al., 2019). According to data from the Northern Finland Birth Cohort 1966, BMI and symptoms of PCOS are significantly correlated at all ages (Ollila et al., 2016). However, it's still unknown how PCOS and BMI are related to one another. Some research suggest that Kisspeptin increases in-

sulin production and blood glucose levels, which raises BMI (Miranda et al., 2013).

In the present research, we reported that patients with higher BMIs had greater blood levels of Kisspeptin than patients with normal BMIs, which was consistent with (Rafique & Latif, 2015). Additionally, there was an association between Kisspeptin levels and serum testosterone and BMI (Umayal et al., 2019). Our findings conflict with those of Rafique and Latif (Rafique & Latif, 2015). who carried out their research on groups of female patients from Saudi Arabia and found that Kisspeptin blood levels were the same in normal and overweight individuals. As compared to individuals with overweight BMI, it has been observed that PCOS patients with normal BMI had higher amounts of Kisspeptin (Akad et al., 2022).

Additionally, in women with polycystic ovarian syndrome, Kisspeptin has been linked to measures of insulin resistance as well as body mass index (Panidis et al., 2006). Kisspeptin enhances the release of insulin from isolated islets that produce insulin in humans and mice, in addition to its effects on the hypothalamus (Hauge-Evans et al., 2006). Kisspeptin's demonstrated capacity to increase insulin secretion, its confirmed mediating role in the influence of changed nutritional status on reproductive function, and the significance of pancreatic β–cells (Bowe et al., 2009). This will clarify the way in which insulin secretion and kisspeptin are related. The methods by which Kisspeptin and many other receptor-operated agonists enhance nutrient-induced secretion while preventing the initiation of a secretory response are similar in that they promote insulin secretion in the presence of a stimulatory concentration of glucose. These experiments were conducted in vitro. Rats with normal nonfasted blood glucose levels, however, showed a substantial increase in plasma insulin when Kisspeptin was administered in vivo (Bowe et al., 2009).

Kisspeptin's effect on modulating the pulsatile release of GnRH and its associated effect on ovulation was very recently discovered. (Azziz, 2016). This discovery makes it possible to explore an additional path of the physio-pathogenesis of chronic anovulation. LH levels are more powerful in PCOS–afflicted women, which might be related to raised kisspeptin activity (Terao et al., 2004), an essential regulating element in the pulsatile release of GnRH, which, as in our study, increases LH production. It is essential to emphasize that, in addition to other variables impacting PCOS traits, such as BMI and insulin disruption, an aberrant GnRH to LH ratio may be an indicator of the physio-pathogenesis of PCOS. According to our study, kisspeptin is connected with both LH and testosterone as well as BMI (Araújo et al., 2020). LH levels are higher in PCOS–afflicted women, which may be related to a raised kisspeptin production (Terao et al., 2004). A significant regulating element in the pulsatile release of GnRH, which in turn increases LH production. It is essential to emphasize that, in addition to other variables impacting PCOS traits, such as BMI and insulin failure, an abnormal GnRH to LH ratio may be a characteristic of the physio-pathogenesis of PCOS (Araújo et al., 2020). Hypothalamic abnormalities in the irregular production of GnRH may have a role in the physiopathology of PCOS. The hypophysis secretes more LH as a result of the alterations. Kisspeptin is a neuropeptide that binds to GPR54, a G-protein-coupled transmembrane receptor that exists in GnRH neurons, causing it to become more active and raising LH levels as an outcome (Lee et al., 1996). Kisspeptin may affect ovarian function through elevated LH secretion (Azziz, 2016), causing theca interna cells of the ovarian follicles to secrete more androgen. Such hyperactivity inhibits the development of follicles, lowers the likelihood of ovulation, and, as a result, minimizes the clinical suppression of GnRH via progesterone during the luteal phase. The testosterone lined with kisspeptin

and the current study's findings show a favorable link (Yilmaz et al., 2014).

However, it was not capable of determining if the rise in kisspeptin was due to hyperandrogenism (Lopes et al., 2014). Kisspeptink's possible effects on PCOS may be mediated through neurokinin B and its receptor. In actuality, the change in LH pulsatility and redution of kisspeptin –mediated LH production were caused by the blockade of NKBNK3R signaling. Another theory is that unusual LH or its receptor is caused by susceptibility genes, as evidenced by the discovery of multiple novel risk loci and candidate genes for PCOS by genome–wide association studies (Crespo et al., 2018). Additionally, because Kisspeptin mRNA is highly abundant in both rat and human gonads, Kisspeptin may directly affect rat ovaries (Terao et al., 2004). It could be a further mechanism for Kisspeptin to affect the pathophysiogenesis of PCOS (Araújo et al., 2020).

The theory that an overactive Kisspeptins system supports greater HPG-axis activity, which in turn causes irregular menstrual cycles and excessive androgen production in PCOS women, is consistent with the overall rise in Kisspeptin levels in the PCOS population (Tang et al., 2019). It was determined through experimental research on humans that supplying patients with kisspeptin causes their LH levels to improve (Dhillo et al., 2007). Administration of GnRH possesses an effect on human beings with KISS1R mutations and hypogonadotropic hypogonadism. We can state with trust that kisspeptin is activating stimulants for the GnRH in the hypothalamic-pituitary-gonadal (HPG) axis as demonstrated by recent research (d'Anglemont de Tassigny, 2007).

Kisspeptin can raise LH levels, but its effects on the FSH take more time to appear and are less prominent. Different gonadotrophin secretory patterns or actions to kisspeptin may have less impact on FSH (McCann et al., 2002). In the 2014 data collection by (Jayasena et al., 2014), kisspeptin treatment increased LH levels by more than twofold while having little to no effect on FSH levels (Jayasena et al., 2014). Numerous investigations have shown that kisspeptin administration directly affects the upstream regulation of the depolarization process by GnRH neurons and the upregulated expression of GnRH mRNA, which raises the LH/FSH ratio (Akad et al., 2022).

CONCLUSION

There was a significant elevation in LH, testosterone, insulin, fasting glucose (FBS), and insulin resistance (HOMA-IR) in $group_2$ and $group_3$ compared to the control group. It was also noted that results show a significant increase at ($P \leq 0.01$) in levels of Betatrophin in $group_2$ (507.99± 102.45) pg/ml compared to the control group (202.94± 23.79) pg/ml with noting that there was a significant difference between $group_2$ and $group_3$ with (193.44± 25.31) pg/ml and a significant increase at ($P \leq 0.01$) in levels of kisspeptin in $group_2$ (193.97± 5.75) ng/ml compared to the control group (150.15± 6.97) ng/ml with noting that there was no significant difference between $group_2$ and $group_3$ with (195± 5.85) ng/ml and significant difference between $group_3$ and the control group.

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ETHICAL

The study was conducted with the approval of the concerned authorities in the Department of Biology, the Deanship of the College of Science, and the Nineveh Health Department, in an official letter dated 2022/6/13 and numbered (3142) to collect samples from hospitals

(Al-Batoul Teaching Hospital and Al-Salam Teaching Hospital) in the city of Mosul, as well as the patient's knowledge and consent to take the sample and with the required health information.

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Non-Compliance of Patients with Antibiotic Prescriptions

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Abstract: Non-compliance with antibiotic treatment instructions is dangerous in complete cure, increasing treatment costs and increasing the number of resistant microbes. This study aimed to assess adherence to antibiotic treatment and its associated factors in patients after visiting Libyan community pharmacies. A cross-sectional study was conducted on patients who visited community pharmacies in Libya. Several reasons for non-compliance have been reported but the main ones were it is not necessary to continue taking prescribed medications once feeling better (27.3%), polypharmacy (19.6%), fear of medication adverse effects (18.7%) and the duration of antibiotic treatment also increased the risk of non-compliance (15%). Appropriate prescribing strategies to increase the public awareness, knowledge, and attitude to ensure appropriate antimicrobial use and minimize the problem of non-compliance.

عدم امتثال المرضى لوصفات المضادات الحيوية

الكلمات المفتاحية : مضادات حيوية؛ المقاومة البكتيرية؛ صيدليات المجتمع؛ التزام المريض؛ ليبيا.

المستخلص: يعد عدم الالتزام بتعليمات العلاج بالمضادات الحيوية أمرًا خطيرًا يؤثر في الشفاء التام، مما يؤدي إلى زيادة تكاليف العلاج وزيادة عدد الميكروبات المقاومة. هدفت هذه الدراسة إلى تقييم مدى الالتزام بالعلاج بالمضادات الحيوية والعوامل المرتبطة به لدى المرضى بعد زيارتهم الصيدليات المجتمعية. أجريت دراسة بمراقبة المرضى الذين زاروا الصيدليات المجتمعية في ليبيا. تم الإبلاغ عن عدة أسباب لعدم الامتثال ولكن أهمها أنه ليس من الضروري الاستمرار في تناول الأدوية الموصوفة بمجرد الشعور بالتحسن(27.3%)، كما أدى تعدد الأدوية (19.6%) والخوف من الآثار الضارة للأدوية (18.7%) ومدة العلاج بالمضادات الحيوية (15%) إلى زيادة احتمال عدم الامتثال. التركيز على استراتيجيات وصف مناسبة لزيادة الوعي العام والمعرفة والسلوك لضمان الاستخدام المناسب لمضادات الميكروبات وتقليل مشكلة عدم الامتثال.

INTRODUCTION

Medical adherence (or compliance) is defined as the extent to which a patient takes medication as prescribed by their health care providers (Llor et al., 2013) In clinical setting it is important for the patient to adhere to prescribed medications. Non-adherence to antibiotics has led to antimicrobial resistance, treatment failure, re-infection, which may create a subsequent require for more aggressive treatments and an elevated costs of care, hospitalization and increased risk of mortality (French, 2005) (Bruyndonckx et al., 2021). Non-adherent patients may show primary or secondary non-adherence. Primary non adherence, in which the prescribed medication is not dispensed or never taken,

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the focus of this study will be on the secondary non adherence, in which patients discontinue taking a medication after starting, without being advised by a health care provider (Jimmy & Jose, 2011)**.** Unlike medicines that only individually harm the patient if used inappropriately, misuse of antibiotics can affect others by growing risks within the community and even worldwide to antibiotics resistance (Thorpe et al., 2018).

Non-adherence to antibiotic treatment for acute community infections with respect to short term antibiotic treatment, were reported globally with significant variation among countries ranged from 44.0% in China to 9.9% in the Netherlands (Pechère et al., 2007). There are several identified variables behind non-adherence to antibiotics, these include factors associated with;1) medication (such as daily dosage regimen), 2) patients attitudes to doctors (patient-physician relationship) and ,3) patients attitudes to antibiotics (knowledge and beliefs) (Fernandes et al., 2014). The above percentages are considered rather high and sound the alarm and the urgent need to perform similar studies to know the rates of compliance with antibiotic treatment in the Libyan society, as Libya was not included in this regard.Importantly, Libya was found to have a significant rate of antibiotic resistance (Shailabi et al., 2022), and antibiotics were widely prescribed with noticeable non-adherence to medication (Mohammed et al., 2016) (Ahmed Atia & Abired, 2017) (Ahmed Atia et al., 2018)

A Libyan study reported higher frequency of inappropriate use of antibiotics for the treatment of undiagnosed diseases with a higher prevalence of bacterial resistance, where the community pharmacists dispensed more antibiotics than general practitioners (Ahmed Atia & Abired, 2017). Another national survey in Benghazi, Libya reported that, medical and nonmedical students had antibiotics without doctor's prescription, just by purchasing in consultation with a pharmacist, with non-adherence to the enclosed leaflet instructions of the antibiotics or took it to ailments that do not require taking antibiotics such as acne (Ghaieth et al., 2015).

In a survey of antibiotic prescribing practices by doctors in Libya, 18% stated that they may prescribe antibiotics in their prescriptions even if they know that the case is not really in need. The majority of those prescribers justified this practice due to the request of patients (Elbabour et al., 2018). In a study conducted at Abusetta hospital in Tripoli, Libya, the antibiotic resistance pattern of bacteria, exhibited resistance to commonly used antibiotics and to at least one antibiotic (Ahmed Atia, Elyounsi, et al., 2020).

Aim: This study aimed to determine the percentage of adherence to a prescribed antibiotic regimen and is considered one of the first studies to explore the potential factors associated with non-adherence to antibiotics in patients after visiting community pharmacies in Libya.

MATERIALS AND METHODS

A cross-sectional study was conducted on a selected sample of patients who visited community pharmacies across Libya. A sample of 83 pharmacies was randomly selected from those licensed to operate by the Libyan authorities to provide pharmaceutical services in 9 different cities. The study was conducted after approval from the Scientific Research Ethics Committee (SREC), Faculty of Dentistry, Benghazi University, Libya (No, 0107) Written approval (Informed consent) was obtained from all study participants.

Eligible pharmacy customers for the study were those ages ranging from 18 and above, who attended the registered community pharmacies, in the 9 cities representing different parts of the country, between January 2022, and September 2022. Those who presented a prescription for an oral

antibiotic (tablet or capsule) for short-term treatment (less than 30 days) were illegible and included.

Data collection: The questionnaire was structured into five parts: 1) demographic data ,2) the prescribed antibiotic, including the name and dose of the drug, the number of units delivered to the patient, the dosing frequency, and the treatment duration 3) Reasons for giving treatment to the patient, 4) have you adhered to the prescribed antibiotic period? 5) Reasons for non-compliance.
The first three parts of the questionnaire were filled in the pharmacy as general information, the treatment completion date was estimated using collected information. Patients were contacted by phone after the end of the treatment period in order to complete the questionnaire.

Data collected during this interview includes the demographic information: age, gender, level of education, marital status, employment status, and family income. Reasons for prescribed antibiotic treatment for the patient: infection with dental and oral, respiratory system, reproductive system, skin and tissues, ear and eyes, etc.

Factors associated with non-adherence to antibiotic include: 1) Taking many types of drugs, forgetting the correct order and dosage, 2) Pharmacists do not specify the method of administration and dose, 3) Difficulty with taking the antibiotic(non-acceptable smell or shape), 4) Long-term treatment, 5) Concern about the adverse effects described in the medication guide, 6) Antibiotic prices too expensive, 7) Too busy with study or work, 8) Less confidence in doctors, 9) Do not know the exact effect of the prescribed medication,10) insufficient knowledge about the disease and passively taking medication, 11) appearance of an allergy or any side effects after taking the drug, 12) Patients consider that their disease does not require medicine, and will recover on their own,13) Medicine information leaflets are too scientific to understand,14) Difficult to comply with prescribed doses (e.g., need to break the tablet into two parts), 15) It is not necessary to continue the prescribed medications once they feel better, 16) Deceit and follow advertising television or promotion of other products, 17) The prescribed drug has been changed by the doctor, 18) Lack of care or attention from family members, 19) Preference for herbal medicine/traditional medicine recipes, 20) sudden social events 21) Participants were also asked to state any other reasons for non-compliance were not mentioned in the questionnaire and In addition, participants were allowed to choose multiple answers for non-adherence reasons.

Preliminary pilot testing and subsequent revisions from experts were conducted to check the reliability and validity of the questionnaire with a sample size of 49 participants before starting the study.

RESULTS

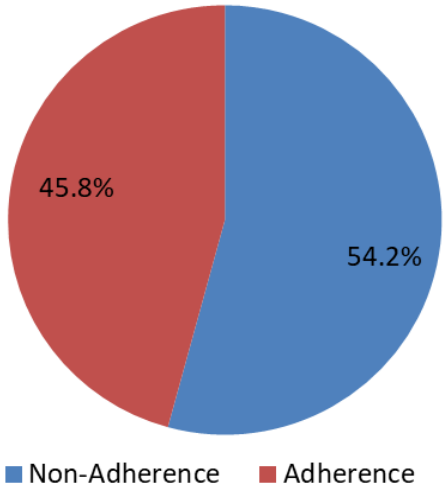

Figure: (1). Proportion of adherence (n = 189) and non-adherence (n = 224) to a prescribed antibiotic regimen among the participants (n 413).

Table:(1). Distribution of Drug Non-Adherence Level Based on Demographic Variables (224 out of 413)

Variables	Drug Adherence (n = 189)	Drug Non-Adherence (n = 224)
Age		
18-24	45(23.8%)	64 (28.5%)
25-34	69(36.5%)	78 (34.8%)
35-44Y	47(24.8%)	47 (21%)
45-54	25(13.2%)	30 (13.4%)
> 54	3(1.6%)	5 (2.2%)
Gender		
Male	58(30.7%)	76 (33.9%)
Female	131(69.3%)	148 (66.1%)
Level Of Education		
Primary school	3(1.6%)	3 (1.3%)
Middle school	5(2.6%)	8 (3.5%)
Secondary school	23(12.2%)	34 (15.2%)
University and above	158(83.6%)	179 (80%)
Marital Status		
Single	74(39.1%)	106(47.3%)
Married	115(60.9%)	118(52.7%)
Employment Status		
Employed	121(64%)	135 (60.3%)
Unemployed	61(32.3%)	79 (35.3%)
Retired	7(3.7%)	10 (4.4%)
Family Income LYD		
750 or less	54(28.6%)	84 (37.5%)
751-1500	74(39.1%)	88 (39.3%)
> 1501-3000	45(23.8%)	42 (18.75%)
3001 or more	16(8.5%)	10 (4.45%)

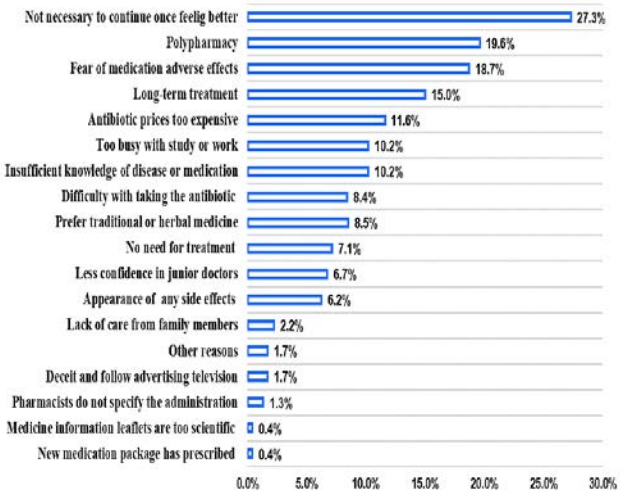

Figure: (2). Reasons for non-adherence to antibiotic therapy

DISCUSSION

The development of bacterial resistance to antibiotics is mainly caused by the inappropriate use of antibiotics, which helps the emergence and spread of resistant bacteria. The resistance development could result in many consequences. A major consequence is that the infection becomes more difficult or even impossible to treat, sometimes turning a common infection into a life-threatening one (Ahmed, 2020) and (World Health Organization, 2012).

Participants in this study reported several reasons for non-compliance but the main reasons were “it is not necessary to continue taking prescribed medications once they feel better” (27.3%), a similar reason was recently reported in a study conducted by (Elzahaf et al., 2021), revealed significant low knowledge, bad attitude and incorrect practice regarding antibiotics use among the Libyan population. where, they also failed to take a full dose, and they directly stopped taking the course of antibiotics once they felt well. Furthermore, (Tong et al., 2018) found that (41.90%) of the participants reported that they didn't need to continue the antimicrobial therapy once symptoms improved.

Polypharmacy was the second most common cause which is “Taking many types of drugs, forgetting the correct order and dosage” (19.6%), and this complexity of the treatment was also reported as a major cause in Chinese community, where 40.5% did not comply to antimicrobial therapy because they took too many drugs varieties (Tong et al., 2018). Polypharmacy was also the main therapy-related factors that had the potential to decrease the adherence to short-term antibiotics with (61.7%) in a recent Jordanian study (Almomani et al., 2022). Nevertheless, polypharmacy could play a negative role in the adherence to the prescribed medications, where the non-polypharmacy patients were significantly more adherent to prescribed

medications than the polypharmacy group (Mohammed et al., 2016).

Fear of adverse effects caused by antibiotic treatment was also noticed as a major cause for non-compliance (18.7%), this fear has been reported in the previous studies with almost 30% (Ahmed, 2020). Additionally, fear of adverse effects is reported as a patient-related factor of non-adherence to short-term antibiotics in adult participants (Almomani et al., 2022). Furthermore, fear of adverse effect again was among the main reasons attributed to poor compliance to antimicrobial therapy in a Chinese study (Tong et al., 2018). Being the fourth highest cause for non-adherence to short-term antibiotics with 15%, the duration can increase the risk of non-adherence, and these findings are consistent with other studies in this regard (Kardas, 2002) and (Fernandes et al., 2014).

The results also showed that the difficulty of purchasing antibiotics due to high prices increased the risk of non-adherence to antibiotics (11.6%), which is consistent with the results of other previous studies (Pechère et al., 2007) and (Kardas, 2002). Practitioners could enhance adherence by emphasizing the value of a patient's regimen and by prescribing and dispensing less expensive medications. Less than one tenth of non-adherents were observed when patients reported difficulty with taking the antibiotic as well as preferring to take traditional or herbal medicine instead. Difficulty with taking the antibiotics were also described in previous researches (Kardas, 2002). This stresses the need to improve antibiotic formulation in order to improve adherence.

Less confidence in junior doctors (6.7%) has been observed to increase the risk of non-adherence to antibiotics, and this finding can be justified by the study conducted on a sample of Junior doctors, employed at primary health centers in the city of Tripoli, Libya, showed that their ready knowledge about proper prescribing of medicines is insufficient and development in prescribing skills is required (Ahmed Atia, Zanned, et al., 2020).

Participants receiving once-daily regimens (42.0%) were more compliant than those receiving twice (25.6%), three times (31.4%) or four times daily regimens (1.1%), which is similar to previous results reported by (Falagas et al., 2015). Similarly, this observation is in accordance with the finding by (Llor et al., 2013) where adherence was inversely related with the daily number and the duration of antibiotics.

Medicines in Libya can be easily obtained without a prescription. This may lead to misuse, unnecessary risk for patients and could promote the development and spread of antibiotic resistance, (Ahmed Atia, 2020a) reported, in 2020, that the self-medication use of medicines among the Libyan population is widespread and involves antibiotics in high proportion in 7 Libyan cities .

A systematic review showed that, it is a common behavior among the general population to request antibiotic prescriptions from physicians (Duan et al., 2021). (Ahmed Atia, 2018), reported that 56.19% of young Libyans bought their antibiotics without a prescription, among them, 98.1% did not use antibiotics for a full treatment period (< 3 days) or at inappropriate dosing intervals. Another national study performed in Al-Bayda, Libya in 2021 reported that 77.3% of participants disposed of their unused medications in the rubbish, which had almost 40% of the uncompleted antibiotic therapy, the misuse of antibiotics by humans and animals is accelerating the process of resistance to antibiotics (Shailabi & Akrim, 2021)

STUDY LIMITATIONS

The study sample may not represent the entire population, as it mainly includes females and participants with a higher educational level. It

is recommended that further studies be conducted to explore other reasons of non-adherence, and that a national study be conducted on awareness of the proper use of antibiotics and the relationship between non-adherence and antimicrobial resistance to antibiotics.

CONCLUSION

It can be concluded from the results that the level of non-adherence to antibiotic treatment was significant in this study. Physicians, pharmacists and nurses should be deeply involved in training all patients to adhere to the treatment regimen**.** The importance of this study was to provide real data on patients' adherence to prescribed antibiotic treatment, this is to be used as a tool for evaluating public health strategies aimed at reducing antibiotic resistance and improving antibiotic prescribing as well as its effects on healthcare resource use and costs.

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

Author contributions: Taher, Elham, Nisreen and Nagat designed the study. Taher, Elham, and Nisreen collected the data. all authors contributed to interpretation the data. Taher, and Elham drafted the manuscript and revised context and all authors contributed to the final version of the manuscript. Taher supervised the project.

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