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Assessment of Oral Hygiene Knowledge, Attitude and Practice Among Children in Makkah City, Saudi Arabia: a Cross-Sectional Study

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ABSTRACT

Background: *Assessment of knowledge, attitude and practice of oral hygiene in children is important for the health of the community. The lack of data in Makkah region initiated the idea of the study.*
Aims: *To assesses the knowledge, attitude, and practice of oral hygiene among Makkah children aged 9-14years.*
Design: *Cross sectional study was conducted to collect data from children in Makkah, using self-administered close-ended validated questionnaire. It contained demographic data about the child and his mother, and included ten items about oral health practice, oral health attitude, and oral health knowledge that were answered by the child and his guardian separately. SPSS version 20 was used for the analysis of the data and descriptive statistics and chi- square tests were applied.*
Results: *405 children, both males and females, and their mothers have participated, 79% of them were Saudi mothers and 57% have had higher education. A significant difference was found regarding the correct tooth brushing frequency among male and female children. 67% had more than two episodes of dental pain during last year. No significant difference was found regarding the knowledge except the effect of the fluoride on teeth. More than 45% of the children who got good score their mothers are University graduates.*
Conclusion: *Most of the children and their mothers have adequate oral hygiene knowledge, practice, and attitude. Children of university graduate mothers are more knowledgeable compared to non-graduate mothers; this necessitates the importance of educating them.*

1. Introduction

Oral health is considered an essential part of the general health and well-being. Dental caries is one of the most common oral diseases affecting the children in the world which is also preventable1. Attitude, knowledge and practice have shown to be factors that improve the dental health standard [2].

Many studies have been conducted to assess the knowledge attitude and practice of oral hygiene among school children and/or their parents in different regions of Saudi Arabia and other countries. More than 40% of children have good knowledge and practice about oral hygiene, but they lack the awareness about the other major factors that can cause dental problems and how it can be prevented2,3. The majority of children knowledge come from their parents4. Evidence showed that parents' knowledge about the oral health care of their infants and children was insufficient5. Unlike the primary school teachers who have acceptable knowledge and attitudes regarding their oral health [6].

The poor oral health knowledge was contributing to the high caries prevalence among the children [7,8].

A study carried out in 2011 about children oral hygiene knowledge in Qatar found that their knowledge was below the satisfactory level. Majority of their children lack the knowledge of the basics of oral hygiene practice. Unaware of the brushing techniques, fluoride effect, dental flossing or the regular dental visits [4].

Most of the previous literature indicated the lack of oral hygiene knowledge and practice among children and was contributing to bad oral health. [2,3,4,5,7,9,10,11,12,13].

This study aimed to assess the knowledge, attitude, and practice of oral hygiene among Makkah children.

2. Materials and Methods

2.1. Study Design and Sample Size

This was a cross-sectional study conducted in Makkah, with a Sample size of 405 participants selected with convince sampling, their age range from 9 – 14 years. The sample size was determined by Sample size calculator's website http://www.sample-size.net/ (Sample size for a descriptive study of a dichotomous variable) according to Confidence level: 95%, Expected proportion (P): 0.5, and a total width of confidence interval (W): 0.1. The samples were collected from the Parks (Alhussainiya, Almu'allim, Umm alkatad park) and the Malls (Alhijaz, Aldiyafa).

2.2. Questionnaire

A validated self-administered close-ended questionnaire9 was used at the study. The questionnaire was translated and its question was modified to accommodate with the aim of the study. The questionnaire was consisted of information related to the children personal information including: Gender and age of the child and his companion (mother or father); Mother's level of education; Nationality of the child and his mother and School type. The questionnaire included 10 items divided into 3 sections; oral health knowledge, oral health attitude, and oral health practice that was answered by both the child

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and his guardian separately. Assessment of participant's oral health-related attitude included items on self-perceived oral health status and the number of visits to the dentist for treatment of dental problems in the last year. Assessment of a participant's oral health-related practices included questions on the frequency of cleaning and cleaning aids used. Assessment of a participant's oral health-related knowledge included questions on benefits of fluoride, the necessity of regular dental visits, the role of sugar in causing dental caries, and the importance of teeth in the body.

Validity of the questionnaire:

The validation was done by distributing the questionnaire after translation and modification among 10 children to test its reliability and clarity of the questions. It was considered approved as 90% of the children reported that the questionnaire was clear and understandable.

2.3. Data Management and Statistical Analysis

SPSS software, version 20 for Mac used for all statistical analyses. Data were presented as frequencies and percentages. The comparison between frequencies was done using Chi-square test. P value less than 0.05 is considered significant.

Ethical Approval

Ethical approval was obtained from Umm Al-Qura University, Faculty of Dentistry Institutional Review Board (IRB). Patient acceptance to fill the questionnaire after a brief explanation about the study was considered as an informed consent.

3. Results

The results of the present study which was conducted to investigate the prevalence of oral hygiene knowledge, attitude and practice among children and their parents was performed through questionnaire with active voice to be clearer. It was distributed in Makkah city to children aged 9 to14 years. A total of 405 children completed all the questionnaires.

Table 1: Demographic characteristics

Demographic characteristics	Percentage (%)
Gender	
Male	47.7%
Female	52.3%
Age group	
9-11 Years old	62%
12-14 Years old	38%
School sector	
Governmental	80.5%
Private	19.5%
Mothers level of education	
University	57.3%
High	28.1%
Intermediate	6.4%
Elementary	6.7%
Non educated	1.5%

Table 2: Prevalence of oral hygiene knowledge among children.

questions	Frequency (%)	Gender		P value
		Male (%)	Female (%)	
Do the teeth plays an important role in the body?				
Yes	381 (94.1%)	180(44.4%)	201(49.6%)	0.716*
No	10 (2.5%)	6(1.5%)	4(1%)	
Don't know	14 (3.5%)	7(1.7%)	7(1.7%)	
Do teeth brushing everyday protects your teeth from caries?				
Yes	390 (96.3%)	186(45.9%)	204(50.4%)	0.194*
No	12 (3%)	7(1.7%)	5(1.2%)	
Don't know	3 (0.7%)	0(0%)	3(0.7%)	
Does regular dental visits helps keeping your teeth in a sound state?				
Yes	347 (92.3%)	173(42.7%)	201(49.6%)	0.065*
No	19 (4.7%)	14(3.5%)	5(1.2%)	
Don't know	12 (3%)	6(1.5%)	6(1.5%)	
Eating sweets, soft drinks or sugary drinks doesn't cause caries?				
Yes	65 (16%)	30(7.4%)	35(8.6%)	0.629*
No	334 (82.5%)	159(39.3%)	175(43.2%)	
Don't know	6 (1.5%)	4(1%)	2(0.5%)	
Using fluoride toothpaste helps protecting teeth from caries?				
Yes	125 (30.9%)	45 (11.1%)	80 (19.8%)	0.004**
No	20 (5%)	8 (2%)	12 (3%)	
Don't know	260 (64.1%)	140(34.5%)	120(29.6%)	

** Significant. * Non-significant.

Table 1, showing the demographic characteristics of the samples. The prevalence of oral hygiene knowledge, oral hygiene attitude and oral hygiene practice among the children were shown respectively in tables 2, 3 and 4. While, figure 1, showing the arrangement of the children knowledge levels (Good, Average and Poor) which were determined by their answers on the questionnaire and related these levels with their mothers' level of education.

Table 3: Prevalence of children's oral hygiene attitude

Questions	Frequency (%)	Gender		P value
		Male (%)	Female (%)	
Have you felt pain in your teeth in the last 12 months?				
Many times	89 (22%)	43(10.6%)	46(11.4%)	0.714*
Sometimes	139 (34.3%)	66(16.3%)	73(18%)	
Never	132 (32.6%)	66(16.3%)	66(16.3%)	
Don't remember	45 (11.1%)	18(4.4%)	27(6.7%)	
How many times have you visited a dentist in the last 12 months?				
Once	78 (19.3%)	33(8.2%)	45(11.1%)	0.575*
Twice	42 (10.4%)	22(5.4%)	20(4.9%)	
More than two	108 (26.7%)	49(12.1%)	59(14.6%)	
Never	177 (43,7%)	89(22%)	88(21.7%)	
If the answer was never then, what are the causes?				
No need	202 (49.9%)	91(22.5%)	111(27.4%)	0.416*
Dental phobia	54 (13.3%)	23(5.7%)	31(7.7%)	
No nearby clinic	27 (6.7%)	13(3.2%)	14(3.4%)	
Costly treatment	16 (4%)	7(1.7%)	9(2.2%)	
No pain	106 (26.2%)	59(14.6%)	47(11.6%)	

** Significant. * Non-significant

Table 4: Prevalence of children's oral hygiene practice.

questions	Frequency (%)	Gender		P value
		Male (%)	Female (%)	
How many times do you brush your teeth				
Once a week	36 (8.9%)	28(6.9%)	8(1.9%)	0.000**
Plenty per week	43 (10.6%)	23(5.7%)	20(4.9%)	
Once a day	100 (24.7%)	46(11.3%)	54(13.3%)	
Plenty per day	196 (48.4%)	77(19%)	119(29.4%)	
Never	30 (.4%)	19(4.7%)	11(2.7%)	
What is the method used to brush				
Using only fingers	1 (0.2%)	1(0.2%)	0(0%)	0.435*
Miswak	3 (0.7%)	2(.5%)	1(0.2%)	
Coal powder	2 (0.5%)	1(0.2%)	1(0.2%)	
Toothbrush and paste	373 (92.1%)	173(42.7%)	200(49.4%)	
Another way	26 (6.4%)	16(4%)	10(2.5%)	

** Significant. * Non-significant.

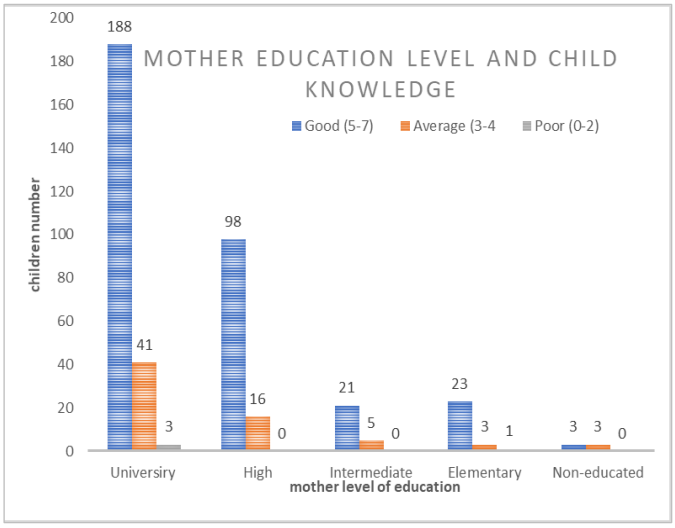

Figure 1: showing the arrangement of the children knowledge levels (Good, Average and Poor) which were determined by their answers on the questionnaire and related these levels with their mothers' level of education.

4. DISCUSSION

The present study showing the prevalence of oral hygiene knowledge, attitude, and practice among the children, using a questionnaire in Makkah city.

Regarding the oral hygiene knowledge, the collected data about the oral hygiene knowledge of Makkah children included five questions in the questionnaire (Table 2). Question about the essential role of teeth in the body, 94.1% were answered yes, that agreed with the studies conducted (Al-Darwish et al 2016) in Qatar and in India 4,9. The daily brushing protects the teeth from caries or not; 96.3% answered yes which agreed with Al-Darwish et al 2016 and Mehta et al 2012 in which showed agreement by (83%). On the other hand the results disagreed with Aljouf study3,4,9. The third question was about the regular dental visits and whether they preserve the teeth or not, which was mostly answered by yes (92.3%) and agreed with the study of Alshehri et al 2015, Togoo et al 2012 which conducted in the rural areas of southern Saudi Arabia. Other study conducted by Mehta et al in Indian showed lowest prevalence (69% girls- 74% boys) [2,5,9].

The fourth question was about whether eating sweets, soft drinks and sugary food wouldn't cause caries, mostly answered by no it causes caries (82.5%), which agrees with Zwiri et al. ,Togoo et al While disagrees with the Mehta et al, conducted in India [2,3,9]. The fifth question was asking about the fluoride and whether it has a protective effect on teeth, it was concluded that fluoride was not well known to children in Makkah. This result was agreed with Al-Darwish et al and Mehta et al [4,9].

Regarding oral hygiene attitude, a total of three questions were asked to investigate the prevalence of the attitude between children (Table 3). The first one was asking about the times they had felt pain in the last 12 months, most of them answered by sometimes (34.3%) and the rest of the answers were distributed between never, many times and didn't remember which agrees with the Indian study of Mehta et al 9. The second question was about the number of visits to the dentist in the last 12 months which were answered mostly by never (43.7%) followed by more than twice (26.7%) and twice (10.4%), and this finding disagrees with the Indian study,9. The third one was about the causes of never visiting a dentist was answered by no need (49%) followed by no pain (26%), and few were complaining about the higher cost of the dental clinics. These findings disagreed with studies conducted by Togoo et al, Mehta et al and Al-Kheraif et al. [2,9,10].

Regarding oral hygiene practice (Table 4), the question about how many times that children brush their teeth showed that a great majority of children are brushing their teeth plenty a day 196 (48.4%) followed by once a day 100 (24.7%), which agreed with Al-Kheraif et al and Zhu, While disagreed with study conducted by Alshehri et al , Zwiri et al., Al-Samadani et al., Togoo et al., and Mehta et al. [2,3,5,7,9,10,11]. Regarding the method of brushing, it revealed that most of the children were using toothbrush and paste while the other methods were not popular between them. Results founded to be agreed with the study conducted in the rural areas of southern Saudi Arabia, Aljouf, Abha, Madinah, and India [2,3,5,7,9].

The Comparison between Mothers' education level and children knowledge (Figure 1), revealed that there is a proportional relationship between the mothers' educational level and the children knowledge which explained as whenever the mother's educational level increases the better knowledge their children get.

Traditionally, good oral hygiene knowledge and practice are leading to good oral health. According to the results of our study we found that a high proportion of children in Makkah have good knowledge of oral hygiene. But they probably might have bad oral health as indicated by previous studies of areas near Makkah. Al-Malik et al. in Jeddah showed that 73% of the children had caries, and another one published by Alhabdan et al. in Riyadh showed that 83% of children had caries12,13. These variations could be due to individual factors including inappropriate brushing technique, getting used to brushing in lately teenage, visiting the dentist only for treatment not prevention, having snacks between meals, fruits not included into their snacks or meals, and high consumption of soft drinks and flavored milk.

5. CONCLUSION

The majority of the children achieved good score in the knowledge of oral hygiene, especially those whom their mothers' education level is university; this necessitate the importance of educating the mothers. Fortunately, most children exhibit a good level of awareness concerning regular teeth brushing, use of fluoride, and dental floss. The sociodemographic factors can influence oral health and hygiene, particularly knowledge, practice, and behavior.

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Double Hematological and Solid Malignancy Diagnosed from Bone Marrow Studies: Case Report, Laboratory View

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ABSTRACT

A 63-year-old male patient was evaluated for cytopenia at King Abdullah Medical City Makkah Al-Mukaramah. Complete blood count and bone marrow studies including flowcytometry revealed acute myeloid leukaemia (AML) with dysplastic changes and according to the WHO criteria published in 2018, it was categorised as AML-M2 not otherwise specified. The patient received azacytidine as a sole line of therapy because of his age and general condition. Ten months later, serum ferritin and prolactin level were elevated and the cytopenia became more severe, prompting new bone marrow studies for proper evaluation. Bone marrow metastasis from the existing non-hematopoietic malignant tumour were detected. A diagnosis of metastasis from adenocarcinoma of the lung was made by immunohistochemistry which showed positivity for cytokeratin 7(CK7), pancytokeratin (CKAE1AE3) and thyroid transcription factor (TTF). Additionally, CEA and CA 19-9 were elevated.

To the best of our knowledge, this is the first case of a dual diagnosis of an haematological and a solid tumour in a patient, originating from the Makkah area, Saudi Arabia to be published. Keeping in mind that the patient was treated with azacytidine therapy, which was not licensed for use until now, for the development of secondary malignancies. Further studies for evaluation of its effect are worthy.

1. Introduction

Double primary malignancies could be divided into synchronous and metachronous, depending on the interval between tumor diagnoses [1]. Synchronous malignancy denotes development of the second tumor either simultaneously [2], or within 6 months after the first malignancy [3]. Metachronous malignancies were secondary tumors that have developed after 6 months, or even more than that from the first malignancy.

Metachronous cancers are not rare and it is expected as a side effect of the primary cancer treatment modalities; chemotherapy or radiotherapy [3]. On the other hand, it is rare to find a synchronous dual malignancy at first presentation and it is even rare to detect synchronous solid tumor with a hematological malignancy [3].

Generally, the pathophysiology of malignancies remains poorly understood where many factors seems to play an imminent role such as immunosuppression, genetic predisposition, environmental exposure to carcinogenic factors like tobacco smoking and alcohol consumption [4], infections, and toxic effects related to treatment by chemotherapy or radiotherapy [1]. Where a metachronous malignancy that develops after receiving chemotherapy for another first malignancy could be considered as an iatrogenic, a synchronous dual malignancy is usually related to genetic instability such as DNA microsatellite instability, mutation in multiple tumor suppressor genes such as p16, p53, PTEN and Rb gene [5] or immunosuppression [6]. With modernization of life, the incidence of malignancies has increased globally. With regards to double malignancy, the improved diagnostic modalities and the scientific level of the clinicians contributes greatly to the increased incidence of the diagnosis of such cases not increasing the incidence of the cases their selves.

2. Aim of the report

To alert the medical community about the careful evaluation of patients with known acute myeloid leukemia with special appreciation to the role of the laboratory investigations.

3. Case report

63 years old male patient was presented to King Abdullah Medical City Makkah Al-Mukaramah, KSA. On July 2015. A bone marrow request was sent for proper evaluation of the patient where his complete blood counts revealed both leukopenia and thrombocytopenia.

4. Laboratory tests on admission

4.1. Serum biochemistry

The liver function tests were normal; GGT 87 U/L (Reference range (RR) 15-85), AST 21 U/K (RR 15-37), ALT 43 U/L (RR 16-61), ALP 81 U/L (RR 45-117), Total Bilirubin 0.3 mg/dL (RR 0.2-1), Direct Bilirubin 0.09 mg/dL (RR 0-0.2), A/G ratio 1.6 (RR 1.1-2.2), Albumin 4.6 g/dL (RR 3.4-5).

The kidney function tests were normal; blood urea nitrogen (BUN), 14mg/dL (RR 7-18), Creatinine 0.8 mg/dL (RR 0.4-1.3), Uric acid 5.8 mg/dL (RR 3.5-7.2), Protein 8.1 g/dL (RR 6.4-8.2). Serum electrolyte levels were within normal range, Phosphorus 3.3 mg/dL (RR 2.5-4.9), Mg 1.9 mg/dL (RR 1.6-2.6), K 4.3 mmol/L (RR 3.5-5.1), Na 136 mmol/L (RR 136-145), Corrected calcium 9.5 mg/dL (RR 8.5-10.1),

Viral markers: the patient had negative markers for HCV, HBV, HIV, and H1N1.

4.2. Complete blood count

Peripheral blood cells were examined by an automated hematologic analyzer (Sysmex XN1000). Peripheral blood smear examination showed normochromic anemia and mild rouleaux formation, the red

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cell indices were Hgb 8.8 g/dL MCV 92 fL, MCH 32 pg and MCHC 34. White blood cells showed leukopenia (1.7 x10^9/L). The leukocyte differential count revealed sever neutropenia; neutrophils 22.8%, lymphocytes 69.1%, and monocytes 6.7%, eosinophil 0.7%, basophile 0.7%. Marked thrombocytopenia was confirmed with no clumps or large platelet forms; Platelets count 51 x10^9/L. No blasts were seen.

4.3. Coagulation studies

Prothrombin time (PT) and partial thromboplastin time (PTT) and fibrinogen were within normal range; PT12.2 sec. (RR 11-16), INR 0.9 (RR 0.8-1.2) , PTT 30.5 Sec. (RR 28-44), and fibrinogen 3.2 g/L(RR1.7- 4.2).

4.4. Bone marrow (BM) studies

BM aspiration

Bone marrow smears were stained with Wright-Giemsa and analyzed according to routine clinical laboratory procedures. The BM sample was particulate with cellular particles and trails. Megakaryocytes were adequate in number with few hypolobated forms denoting dysmegakaryopoietic changes. Granulopoiesis was adequate but revealed mainly early granulocytic precursors and some dysplastic changes (degranulation). Active erythropoiesis was noticed with dyserythropoietic changes in the form of binucleated and megablastoid changes. Plasma cells were mildly prominent (2-4%). Some histiocytes were seen but with no evidence of phagocytic activity. The blasts were increased; around 24-30%, and they were variable in size, with large nucleus and multiple nucleoli. The blasts had abundant basophilic granulated cytoplasm. Apart from morphology, more than 5% of the blasts were positive for myeloperoxidase by immunohistochemistry denoting their myeloid nature (Figure 1).

4.5. Bone marrow biopsy:

The BM biopsy revealed 50% cellularity and interstitial involvement by immature looking mononuclear cells; blasts in the background of trilineage hematopoiesis. Some dysplastic megakaryocytes were seen (Figure 1).

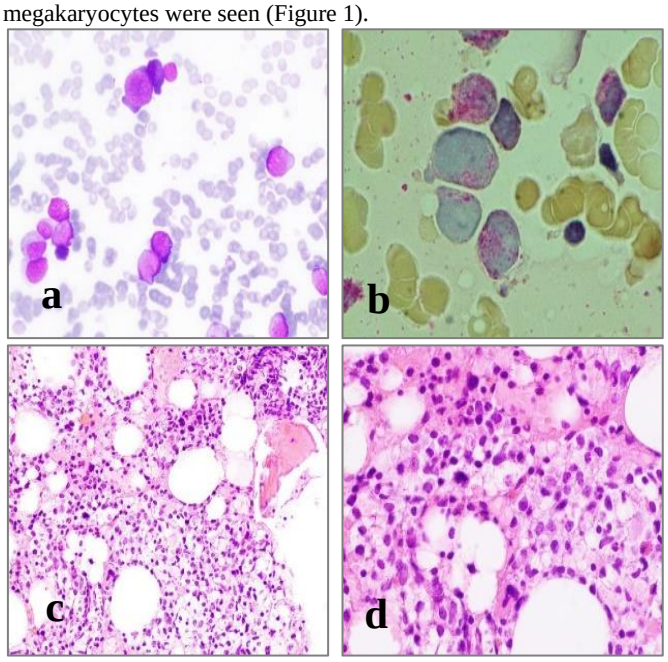

Figure 1: Represents bone marrow (BM) studies at presentation. (a) myeloblasts present in the BM aspiration stained by Wright-Giemsa stain (X 40). (b) Positive myeloperoxidase staining of the blasts. (c & d) BM biopsy reveals normocellular BM with diffuse infiltration by immature cells

4.6. Cytogenetic results

Revealed normal chromosomal count (46, XY [20]) without apparent clonal abnormality. Also, Fluorescence in situ hyperdization (FISH) did not reveal any clonal abnormalities.

4.7. Molecular studies

Revealed negative FLT3, NPM, BCR-ABL, and PML-RARA**.**

4.8. Flowcytometry analysis

Using BD-FACS-Canto II System (BD - Bio Science) and comprehensive panel of monoclonal antibodies labelled with fluorescein stain. The dim CD45 positive cells in blast gated area ranged from 20 to 25%. The blasts expressed the myeloid markers: cytoplasmic MPO, CD13, CD33, CD15, and the stem cell markers CD34 and CD117 and the non-lineage specific marker HLADR in addition to CD9, CD38, and CD58. The blasts were negative to CD11c, CD14, CD16, CD36, CD64, CD66, CD41a, CD2, CD3 surface or cytoplasmic, CD5, CD7, CD8, CD4, CD56, CD10, CD19, CD20, CD22, cytoplasmic CD 79a, TdT, CD25, or CD110. Figure 2 represents some of the positive marker expression.

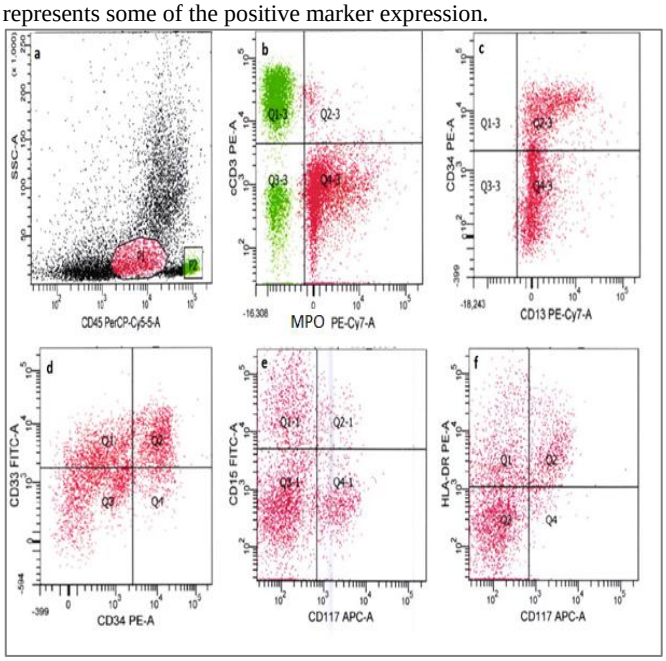

Figure 2: flowcytometry analysis of the BM at presentation. (a) Dim CD45 population; P1 gate represents the leukemic cells. (b) Myeloperoxidase (MPO) expression by the leukemic cells. (c) represents CD13 positive leukemic cells with partial co-expression of CD34. (d) represents CD33 positive leukemic cells with partial co-expression of CD34 (e) CD15 and CD117 expression by the malignant population. (f) Partial expression of HLADR and CD117 by the leukemic population.

The performed bone marrow aspiration / biopsy and immunophenotyping support the diagnosis of acute myeloid leukemia with dysplastic changes and according to the WHO criteria 2018 it is AML-M2 not otherwise specified. Note no WHO reference in list

Being an older patient with generally poor health condition, the only treatment option for this patient was Azacytidine, to reduce the blast count. He did not receive the AML chemotherapy protocol.

The patient was followed up and his investigations were within accepted range for his condition. On May 2016, the serum ferritin level was very high; 2382ng/ml (RR 8-388 ng/ml) and the prolactin level was elevated; 22(0-0.1ng/ml). On the next follow up samples, the cytopenia was very pronounced, necessitating another BM study for better evaluation of the patient condition. The positive laboratory investigations were very high serum Ferritin level; 8109.8 ng/mL, elevated CEA; 48.59 ng/dL (RR 0-5) and CA19-9; 270.08 U/mL (R.R 0-30.9).

In contrast to the PB examination at presentation, the myeloblasts were seen in the PB; 14% and in the BM as well (Figure 3 (b)).

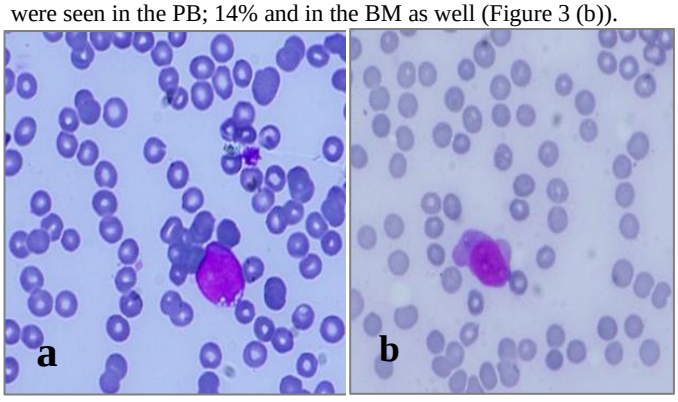

Figure 3: (a) represents the presence of myeloblasts in the peripheral blood. (b) represents myeloblasts in the BM despite being diluted sample.

The examination of the touch print revealed the presence of sheets of malignant non- hematopoietic cells that were obvious also in the BM biopsy with the presence of rosette shaped malignant cells (Figure 4).

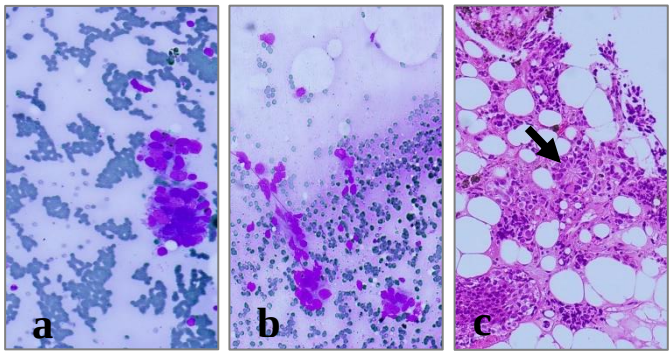

Figure 4: (a) Represents sheets of malignant non-hematopoietic cells in the BM. (b) sheets of malignant non-hematopoietic cells in the touch imprint. (c) the BM biopsy represents malignant non- hematopoietic cells forming rosette (black arrow).

Immunohistochemistry studies on BM biopsy reveals negative reaction for CD45, CD34, prostatic specific antigen (PSA) and anti-IL-7 receptors (CDX2). On the other hand, the malignant non hematopoietic sheets showed positive reaction for cytokeratin 7(CK7), pancytokeratin (CKAE1AE3) and Thyroid transcription factor (TTF) (Figure 5). The expression of TTF is linked to either adenocarcinoma of the lung or the thyroid gland, however the adenocarcinoma of the thyroid gland rarely metastasizes in the BM. Additionally the co-expression of TTF together with CK7 supports the diagnosis of BM secondaries due to lung adenocarcinoma [7].

The patient received palliative therapy and died two months later.

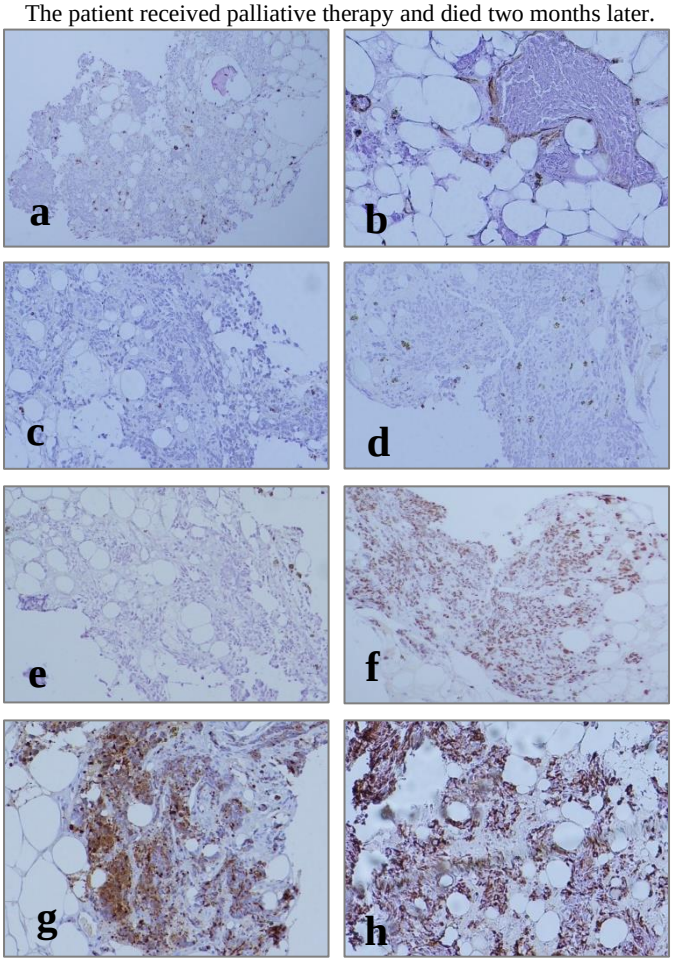

Figure 5: Immunohistochemical staining of the BM biopsy (a) CD45 staining negative. (b) CD34 negative. (c) CD20 negative. (d) anti-interleukin 17 (CDX2) negative. (e) Prostate specific antigen (PSA) negative. (f) Thyroid transfection factor (TTF) positive. (g) cytokeratin 7 positive. (h) Pancytokeratin (CKAE1AE3) positive.

5. Discussion

The criteria used for the diagnosis of multiple primary cancers were first conceived by Warren and Gates in 1932 [8] which stated that:

1. Each of the tumors must be a malignancy confirmed by histology
2. Each must be geographically separate and distinct.
3. Probability of one being the metastasis of the other must be excluded.

These criteria are fulfilled in this case where the AML is confirmed by morphology, IHC and flowcytometry analysis and the non-hematological malignancy is confirmed by being negative for CD45 and positive for TTF, Pan cytokeratin and cytokeratin [7].

According to the duration between the diagnosis of first and second malignancy, this is a case of metachronous second malignancy. It could be explained by the presence of a predisposing factor for the development of the first malignancy that makes the patient a higher risk candidate for a second de novo malignancy [5]. It is well known that metachronous malignancy occurs as a side effect of exposure to chemotherapy. In this report, the patient received azacytidine which is a cytosine analogue that functions as a DNA methyltransferase inhibitor, and is known to show effective potency in reactivating epigenetically silenced tumor suppressor genes, in vitro [9]. However, it has been difficult to define the mode of action in vivo, and it appears that clinical responses are affected by epigenetic alterations and induction of apoptosis [10]. In addition, the drug can induce epigenetic side effects, like the activation of silenced retroelements [11] that needs further studies of their effects and whether they do or do not induce second malignancy.

The incidence of multiple primary malignancy is variable among different studies. In a recently published four year study, in a geographically close Regional Cancer Institute, a metachronous malignancy incidence rate of 0.18% was recorded; a rate which is around four-fold greater than that for synchronous malignancy [12]. To the best of our knowledge, no reports from Saudi Arabia regarding the incidence of double malignancy exist and this is the first reported case to be published.

The incidence of development of solid tumor with a hematological malignancy is very rare. The implicated solid tumors reported are cancer in the esophagus, stomach, colon, and breast; with the major hematological malignancies reported being MM, MDS, NHL and CML [13]. Herein we report a case of initial AML followed by adenocarcinoma of the lung. According to a retrospective study published in 2014, lung cancer was the most common solid malignancy developed in patients with hematological malignancies [14].

This case was diagnosed in an elderly male with an unrelated positive family history. Being a patient above the age of 60 years old has been confirmed in other publications as increasing risk of malignancy, with most cases of double primary malignancy belonging to adults in the 5th to 6th age decades of life and beyond [4 &15]. Additionally, a Japanese study reported that elderly patients with hematologic malignancies are more likely to have multiple malignant neoplasms than younger patients [16]. Also, a predominance of malignancy was reported for males in the reviewed literature [6 &17].

In the current report, ten months after the patients' initial diagnosis, serum ferritin were noted to be elevated and continued to show a higher value till the diagnosis of the second malignancy. It is widely accepted that ferritin is an acute-phase reactant and can be nonspecifically elevated in a wide variety of inflammatory states including infection, malignancy, and autoimmune diseases [18]. These data should alert the attention of the clinician to consider the presence of a further clinical issue. Furthermore, the prolactin level was elevated which is highly suspicious of ectopic hormone production and should be considered as an early negative poor predictive factor for clinical outcome [19].

The elevated levels of the tumor markers; CA19-9 and CEA were highly suspicious for a second solid malignancy irrespective of its type. The CA 19-9 antigen is a glycoprotein synthesized in several epithelia that is typically high in the serum of patients with pancreatic tumors. Although uncommon, it is a fact that serum levels of CA 19-9 are increased in lung tumors, especially in adenocarcinomas [20 and 21]. The dual elevation of both CEA and CA19-9 has been reported in malignant lung lesions and provides a useful tool for diagnosis of lung cancer, especially if the tumor cannot be visualized by bronchofibroscopy [22]

In this case the second tumor presented in the form of metastasis in the BM denoting the advanced stage of the disease and the patient died two months after the diagnosis of the 2nd malignancy. The prognosis of double malignancy can be speculated based on the stage of each cancer, with previous reports indicating that prognosis would be good if the tumors were at early stage, otherwise the prognosis was likely poor [11].

6. Conclusion

With recent advances in diagnostic and staging modalities, as well as progress in the management of common cancer, the possibility of the existence of a second primary malignancies should always be considered. This is especially the case, whenever a novel laboratory finding emerges, that could be an early indicator in the detection of associated tumors, preferably before clinical manifestations occurrence. Generally, thorough regular follow up with proper result interpretation could detect most of the metachronous second primary malignancies at an early stage.

- **Acknowledgement**

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Hepatitis B: Knowledge, Preventive Attitude and Vaccine Status of Dental Students and Interns at Umm Al-Qura University, Makkah, Saudi Arabia

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ABSTRACT

Background: Cross infection of blood-borne pathogens during dental practice is a major infection control problem among dental health care workers. Of these infections, Hepatitis B causes significant morbidity and mortality, and imposes a great burden on the Saudi Arabia healthcare system. Therefore, awareness of Hepatitis B virus (HBV) infection, routes of transmission, preventive practices and vaccination are very important for the protection of dental students.

Aim: Determination of the level of knowledge, attitude and practice of preventive measures regarding the HBV as well as vaccine status among Umm Al-Qura University (UQU) dental students and interns.

Method: A descriptive cross sectional study was conducted on 182 undergraduate students in fourth, fifth and sixth grades as well as on interns at Umm Al-Qura College of Dentistry. A self-structured questionnaire of 31 questions was distributed among participants to determine knowledge, attitude, preventive measures about hepatitis B during dental practice as well as the vaccine status. Data were collected, tabulated and statistically analyzed by SPSS v.20.

Results: 182 questionnaires were answered, total males were 88 (48.4%) and females were 94 (51.6%). Among all students in 4th, 5th, 6th grades and interns, the overall knowledge was high (>70%). Also the scores for total attitude and knowledge about HBV infection were significantly higher in females than males ($p<0.001$). All participants were vaccinated against HBV and received 1-3 doses. However, 10.9% of them did not complete their doses (they received only 2 doses).

Conclusions: UQU dental students and interns showed high knowledge and attitude about many aspects of HBV infection, but also there are some aspects in which they showed poor knowledge. So, sustained training and awareness about HBV infection and its preventive measures are recommended.

1. Introduction

Hepatitis is an inflammatory disease of the liver and a major health concern worldwide [1]. Among different types of hepatitis, Hepatitis B Virus (HBV) infection affects more than 350 million people worldwide of which approximately 75% are Asian [2].

Recent data reported that there are an estimated 257 million people living with HBV, resulting in 887,000 deaths due to its complications, which include cirrhosis and hepatocellular carcinoma [3]. The Center of Disease Control [4] estimated the prevalence of HBV by country and constructed a map of the data collected, showing that HBV prevalence in Saudi Arabia was low to intermediate (2-4%).

The HBV is a partially double-stranded DNA virus that belongs to the hepadnaviridae family [5]. Generally, it is transmitted by blood transfusion, unsafe use of therapeutic injections, tattooing, vertically from mother-to-child transmission and by sexual intercourse [6].

Occupational injuries which expose health care professionals to blood-borne pathogens are an important public health concern. Dental health-care workers (DHCWs) are at particularly high risk of exposure to cross infection with blood-borne pathogens including hepatitis B virus (HBV) [7,8].

Transmission of infections during dental procedures could occur through direct contact with blood, saliva or other secretions, injury with an anesthetic needle or splash exposure of the mucous membranes, droplets and aerosols as well as indirect contact with contaminated instruments, operatory equipment or environmental services [2,7,9,10].

As the dental clinic is an environment where cross infection occurs easily, preventing the spread of Hepatitis B infection is very important in dental practice and dental student should be aware of the risks involved in performing dental procedures, and must take appropriate steps while practising [11].

Accidental exposure to infections in dental settings can be avoided by using standard safety precautions including activities such as hand hygiene, proper disposal of sharps and wearing of personal protective equipment (gloves, gowns, goggles, cap wearing) [12]. However, because some exposures cannot be prevented, vaccination and proper post-exposure management are the main forms of protection [13]. Therefore, an awareness of HBV infection, routes of transmission, preventive practices are very important for protection of future dentists [14,15] .

In southeast Asian region, there are estimated to be 80 million HBV carriers [16], and because the majority of infected diseases

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carriers cannot be identified, implementation of standard precautions in dental schools is the most effective way to control cross infection [17,18].

Despite the reduced prevalence of Hepatitis B in Saudi Arabia by a planned vaccination program that was started in 1989 [19], the disease still causes significant morbidity and mortality, and imposes a significant burden on the country's healthcare system [20].

To the best of our knowledge, there have been few attempts to evaluate the knowledge, attitudes and preventive practice of HCWs in Saudi Arabia regarding occupational risks of HBV. Previously Al-Hazmi, 2015 performed two studies on primary healthcare physicians [21] and medical students at Al Jouf University College of Medicine [14] and concluded that there were inappropriate practices and lack of knowledge about infectious and occupational risk of HBV. With regard to dentists and dental students in Saudi Arabia, three studies were published; the first concerned 41 dentists at Al Jouf [22], the second and third were performed at Riyadh and Abha on 420 and 723 participants respectively [22,24]. These studies on dental practitioners revealed unsatisfactory findings in which overall knowledge was below average. Although questionnaires used in the last two studies involved the practices of students regarding protective measures against HBV as well as their HBV vaccination status, there was no data on post vaccination antibody response among vaccinated dental students and interns.

This study was conducted with the aim of determining the level of knowledge about HBV infection, attitude and day-to-day practices that help to prevent its spread. The study also aimed to assess the existing status and response to hepatitis B virus vaccination among Umm Al-Qura University dental students and interns.

2. Materials and methods

A descriptive cross-sectional study was conducted on undergraduate students in fourth, fifth and sixth grades as well as on interns at Umm Al-Qura College of Dentistry, Makkah. The duration of the study was 3 months from December 2017 to February 2018. Students at grades 1-3 were excluded as the dental curriculum at the college includes six academic years of undergraduate study followed by one year of internship. Clinical sessions and patient contact start from the fourth grade onwards.

Ethical approval was taken from the college IRB and no study activities were started until the IRB approval was obtained. The questionnaire used for data collection in this study was self-structured with an introductory paragraph about the aim of the study and an endorsement that it did not include any personal information and so answering its questions was considered as an acceptance to participate in the study.

The questionnaire included 32 questions divided into 5 groups; first group of questions covered demographic characteristics of participant (gender and grade), the second group consisted of 10 questions explaining knowledge about Hepatitis B infection. The third group contained 12 questions about believes, attitude and behavior during dental procedures and the fourth group contained 7 questions to evaluate students' knowledge about Hepatitis B prevention. The fifth group composed of 3 open ended questions to evaluate the vaccine status and Anti-HBs level of participant.

An electronic form of prepared questionnaire was created through Google form for online surveys. Study subjects of different selected grades and of both sexes were invited to participate in the study through social media networks after testing framed questions on 10 participants to confirm their clarity. Printed copies of the questionnaire were also distributed among study participants who did not respond to the electronic form.

The target sample size (n=196) was the total number of students in the three clinical grades together with the interns undergoing their training rotation at the college during the study period (n=16). The total number of participants who responded was 182 subjects with response rate of 84.5% (70 in 4th grade, 57 in 5th grade & 39 in 6th grade) and 16 interns. Female participants were 94 (51.6%) while male participants were 88 (48.4%).

After collecting data, an incorrect answer to the 29 close ended questions was given a zero score while the correct answer was given a score of 1. The total score obtained by each participant was converted to a percentage and was evaluated by considering a score less than 40% as low, 40-70% as average, and a score of 70-100% as high according to Sharif et al., 2016 [25]. Finally, the mean knowledge and practice scores (%) for all the participants were calculated.

Data were collected, tabulated and statistically analyzed using Statistical Package for Social Science (SPSS v.20). $P < 0.05$ was considered as significant; chi-square tests were used for the analysis of categorical data. T-tests were conducted to compare total knowledge between different grades.

3. Results

182 questionnaires responded with a return rate of 84.5%, total males were 88 (48.4%) and females were 94 (51.6%). Table 1 illustrates the respondents' characteristics. The majority of participants were in fourth year (n=70; 38%).

Table 1: Respondents' characteristics

Gender	Academic year				Total
	4th	5th	6th	Intern	
Female	33 35.1%	31 33%	20 21.3%	10 10.6%	94 100%
Male	37 42%	26 29.5%	19 21.6%	6 6.8%	88 100%

3.1. *Knowledge about Hepatitis B virus*

Among undergraduate students in 4th, 5th and 6th grades, there was good knowledge (70<%) regarding their highest risk of acquiring infection, possibility of infection transmission to and from the patients, the fact that patients may or may not have jaundice and a risk of transmission of infection by needle stick injury. On the other hand, an average level of knowledge was found about HBV ability to cause infection outside the body and its incubation period and infectivity in comparison with HIV.

The knowledge was different among undergraduate students in different grades regarding survival of HBV on unsterilized surfaces where the knowledge was average (40-70%) except for grade 4 where the knowledge was high; and knowledge that HBV infection is not always symptomatic was high in 6th grade students while it was average in 4th and 5th grades.

Among interns participating in the study, high knowledge was detected for all questions except for infectivity of HBV by comparison with HIV where the knowledge was low (< 40%) (Table 2).

Table 2: Students' knowledge about HBV distributed by their grade (correct answer rates)

Question item: Correct answer	% of Students with correct answers				
	4th grade	5th grade	6th grade	Interns	X2 (P value)
-Dentists are at higher risk of HBV infection than general population(Yes)	98.6	100	100	100	1.609 (0.657)
-HBV can be transmitted from dentist to patient (Yes)	74.3	73.7	74.4	100	5.921 (0.432)
-HBV can be transmitted from patient to dentist (Yes)	94.3	98	100	100	8.705 (0.191)
-HBV is not infectious outside the body (in the environment) (No)	42.9	40.4	53.8	75	10.466 (0.314)
-HBV can survive for prolonged time on unsterilized surfaces (Yes)	75.7	68.4	69.2	100	12.0 (0.213)
-The incubation period of HBV ranges from weeks to months (Yes)	62.9	52.6	61.5	75	7.381(0.598)
-Hepatitis B infection is always symptomatic (No)	57.1	63.2	74.4	87.5	1.763 (0.227)
-People with hepatitis B may or may not having jaundice. (Yes)	67.1	70.2	74.4	87.5	4.835 (0.848)
-Hepatitis B virus can be transmitted by needle stick during dental treatment (Yes)	91.4	98.2	94.9	100	10.551(0.308)
-HBV is less infectious when compared with HIV (No)	58.6	64.9	53.8	37.5	7.704 (0.564)

Table 3: Students' Believes, Attitude and behavior during patient treatment (correct answer rates)

Question item: Correct answer	% of Students with correct answers				
	4th grade	5th grade	6th grade	Interns	X2 (P value)
-Dentists have rights to know their patients' HBV infection status (Yes)	94.3	93	97.4	100	3.663 (0.722)
- You can safely treat patients with HBV infection (Yes)	77.1	82.5	87.2	100	7.820 (0.252)
-Shaking hands with hepatitis B patient makes you uncomfortable (No)	48.6	43.9	53.8	31.3	4.738 (0.578)
-You wear gloves before touching membranes and non -intact skin of patient (Yes)	92.9	94.7	94.9	100	3.98(0.679)
-You wash your hands before and after treatment procedure. (Yes)	97.1	93	87.2	93.8	4.715(0.581)
-You wear goggles during treatment procedures of patients. (Yes)	67.1	70.2	64.1	81.3	9.477(0.372)
-You wash your hands after contact with patient's body fluids(Yes)	97.1	96.5	100	100	3.764(0.709)
-You use gowns to protect yourself when treating patient (Yes)	97.1	96.5	100	93.8	4.489(0.611)
-You use mask to protect yourself when treating patient. (Yes)	97.1	98.2	100	100	5.419(0.491)
-You bend needles after injections of your patients (No)	54.3	59.6	71.8	68.8	8.121(0.229)
-You discard used needles into a medical waste container(No)	10	17.5	10.3	12.5	4.206(0.649)
-You check the indicator showing whether or not instruments have been sterilized before using them in a procedure(Yes)	84.3	75.4	61.5	93.8	11.889(0.064)

3.2. *Believes, attitude and behavior regarding HBV during dental procedure*

Table 3 shows the percentage of correct answers to sample questions of the questionnaire regarding attitude and behavior towards HBV and standard infection control precautions. High percentages of participants have the correct attitude, however, all of them reported unaccepted attitude regarding discarding used needles into a medical waste container (with low score< 40%), bending needles after injections (high score in grade 6 and average scores in other grades and interns), using goggles during patient treatment (average scores in 4th and 6th grades and high in the other participants) and being uncomfortable when shaking hand with HBV patient (low score in interns and average scores in all undergraduate grades). Significant differences were observed between percentages of students checking the indicator for efficient sterilization with the average score among 6th grade students and high scores among the other groups.

3.3. *Prevention*

With regard to knowledge about HBV prevention, all participants had high knowledge (<70%) except for 6th year students who had average knowledge (40-70%) about the recommendation that Hepatitis B immune globulin (HBIG) be used in emergency prevention (Table 4). Figure 1 shows the relationship between knowledge about HBV, attitude and prevention scores and grades. Higher knowledge about HBV (86%), prevention (90%) and better attitude (81%) were among interns. Figure 2 shows that scores of total attitude and knowledge about HBV infection were higher in females than males with a significant difference (p< 0.001). Nevertheless, the knowledge about HBV prevention was not statistically significant between male and female scores.

Table 4: Students' Knowledge about Hepatitis B prevention (correct answer rates)

Question item: Correct answer	% of Students with correct answers				
	4th grade	5th grade	6th grade	Interns	X2 (P value)
- Is HBV infection preventable? (yes)	78.6	89.5	100	87.5	18.437(0.030)
- Is HBV vaccine available? (yes)	95.7	96.5	94.9	100	5.278(0.809)
-Is it necessary to all dental staff to be vaccinated? (yes)	97.1	98.2	100	100	5.419(0.796)
-Is vaccine for hepatitis B safe and effective for all ages? (yes)	72.9	87.7	79.5	75	12.392(0.192)
- Is it necessary to measure the Anti-HBs level after having vaccine? (yes)	95.7	84.2	79.5	93.8	12.327(0.196)
- Can Hepatitis B immune globulin (HBIG) be used in emergency prevention? (yes)	75.7	75.4	69.2	81.3	11.940(0.217)
- Do you believe that HBV vaccination should be mandatory for all the dental healthcare workers in Saudi Arabia? (yes)	94.3	96.5	92.3	93.8	5.891(0.751)

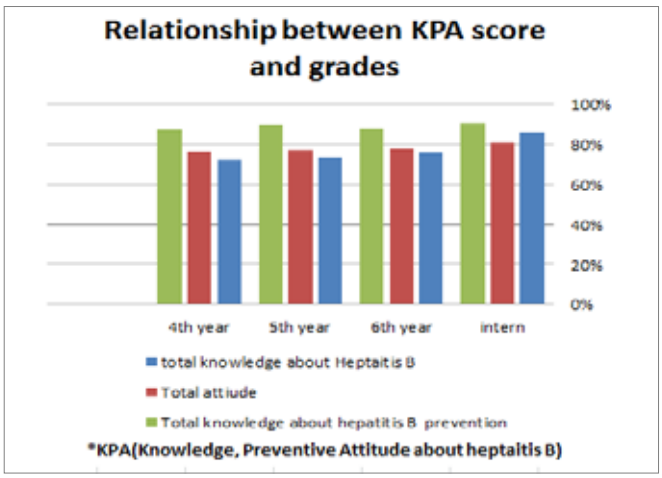

Figure 1: Comparison between different grades according to scores of total knowledge & attitude

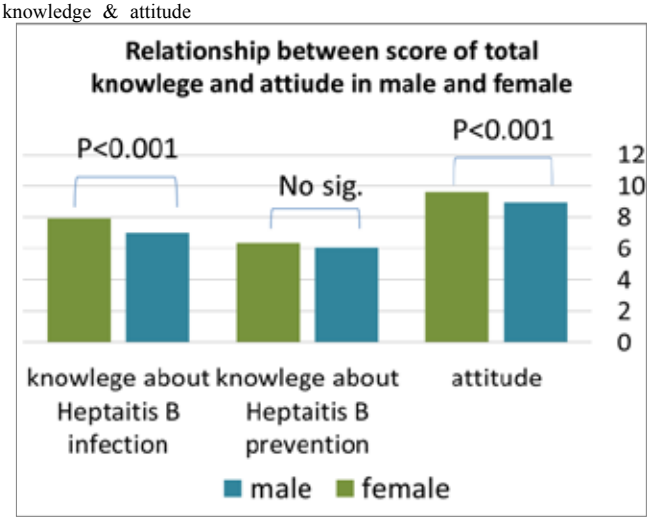

Figure 2: Comparison of males and females according to scores of total knowledge & attitude

Vaccine status

All participants were vaccinated against HBV and received 1-3 doses according to their Ab levels. 10.9% of them did not complete their doses (received only 2 doses)

Measuring Ab levels after vaccination was done by 145 (79.7%) of participants but 91 (62.76% out of them) did not know its level. On the other hand, 14 (0.77% out of all study participants) did not measure it (Figure 3).

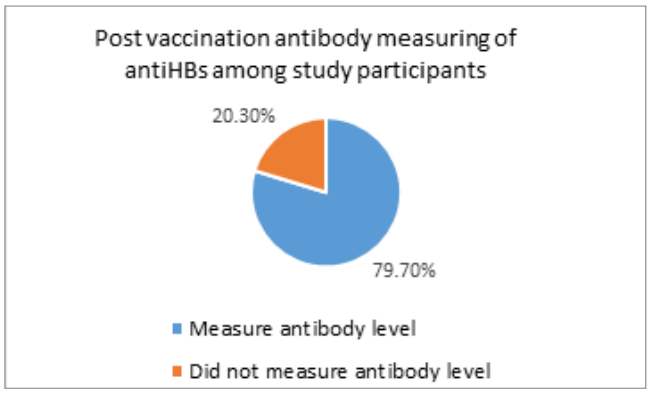

Figure 3: Percentages of study participants according to post vaccination antibody measurement

4. Discussion

Dental professionals have a significant occupational hazard for infection by Hepatitis B virus as they are frequently dealing with blood, saliva, and commonly exposed to needle stick injuries [23]. Data about HBV knowledge, preventive attitude and vaccine of health care workers including dental students and interns, as future dentists, are important in designing health intervention methods and public health policies [26].

Among undergraduate students in the present study, there was high knowledge (70<%) regarding modes of infection and clinical picture of HBV. These results are similar to previous studies done in Saudi Arabia by Al-Shamiri et al., 2018 [23] and Alrahmah et al. 2018 [27] but higher than those recorded by Saquib et al., 2019 [24] for their study on dental students randomly selected from the various dental colleges across Saudi Arabia. In the study of Saquib et al., 2019 [24] only about 20% of their study participants were aware of how the virus is spread and 13.6% knew that most of the patients have no prominent symptoms. The high level of knowledge in our college may be attributed to the annual reinforcement sessions for infection control practice in dental clinics and periodic auditing of students and interns during clinical sessions. According to previous studies from other parts of the world, knowledge rates about these items vary from low in India [15] to high in Turkey [28], China [29] and Libia [30] which reflects the worldwide awareness about Hepatitis B virus.

Although the majority of Umm Al-Qura university dental students were aware of the common transmission routes for HBV and its clinical picture, their knowledge was less accurate (40-70%) with respect to the virus incubation period and its ability to cause infection outside the body. These results are similar to those of Al-Hazmi, 2015[22] at Aljouf, Saudi Arabia (53.7% and 51.2% respectively). Controversially, only 28.2% of study participants by Saquib et al., 2019 [24] knew the duration of HBV incubation period and 75.7% of dental students participated in study of Al-Shamiri et al., 2018 [23] reported that HBV is infectious outside the body.

Regarding HBV transmission by comparison with HIV, knowledge was average in our study which was similar to that of Saquib et al., 2019 [24] (53.8%), lower than that of Al-Hazmi, 2015 [22] (70.7%) Alsamghan,2012 [31] (79.3%) and higher than that of Al-Shamiri et al., 2018 [23] (less than 40%). The difference may be attributed to different numbers of study participants and the curriculum of basic medical sciences studied in different universities and due to the fact that senior students may forget some basic knowledge delivered at earlier levels of college.

Higher knowledge about the virus's ability to cause infection outside the body of 4th grade students than other undergraduates participating in the present study, could be explained by their more recent study of basic medical sciences.

Among interns, high knowledge was detected for all questions except for infectivity of HBV by comparison with HIV where the knowledge was low (< 40%). High knowledge among them could account for the fact that graduate students who had clinical experience became more aware of HBV problem as well as the fact that they prepared themselves for the Saudi dental license examination which is an exam that assesses readiness of student to practice and proceed to post graduate training.

All participants in the current study reported unaccepted attitude regarding to discarding used needles into a medical waste container and bending needles after injections. Similarly, Haridi et al., 2016 [32] recorded that 22.1% of their study participants wrongly believed that bending needles helps them to avoid needle stick injuries. The percentage of students who agreed on discarding used needles into a medical waste container in the study of Akbulut et al. 2011 [28] is similar to our finding and may indicate a lack of knowledge regarding the differences between waste containers. The correct attitude of using goggles

during patient treatment ranged from average to high scores in our study participants which is similar to Al-Shamiri et al., 2018 [23], where 80.9 % of their participants reported that they use goggles during treatment.

In our study, high percentages of participants reported checking the indicator for efficient sterilization. Consistent with these results, Shigri et al. 2015 [33] reported similar percentages whereas in the Akbulut et al. 2011 [28] study only 14% among their dental clinical students used to check it.

Findings of the current study concluded that the highest knowledge about HBV, its preventive measures and attitude among study participants were detected in interns, higher in females than males. This result partially agrees with those recorded by Al-Shamiri et al., 2018 [23], and Saquib et al., 2019 [24], where both reported that females showed better attitude than males while in the Al-Shamiri et al., 2018 [23] study, final year students showed significantly better attitudes than interns and in the Al-Shamiri et al., 2018 [23] study, interns showed the highest level of attitude, a good level of practice but low level of knowledge.

With regard to knowledge about HBV prevention, all participants had high knowledge; this agrees with those of Saquib et al., 2019 [24] where 66.3% of study participants knew that a vaccine against HBV infection is available. Concerning knowledge about whether or not Hepatitis B Immunoglobulin (HBIG) can be used in emergency as prevention or not, the sixth grade students showed moderate knowledge (69%) which is slightly lower than the findings of a Chinese study which reported that sixth grade dental students have high knowledge (75.45%) [29].

All participants in the present study were vaccinated against HBV with 89.9% out of them completed their recommended doses. This rate is close to that reported in Canada (100%) [13]; comparable to that reported by other studies in United Arab Emirates 95.8% vaccinated; with 64.45% of them completed the required dosage [34] and in Saudi Arabia (89.9% vaccinated; with 74% completed their doses) [35], and (91.4 %; with 41% completed doses) [23]. The present study findings could be attributed to the college infection control policy that vaccination is an obligatory requirement for students before clinical sessions.

However, lower percentages were detected in Brazil (79.9.8%) [36], Yemen (70.7% were vaccinated; 50.0% completed doses) [37] and Central India [11] where only 38.8% of undergraduate students were vaccinated. These low vaccination rates could be explained by the high cost of the vaccination and a lack of awareness about the significance of vaccination in dental practice.

Similar to our study results, where 79.7% of students knew their post vaccination anti-HBs levels, Al-Hazmi, 2015[22] found that 85.8% of his study participants knew their status regarding anti-HBs. Because HBV vaccination does not always produce adequate antibody level for protection and it might give students a false sensation of safety, it is recommended for all vaccinated dental practitioners to know their post vaccination antibody levels.

Hepatitis B virus vaccine response confirmation, by measuring anti-HBs level within one to three months of primary vaccination, is recommended by Occupational Health and Safety Administration (OHSA) in all health care workers [38]. Antibody levels were measured after vaccination in 79.7% of the present study participants where this finding is much higher than those reported by Balcheva et al., 2015 [39] (34.3%) and Halboub et al., 2015 [37] (9.5%).

Limitations of the study are the small sample size, few number of interns by comparison with other groups may lead to misinterpretation of the level of knowledge and attitude. Also, the data collected by questionaires were self reported and so answers depended on the responder honesty.

5. Conclusions

Although dental students and interns in the present study showed high knowledge and attitude about many aspects of HBV infection and its prevention, average knowledge and/or low to average attitude about other aspects were recorded. Among study participants, higher attitude and knowledge were recorded among females and interns. With regard to their current HB vaccine status, all the participants received HBV vaccine but 9.7% of them did not measure HBs Ab levels after vaccination.

So, there is a need for sustained training and awareness about HBV infection and its preventive measures with periodic evaluation to overcome the gap of knowledge and to improve attitude. Students with incomplete vaccination doses should complete their programme of vaccination and measure post vaccination antibody levels to ensure adequate response to vaccine and protect UQU students and interns from HBV.

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- Conflict of interest

The authors declared that there is no conflict of interest.

- Funding

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- Ethical statement

- Ethical approval was taken from the college IRB and no study activities were started until the IRB approval was obtained.
- The questionnaire used for data collection in this study was self-structured with introductory paragraph about the aim of the study and an endorsement that it did not include any personal information and so answering its questions was considered as an acceptance to participate in the study.
- The manuscript is not currently being considered for publication in another journal

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Knowledge and Attitude Towards Total Knee Arthroplasty Among Health Education Students in Saudi Arabia

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ABSTRACT

Objectives: Total knee Arthroplasty (TKA) is considered the treatment of choice for knee osteoarthritis, and health educators can assist patients to choose the most appropriate treatment plan. This research aims to measure knowledge of and attitudevs towards TKA among health education students, as they are the health educators of the future.
Methods: A cross sectional design was used to study 227 health education students from four Saudi universities that offered a health education program. A questionnaire was distributed to eligible participants during the 7-month study period (20 September 2017 until 17 April 2018). Data were analyzed using SPSS. A chi-square test to measure the association between the different groups in terms of categorical variables and a Spearman's correlation test were conducted. Poor knowledge rated as 0-49%; fair knowledge 50-74%; and good knowledge 75-100%. A total score of more than 7.2 indicated positive attitude, more than 4.6-7.2 neutral attitude and less than 4.6 negative attitude.
Results: Of all participants, 71.40% had poor knowledge of TKA, while 76.20% had a neutral attitude towards it. Participant's level of knowledge was significantly associated with having previously heard about TKA and having a family member who had undergone surgery. Participants' attitude was significantly associated with having previously heard about TKA.
Conclusions: Overall, knowledge of TKA was poor, and attitude towards it was neutral. A correlation was found between participants' level of knowledge and attitude. Further studies for exploration are needed among health-care professionals including orthopedic surgeons and health educators.

1. Background

Knee pain is a public health concern as it affects mobility and leads to disruption of many daily life activities [1]. Although knee pain might be caused by a variety of conditions, in many cases it could be a sign of osteoarthritis (OA) which is a common disease worldwide [2]. A study conducted in Al-Qassim, which is a province located in Saudi Arabia, showed that OA is prevalent in the community as 53.3% of male patients and 60.9% of female patients are suffering with OA [3]. Nonsurgical interventions such as; weight loss, physical therapy and local injections could be useful for pain relief. Nevertheless, these treatments have not been successful in treating severe OA or in stopping the development of the disease. For such severe and progressive OA cases, Total Knee Arthroplasty (TKA) is considered to be an effective treatment of choice because it improves patients' conditions by relieving the pain [4]. TKA is an orthopedic surgical surgery which takes around 1 to 2 hours. During the surgery, a damaged knee-joint is replaced with an artificial one [5]. A patient is considered a candidate for TKA upon the existence of three indications; knee pain, functional limitation and stiffens [3]. In most cases these indications are caused by arthritis of the knee, specifically; OA rheumatoid arthritis, and post-traumatic arthritis [6]. Furthermore, obesity was found to increase the risk of OA leading to a demand for TKA [7].

TKA is considered as a reliable surgery that is effective on various aspects [8]. It is found to be a safe and cost-effective surgery [9]. Additionally, TKA effectively relieve pain and improve function [10]. TKA was also found to improve the Quality of Life (QOL) one study assessed the effectiveness of TKA by measuring QOL of patient pre-post undergoing TKA and the results showed a significant improvement in the participants' QOL as the pain was relieved and there were improvements on participants' mobility [11]. By abstaining from undergoing TKA when needed, patients' QOL is hindered resulting to unhealthy life which does not support one of the strategic objectives of the Saudi vision 2030, that is offering a fulfilling and healthy life [12].

Ever since TKA has been introduced in 1960's and 1970's, there has been a dramatic increase in the numbers of TKAs performed [13]. A study stated that almost 600 thousand TKA surgeries are performed yearly in the USA, also the same study anticipated a rise in TKA rates up to 673% by 2030 based on the fact that there is an increase in age and obesity rates [14]. In the Middle East and Gulf region there is a low rate of up-taking the surgery due to the lack of having the right perception and understanding regarding TKA, its benefits and complications [1]. TKA has generally proven to show satisfactory outcomes [7,11]. A study was conducted in the context of Saudi Arabia showing that the satisfaction rates among Saudi patients who underwent the procedure reached as high as 93%, however 67% of Saudi patients, who needed to undergo TKA refused the procedure because of their misconceptions and negative attitude which included a fear of disability and death [15]. Although age is not considered as a condition to undergo the TKA, but it is mostly performed on people ageing between 60 and 80 [2]. With regard to gender it was found that the number of women undergoing TKA is higher than men [16].

The refusal to undergo the procedure could be driven by the beliefs, concerns, familiarity with the procedure, and patients' expectations (8,15). A previous study conducted by Al-Mohrej et al. (2017), found that only 29.7% of the general Saudi population have had information about TKA with many misconceptions among the public concerning

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TKA, and its indications. These findings were presented in the first national and international study which examined the knowledge and attitude regarding total knee Arthroplasty among the public [17]. On the other hand, a study that measured knowledge of patients about total joint Arthroplasty including TKA in Hong Kong showed a good knowledge about the benefits and outcome of the surgery [18].

The KAB model was used as a framework to guide our study. This model suggests that there is a relation between level of knowledge, attitude and adopting a behavior [19]. An application of the KAB model to guide healthy behavior was effective in several instances [20]. Thus, this study tends to understand the knowledge and attitude of health education students, on the assumption that knowledge and attitudes will affect the educators' behaviors.

Patients' decision and choices related to undergoing TKA are affected by their knowledge, attitudes, as suggested by Knowledge, Attitude and Behavior (KAB) model, and by their socio-cultural factors [17,20]. Patient education should be provided by the multidisciplinary team, including health educators to improve knowledge, change preconceived beliefs, and misconceptions, which may ultimately affect decision-making [21]. Based on the literature review, this study is the first to assess the knowledge and attitudes of health education students towards TKA. By understanding of the indications, complications and outcomes of TKA, health educator can help in filling the gaps in patients' knowledge and overcoming communication barriers between the patients and doctors [22]. Health educators aim to change health beliefs and improve practices in the public and through the process of achieving their aim, some aspects have to be considered including; knowledge, attitudes, goals, perceptions, social status, cultural traditions and other aspects [23]. Health educators influence the process of decision making of options related to health among society as they educate individuals and communities about the behaviors that boosts their willingness and they can help by teaching them how to deal with their health conditions [22]. Health educators are responsible to give patients all information related to their health status, explaining the diagnosis and required surgery and tests pre-operative and give instructions post-operative [24].

So, this study aims to measure the level of knowledge and attitude of health education students regarding TKA as they are the future health educators, and its objectives are to assess the level of knowledge about total knee Arthroplasty among health education students', to assess health education students' attitude toward total knee Arthroplasty, and to identify the relationship between the socio-demographic characteristics and level of knowledge and attitude among the study participants.

2. Methodology

2.1. Study design

A cross-sectional study was conducted, in four Saudi universities. The population of the study were health education students who are enrolled in universities which offer health education bachelor's degree. Health education undergraduate students were included to the study. Health education interns were excluded as the study was targeting students who are still in the process of learning.

2.2. Ethical consideration

Prior to conducting the study ethical approval was obtained from Princess Nora University (PNU) IRB (H-01-R-059) and approval was obtained from all concerned universities. Informed consent was distributed along with the questionnaire.

2.3. Sampling

Stratified random sampling technique was used. The number of total population equals 764 students divided between the four universities. 82 of which are students of PNU, 342 students of Jazan University, 147 students of King Saud University (KSU) and 193 students of Umm Al-Qura University. proportional allocation was applied in each stratum using a sampling fraction of how they are represented of the total population.

OpenEpi web application was used for calculating the sample size [25]. Based on assuming that 29.7% has the right knowledge about TKA from a nation-wide study among Saudi population [17]. And considering an error margin of 5%, a minimum sample size of 227 was considered as sufficient for this study. The sample size consisted of 11% (n=25) PNU students, 45% (n=102) Jazan students, 19% (n=43) KSU students and 25% (n=57) Umm Al-Qura students.

2.4. Data collection

An English questionnaire was developed, validated and then translated to Arabic. The Arabic version was sent to the students via WhatsApp. The questionnaire consisted of 15 questions and divided into 3 sections, which were; socio-demographic characteristics, level of knowledge and attitude toward TKA. All the questions were mandatory to answer in order not to have missing data. The questionnaire took approximately one to two minutes to complete.

2.5. Socio-demographic Characteristics

The first section identified five socio-demographic characteristics, which were; sex (Male/Female), university (PNU/KSU/Umm Al-Qura/Jazan), level of education (1st year/2nd year/ 3rd year), whether they had heard of the surgery previously (yes/no) and if they had a family member who underwent the surgery (yes/no).

2.6. Knowledge

The second section assessed the level of knowledge including eight questions. The questions were True/ False/ Don't know (TFD) statements, which were; the surgery is common in Saudi Arabia, number of female patients is higher than male, arthritis is the main risk factor, age does not affect the surgery, BMI affects the outcome of surgery, the surgery is irreplaceable with non-surgical treatments, refusing the surgery leads to disability and the chance of complications occurrence after the surgery is low.

Following the Angoff method to set cutoff scores, each correct answer was awarded a score of one and each incorrect answer was awarded a score of zero [26]. All the awarded scores were summed to calculate the total score which was then converted to a percentage. Level of knowledge was classified into three categories based on standards previously used by knowledge, attitude and behavior (KAB) study; poor knowledge rated as percentages from 0-49%; fair knowledge rated as 50-74%; and good knowledge rated as 75-100% [27].

2.7. Attitude

The third section assessed the attitude towards TKA with two questions using a Likert scale, which were; (strongly disagree, disagree, neutral, agree and strongly agree) if they as health educators thought that the surgery is dangerous and their attitude towards TKA.

The strongly favorable attitude was awarded a scoring of 5 points and the strongly unfavorable attitude was awarded a scoring of 1 point. A total score of more than 7.2 indicated positive attitude, more than 4.6 to 7.2 indicated neutral attitude and less than 4.6 indicated negative attitude [28].

2.8. Validity and reliability

The questionnaire was translated into Arabic by experts using the forward-backward method [29]. Face validity was done by an orthopaedic consultant who evaluated whether our study instrument measures what it is supposed to measure (30). Cronbach's α test result from our questionnaire was (0.804).

2.9. Feasibility

A pilot test was used to test the study feasibility, reliability and if any modifications are needed [31]. 10% of the sample size was selected from health education students randomly for the pilot test. These students were excluded from the participants since the questionnaire was modified according to their given feedback [32]. Based on the piloting result, the questionnaire was modified, one unclear question was removed, and the questionnaire was translated into Arabic.

2.10.Statistical analysis

Data analysis was done using the Statistical Package for Social Sciences (SPSS, version 23 for Mac). The data were assessed for normality by Shapiro-Wilk. Bivariate analysis was chosen to assess socio-demographic characteristics, knowledge and attitude provided by the study questionnaire [32]. Chi-square ($\chi 2$) test was used to analyze the data and Spearman's Correlation Coefficient was used between the total knowledge and attitude. The test is considered significant if the p-value is less than 0.05.

3. Results

The questionnaire was distributed to measure knowledge and attitude towards TKA of health education students in Saudi Universities. The response rate was 100% as 227 of these students responded to the questionnaire and participated in the study.

3.1. Socio-demographic characteristics and hearing about TKA previously

Table (1) provides an overview of the socio-demographic characteristics of the participants. The participants were recruited from four Saudi universities 11% from PNU, 18.9% KSU, 25.1% Umm al Qura and 44.9% Jazan. Of these participants, the number of female participants was significantly higher making around 77%. The percentage of participants from first, second and third year students were 33.9%, 35.7% and 30.4% respectively. However, only 8% had a personal experience with a family member who underwent the surgery. With regard to their previous knowledge and experience with TKA, table (2) shows that 55.9% of the participants reported that they had heard about TKA previously.

Table 1: Socio-demographic characteristics of the participants

Variable	Categories	No. (227)	Percent (%)
Sex	Male	52	22.9
	Female	175	77.1
University	†PNU	25	11
	‡KSU	43	18.9
	Umm Al Qura	57	25.1
	Jazan	102	44.9
Level of education	1st year	77	33.9
	2nd year	81	35.7
	3rd year	69	30.4
Have you heard about total knee Arthroplasty?	Yes	127	55.9
	No	100	44.1
Do you have a family member who underwent the surgery of total knee Arthroplasty?	Yes	19	8.4
	No	208	91.6

†PNU- Princess Nora University
‡KSU- King Saud University

Table 2: Hearing about Total Knee Arthroplasty previously

Variable	Categories	No. (227)	Percent (%)
Have you heard about total knee Arthroplasty?	Yes	127	55.9
	No	100	44.1

3.2. Participants' knowledge about TKA

Table 3: Frequency distribution of participants' answers of knowledge about Total Knee Arthroplasty

Variable	Categories	No. (227)	Percent (%)
Total knee Arthroplasty surgery is common in Saudi Arabia.	True	49	21.6
	False	14	6.2
	Don't know	164	72.2
The number of females who undergo Total knee Arthroplasty is higher than the number of males.	True	54	23.8
	False	8	3.5
	Don't know	165	72.7
Arthritis is the main risk factor for total knee Arthroplasty.	True	83	36.6
	False	8	3.5
	Don't know	136	59.9
Total knee Arthroplasty surgery is not affected by age.	True	33	14.5
	False	76	33.5
	Don't know	118	52
Total knee Arthroplasty outcome is affected by body mass index.	True	105	46.3
	False	1	.4
	Don't know	121	53.3
Total knee Arthroplasty is irreplaceable with the available non-surgical treatments. Such as physical therapy, injections and weight loss.	True	26	11.5
	False	73	32.2
	Don't know	128	56.4
Refusing total knee Arthroplasty could lead to disability.	True	57	25.1
	False	21	9.3
	Don't know	149	65.6
The chance of complications occurrence after undergoing total knee Arthroplasty is low.	True	43	18.9
	False	26	11.5
	Don't know	158	18.9

What stands out in figure (1) is that the majority of participants (71.4%) had poor knowledge about TKA and only 5.7% had good knowledge. A closer inspection of Table (3), which presents an overview of the knowledge of participants, shows the limited knowledge about TKA. To be explicit, only 11.5% knew that TKA surgery is irreplaceable with nonsurgical treatments, 14.5% knew TKA is not affected by age, 18.9% knew that the complications rate after TKA is low, 21.6% knew TKA is common in Saudi Arabia, 23.8% knew the number of females who underwent the surgery is higher than males, 25.1% knew that refusing the surgery when needed could lead to disability. Higher rates were reported in term of the causes as 36.6% knew that arthritis is the main risk factor for the surgery and 46.3% knew BMI affects the surgery outcome. Finally, remarkably high rates of the participants (52%-72.7%) answered don't know to different questionnaire statements.

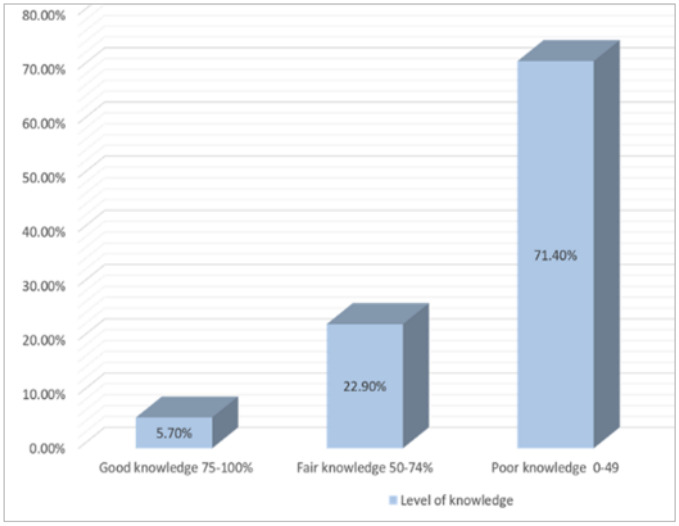

Figure 1: [Participants' level of knowledge about Total Knee Arthroplasty]

3.3. Participants' attitude towards TKA

Table (4) demonstrates participants' attitude towards TKA. Our result showed that 76.2% of the participants had a neutral attitude towards the surgery and its seriousness as shown in Figure (2).

Nearly a third of the participants consider TKA as a dangerous procedure, while a minority (8.8%) did not. Nevertheless, almost half the participants had a positive attitude toward the procedure and a minority (0.9%) had a negative attitude.

Table 4: Participant's attitude towards Total Knee Arthroplasty

Variable	Categories	No. (227)	Percent (%)
As a health educator, do you think that total knee Arthroplasty is dangerous surgery?	Strongly disagree	2	.9
	Disagree	36	15.9
	Neutral	102	44.9
	Agree	67	29.5
	Strongly agree	20	8.8
As a health educator, what is your attitude toward total knee Arthroplasty?	Strongly disagree	2	.9
	Disagree	13	5.7
	Neutral	102	44.9
	Agree	93	41
	Strongly agree	17	7.5

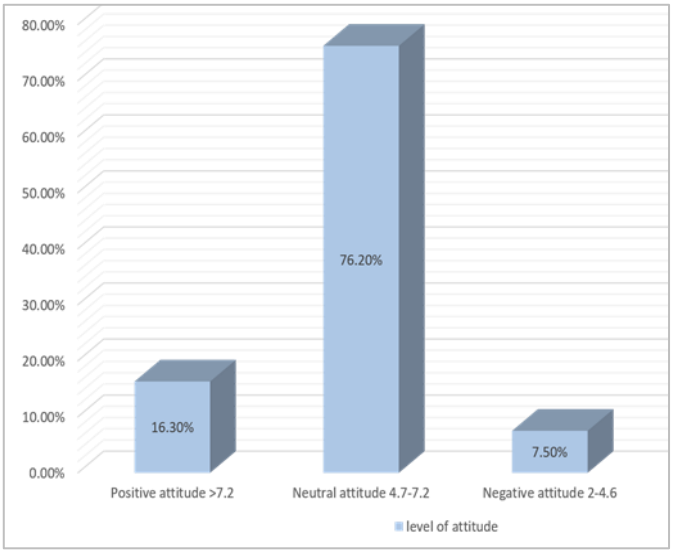

Figure 2: [Participants' level of attitude towards Total Knee Arthroplasty]

3.4. Relationships to the level of knowledge

Table (5) shows a relationship between socio-demographic characteristics and level of knowledge about TKA. A highly significant association was found between having a family member

who underwent TKA surgery and the level of knowledge ($p < 0.001$). Also, as shown in table (6), a highly significant association was found between hearing about TKA previously and the level of knowledge ($p < 0.001$)

Table 5: Relationship between Socio-demographic characteristics and level of knowledge about Total Knee Arthroplasty

Socio-demographic characteristics	No. (227)	Knowledge			*P-value* by chi-square test
		Good %	Fair %	Poor %	
Sex					.644
Male	52	7.7	19.2	73.1	
Female	175	5.1	24	70.9	
University					.944
†PNU	25	4	32	64	
‡KSU	43	7	18.6	74.4	
Umm AlQura	57	5.3	22.8	71.9	
Jazan	102	5.9	22.5	71.6	
Level of education					.191
1st year	77	6.5	18.2	75.3	
2nd year	81	8.6	25.9	65.4	
3rd year	69	1.4	24.6	73.9	
Do you have a family member who underwent the surgery of total knee Arthroplasty?					.000**
Yes	19	31.6	42.1	26.3	
No	208	3.4	21.2	75.5	

** *P-value* ≤ .001
†PNU- Princess Nora University
‡KSU- King Saud University

Table 6: Relationship between hearing about Total Knee Arthroplasty previously and the level of knowledge

Variable	No. (227)	Knowledge			*p-value* by chi-square test
		Good %	Fair %	Poor %	
Have you heard about total knee Arthroplasty?					.000**
Yes	127	9.4	40.2	50.4	
No	100	1	1	98	

3.5. Relationship between hearing about TKA previously and the attitude

Table (7) shows the relationship between hearing about TKA previously and the attitude towards TKA. A significant association was found between hearing about TKA before and the participants' attitude towards TKA ($p < 0.05$)

Table 7: Relationship hearing about Total Knee Arthroplasty and the attitude

Variable	No. 227	Attitude			p-value by chi-square test
		Positive %	Neutral %	Negative %	
Have you heard about total knee Arthroplasty?					.026*
Yes	127	22	70.1	7.9	
No	100	9	84	7	

* $p < .05$

3.6. Spearman's Correlation between knowledge and attitude

Table (8) presents the correlation between level of knowledge and attitude towards TKA. A positive correlation was found between level of knowledge and attitude, r=0.228, p =.001.

Table 8: Correlation between knowledge and attitude towards Total Knee Arthroplasty

		Knowledge	Attitude
Knowledge	R	1.000	.228
	P-value by correlation test	.	.001**
	N	227	227
Attitude	R	.228	1.000
	P-value by correlation test	.001**	.
	N	227	227

** *p-value* ≤ *.001*
†r = Correlation
‡N = Number of sample size

4. Discussion

Our study attempted to find out the relation between health education students' socio-demographic characteristics, knowledge and attitude towards TKA in Saudi universities. Numbers of female participants were significantly higher than male participants due to the fact that PNU is a female-only university and the number of male students enrolled in health education program in other universities is less than females. More than half of the participants had heard about TKA previously and few of the participants had a family member who underwent TKA.

Most participants had a poor level of knowledge about TKA, although more than half of the participants had heard about TKA previously, which is consistent to a previous study conducted by Al-Mohrej et al. (2017) in Saudi Arabia which reported similar findings [17]. The minority of the participants had good level of knowledge which is contradictory with the findings of a previous study conducted in Hong Kong where the majority of their participants had a good level of knowledge about TKA [18]. This inconsistency could be explained by the fact the Hong Kong study was conducted in OA or inflammatory arthritis patients, who have already got information about the surgery from their physicians while our study was on randomly selected students.

High rates of participants were not knowledgeable about TKA indicating a gap in educational sessions and campaigns. More than half of the participants did not know if non-surgical treatments such as physical therapy and injections can replace TKA surgery. This finding is consistent with Al-Mohrej et al. [17]. The number of participants who thought TKA is affected by age is more than the ones who did not. A possible explanation for this is that in Saudi Arabia most TKA surgeries are performed on patients with age mean 65.7 [33].

Overall, a low rate of participant had a positive attitude towards TKA and its seriousness. This could be attributed to their poor level of knowledge as KAB model suggests that attitude is driven by knowledge [19].

Our findings indicated a highly significant association between hearing about TKA previously and having a member who underwent TKA surgery with the level of knowledge, and it seems possible to explain this by taking the member who underwent the surgery as their source of information about TKA. Also, a significant association was found between hearing about TKA previously and their attitude toward it as they have acquired a perception about TKA, which is also be explained by the KAB model [20].

Finally, our study found a correlation between the participants' knowledge about TKA and their attitude toward it. This correlation can be interpreted by the KAB model as it proposes that knowledge affects attitude contributing to a change in behavior [19].

5. Conclusion

Based on our study findings, we conclude that, the level of knowledge of most participants was poor. Most of the participants had a neutral attitude towards TKA. Also, more than half of the participants had heard about TKA previously, which was found to have a significant association with their attitude. Additionally, a significant association was found between having a family member who underwent TKA surgery and hearing about TKA before with the level of knowledge. Finally, a correlation was found between level of knowledge and attitude.

6. Recommendations

The following strategies were recommended to raise knowledge and attitude towards TKA among health education students and public;

- Integration of the most common diseases in Saudi Arabia and their treatments in the curriculum of health education programs.
- Public education about TKA through media and health education campaigns.
- Further researches are needed to assess the knowledge and attitude towards TKA and its benefits in order to promote the awareness of the community and increase the utilization of TKA
- Further studies are needed among orthopaedic surgeons, health care professions involved in OA treatment and OA patients to identify the gap in doctor-patient communication to provide a more comprehensive result and to explore reasons for refusal to utilize this surgery

- Further studies are needed among patient educators working in hospitals and healthcare centres to identify their role in providing and correcting information about TKA

- **Acknowledgement**

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- **Declaration**

The authors declare that there are no conflicts of interest. The authors are exclusively responsible for the content and the writing of this research paper.

- **Limitations**

While this study met the intended objectives, it has some limitations. First there are few studies measuring knowledge and attitude towards TKA, resulting in unavailability of a standard questionnaire that measures the study objectives requiring development and validation of a questionnaire. So a questionnaire was developed, validated and used for this study. Second, the number of female participants is higher than the males. However, this is understandable as the number of female students enrolled in health education program is higher than male students which makes the sample representative of the population of the study.

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Preferred Obstetrician-gynaecologist Gender among Female Residents in Jeddah, Saudi Arabia

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ABSTRACT

Background: A number of studies have investigated female patient's preference for their obstetrician/gynecologist (OG) physician. This topic was rarely investigated in Saudi Arabia.
Aims: This study investigated OG gender preference among female resident in Jeddah, Saudi Arabia and factors that influence their choice.
Methods: In this cross-sectional study, 596 female participants answered a self-reported questionnaire. Participants were recruited from shopping malls in four different areas in Jeddah using convenient sampling. The participants were asked or questioned about their OG gender preference and factors affecting it.
Results: OG gender preference was found to be an important issue among females in Jeddah, as 66.6% of the participants do ask about the OG gender before their visit, and 39.4% would feel uncomfortable if the preferred OG gender is not available. The results showed that 57.89% preferred female OG, 20.47% preferred male and 21.64% had no preference. Participants answered that for presence at delivery, 50.34% preferred female OG, 27.35% preferred male OG and 22.32% had no preference. Also, in the case of OG emergency and critical surgery, 9.2% reported they would refuse surgery if the preferred OG was not available and seek an alternative solution such as changing hospital. The most important factors that influenced the participant's choice were: the ability to handle OG emergencies situations with confidence (53.40%), OG knowledge about women health (45%), and OG sympathy (42%).
Conclusions: OG gender is an important issue for Saudi female, as the majority prefer female OG in general, but prefer male OG in critical conditions.

1. Introduction

Many studies were conducted to know the patients preference in choosing their physician [1-6]. In fact, a recent systematic review highlighted patient preference as a growing topic of interest and indicated that there are many psychological constructs affecting that including: mood, health beliefs, individual differences, motivation and cognitive factors [7].

Some studies have focused on the investigation of female patients' preference for their obstetrician/gynecologist (OG) physician [8-10]. Another systemic review indicated that most females prefer female OG because female OG tends to communicate in patient-centered style [11]. Also, the review could not find a clear association for female age or ethnicity toward patient gender preference. Such topic is important as it is patient right to choose the health care provider [12], which fulfil patient's autonomy and improve patient's satisfaction of treatment [13].

A number of studies investigated female patients' preference for their obstetrician/gynecologist (OG) physician in different countries around the world. For example, studies in Emirates [9, 14] and Iraq [15] indicated that the majority of females preferred female OG (96.8-86.4% and 73% respectively). Also, there is a small percentage that prefers male OG and others who have no gender preference. However, a study in Nigeria [16] had around 36.7% preferred female OG, 30.3% preferred male OG, and 33% had no preference. Also, a Turkish study found that 32.3% of the participants preferred female OG, while 53.5% of them had no preference [17]. A study in the United States (USA) reported that the majority 66.6% have no gender preference, and 80.8% believe that gender does not influence the quality of treatment [18]. This can reflect the diversity of choices available based on their culture.

Nevertheless, preferences noted in studies, might be more accentuated in other countries where religion and culture are very crucial in the patient's behavior. In religious countries, such as Saudi Arabia, females are known to wear an ankle-length over-dress (Abaya), and most of the women avoid physical contact with unrelated male following Islamic teachings, as the majority of the Saudi are Muslim. So, this is important for women with deep religion cultural roots, because OG visits can include intimate examination, involving breast and genitalia [19-21]. Therefore, it is very important to understand the Saudi cultural dimension in order to improve the quality of care provided [22]. In light of the Government reforming plan in Saudi Arabia, the health workforce is projected by the Saudi Health Commission for Health Specialties report [23], to meet Saudi Arabia vision 2030.

According to the best of our knowledge, there were two studies which have investigated this topic in Saudi Arabia; in Jeddah [24] and Riyadh [22]. The study conducted in Jeddah included a sample of 418 university hospital's patients. The results showed that the percentage of patients who preferred to be examined by a female OG, during pelvic examination was 77.1% but for major OG surgery 47%. The study also showed that participants preferred female OG due to being comfortable with a female, in line with their husband preference and their feeling that a female OG is more knowledgeable. Nevertheless,

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the study had a small percentage of participants from a high income category (7.9%), and most of them were unemployed (70.7%). This might affect the results as another Turkish study, who have similar Islamic culture, indicated that the participants who preferred female were mostly with lower income and were unemployed [17].

The other study in Riyadh was a qualitative study and indicated that female patients tend to choose a female OG, and highlighted that religion was an important factor in their choosing the preferred OG gender [22]. However, the data from those two studies are not enough and there is need for further studies to be generalized over the Saudi population.

Due to the paucity of research and the lacked information, this study aims to identify OG gender preference levels among the general population of female residents in Jeddah and to identify the factors that affect their choices. The study also aims to identify the female population choices during an emergency time, as a decision in a life-threatening situation may be different.

2. Material and Methods

This cross-sectional study investigates the preferred OG gender among the general population of female residents in Jeddah, Saudi Arabia, which is the target population for this study. The inclusion criteria include female individuals who are aged more than 18 years old. All participants who did not sign the study consent were excluded from the study. Using a confidence level of 95%, the accuracy level of 5%, and estimated prevalence of 50%, 385 participants were needed in this study as the minimum number. Sample size was calculated using G*power software.

To have a more representative sample, data were collected by dividing Jeddah city into four geographic areas: South, North, Center, and East, and a shopping mall was selected from each area that represents different socio-economic status in convenient way.

A convenience sampling technique was used in this study. Research team member approached shopping mall visitors face to face and asked them if they were willing to participant in this study. Consent approvals were obtained from the participants from shopping malls before starting data collection.

Each participant was required to complete a self- administrated questionnaire that was provided in hard copy. Each participant answered the questionnaire voluntarily. The questionnaire was developed from a similar previous study [18], with some modifications. The questionnaires were distributed by the research team, and participants signed the consent form before answering the questionnaire. Each questionnaire took approximately 5 to 10 minutes to answer. The questionnaire was administrated in the Arabic language as it is the formal language of the country. The questionnaire composed of three sections: Section one measured participant's gender preference via eight questions: the preferred doctor gender in general; the preferred OG gender, the preferred OG gender to perform vaginal delivery; the preferred surgeon in general; the preferred OG gender to perform cesarean section; and the preferred OG gender to manage OG emergencies. Also, there were two questions to ask about the action taken when the preferred OG gender is not available, and if the participant usually asks about the OG available gender. In Section two, factors that could lead the participant to prefer OG gender over another were explored. These factors included the following: religion, parents/husband influence, tradition, OG ability to handle emergency situation with confidence, OG knowledge about women health, OG sympathy, previous experience with OG, family and friends experience with OG, shyness, religion, parents/husband influence, tradition, and OG nationality. Also in this section, participants were asked if they had previously visited a non-preferred OG gender, and if they were uncomfortable when they didn't find their preferred OG gender. The last section contained nine questions about demographic data including: age, marital status, number of children, previous cesarean section, level of education, occupation, income, nationality, and residency.

Data analysis was conducted using SPSS v.21. Frequency tables and descriptive statistics were generated. Chi-square test was used to test for difference in the data responses. All assumptions for chi-square were fulfilled before conducting the test. Statistical significance levels were detected by using the P-value of 0.05

Informed consent was obtained from each participant before starting the questionnaire, all data were treated anonymously, and any identifiable data would be destroyed. The Ethical Committee at Faculty of Public Health and Health Informatics approved this study.

3. Results

A total of 596 female participants completed the study questionnaire. The mean (m) age of participants was 31.36 years and standard deviation (sd) was 10.45. Also, the median of participants' children was 1. The number of children ranged from 0 to a maximum of 12. Other demographic variables are shown in table 1.

Table 1: Demographic categories of participants

Variable		Count (%)
Marital status	Married	307 (51.50%)
	Single	237 (39.80%)
	Divorce	42 (7.00%)
	Widow	10 (1.70%)
Nationality	Saudi	515 (86.40%)
	Non-Saudi	81 (13.60%)
Occupation	Employee	340 (57.00%)
	Non-employee	256 (43.00%)
Family financial status	Less than 5000 SR	122 (20.50%)
	5000 - 15000 SR	284 (47.70%)
	More than 15000 SR	190 (31.90%)
Educational level	Less than college	388 (65.10%)
	Completed college	208 (34.90%)
Have had a cesarean section	Yes	136 (22.80%)
	No	460 (77.20%)

SR: Saudi Riyal

The participants' gender preference for general physician and OG is displayed in Table 2 according to different situation. This was also was displayed in figure 1.

Table 2: The participants' gender preference for general physician and OG.

Question	Preference		
	male	female	no preference
preferred general physician	185 (31.04%)	158 (26.51%)	253 (42.45%)
preferred OG	122 (20.47%)	345 (57.89%)	129 (21.64)
preferred OG in delivery	163 (27.35%)	300 (50.34%)	133 (22.32%)
preferred general surgeon	335 (56.21%)	58 (9.73%)	203 (34.06%)
preferred OG in cesarean section	278 (46.64%)	142 (23.83%)	176 (29.53%)
preferred OG in in emergency settings	303 (50.84%)	83 (13.93%)	210 (35.23%)

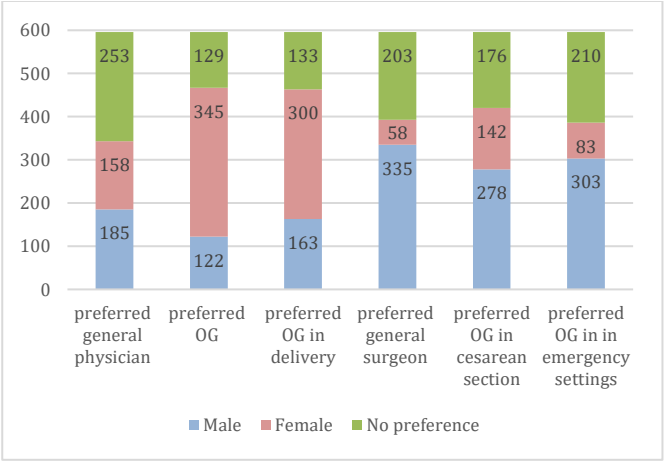

Figure 1: The participants' gender preference for general physician and OG.

A total of 90.8% of the participants answered that they will go with the available OG gender in case of emergency during delivery, however, 55 participants (9.2%) chose not to conduct the surgery and seek alternative solution including transferred to another hospital in

the emergency situation, (49 preferred male, and 9 preferred female in emergency situation)

Results showed that 397 (66.6%) of the participants do usually ask about the OG gender before their visit. Also, 269 (45.1%) reported that they become uncomfortable when the preferred OG gender is not available. There was 235 (39.4%) who answered that they had visited OG who is not their preferred gender in the past.

Table 3: Factors affecting participant's OG gender preference.

Variable		Preferred OG gender		
		Male n (%)	Female n (%)	No preference n (%)
Age	18 - 30 years	65 (19.2%)	194 (57.2%)	80 (23.6%)
	31 - 50 years	44 (20.6%)	131 (61.2%)	39 (18.2%)
	More than 50 years	13 (30.2%)	20 (46.5%)	10 (23.3%)
Marital status	Married	54 (18.7%)	162 (56.1%)	73 (25.3%)
	Not married (singe, divorced, widow)	68 (22.1%)	183 (59.6%)	56 (18.2%)
Nationality	Saudi	106 (20.6%)	293 (56.9%)	116 (22.5%)
	Non-Saudi	16 (19.8%)	52 (64.2%)	13 (16.0%)
Occupation	Employee	60 (17.6%)	208 (61.2%)	72 (21.2%)
	Non-employee	62 (24.2%)	137 (53.5%)	57 (22.3%)
Family financial status	Less than 5,000 SR	22 (18.0%)	73 (59.8%)	27 (22.1%)
	5,000 – 15,000 SR	57 (20.1%)	181 (63.7%)	46 (16.2%)
	More than 15,000 SR	43 (22.6%)	91 (47.9%)	56 (29.5%)*
Educational level	Complete college	41 (19.7%)	126 (60.6%)	41 (19.7%)
	No college	81 (20.9%)	219 (56.4%)	88 (22.7%)
Preferred general physician	Male	101 (54.6%)	65 (35.1%)	19 (10.3%)
	Female	4 (2.5%)	149 (94.3%)	5 (3.2%)
	No preference	17 (6.7%)	131 (51.8%)	105 (41.5%)*
Had previous cesarean section	Yes	45 (33.1%)	57 (41.9%)	34 (25.0%)
	No	77 (16.7%)	288 (62.6%)	95 (20.7%)*

***p <0.05**

Using chi-square, there was three variables that had a significant relationship with preferred OG gender, which is: family financial status, preferred general physician gender and previous cesarean section experience, which are detailed in Table 3. According to the table, participants with different family income have different preferences. The OG gender preference for participants with low and high family income have a similar pattern, as majority prefer female, no gender preference, and then male OG. While the majority middle family income participants chose female, male, then no preference choice. Also, the majority (94.3%) of participants who preferred female physicians preferred female OG. There were 51.8% of participants who have no physician gender preference in general, but preferred female OG. Despite that 45.6% of participants who preferred male physician were found to prefer also male OG, other 35.1% of them prefer female OG. The percentage of participants who prefer male OG was significantly higher among participants who had a cesarean section, compared to those who did not. Age, marital status, nationality, occupation, and educational level were found to be not significantly related to participants' choice for their preferred OG gender.

As participants were asked to choose all factors to determine the factors that lead them to prefer specific gender over another, the participants' factors are displayed in Table 4, bearing in mind that many of them choose more than a factor.

Table 4: Factors that influence participants to prefer OB gender over another.

Factor	Count (%)
OG handle the emergency situation with confidence	318 (53.4%)
OG knowledge about women health	268 (45.0%)
OG sympathy	253 (42.4%)
Previous experience with OG	228 (38.3%)
Family and friends previous experience with OG	209 (35.1%)
Shyness	179 (30.0%)
Religion	137 (23.0%)
Parents/ husband influence	137 (23.0%)
Tradition	84 (14.1%)
Nothing effect on my choice	50 (8.4%)
OG nationality	41 (6.9%)
Community influence	37 (6.2%)

4. Discussion

This study investigated Jeddah females preference toward OG gender. In fact, OG gender preference seems to be a point of concern for Jeddah females, as this study results showed that more than two-thirds of the participants ask about the OG gender during taking an appointment, and around half of participant reported they would be uncomfortable when their preferred OG gender is not available.

The results obtained indicated that female OG, in general, was the most preferred by 57% of the participants, while other participants were divided between preferring male OG, or having no preference. However, this preference changes slightly in a different situation. For example, half the participants preferred to deliver their babies by a female, while 27% preferred to be delivered by male OG. So, the percentage that preferred female OG decreased and the percentage that preferred male OG increased in case of delivery. Furthermore, when there is a cesarean section, 23% preferred female OG, while 46% preferred male OG. In emergency and life-threatening setting, male OG was preferred by 50%, followed by no preference choice (35%), and only 13% preferred to be handled by 13%. This trend might indicate that female OG is preferred in a regular situation and in delivery. However, during cesarean section and emergency setting male OG was preferred.

When comparing the results of this study with the results to previous studies conducted out Saudi Arabia, it is found that this study and other studies conducted in Islamic and Arabian countries such as the Emirates and Iraq [9, 14, 15], indicated that the majority of females prefer female OG. However, this was different than those studies in USA, Turkey and Nigeria [16-18]. When comparing this study with the only other study conducted in Saudi Arabia, it is noticed that there is a similarity in the percentages for female OG preference in general [24]. However, this study results show a difference in the preference during critical surgical condition related to OG, as this study result indicated that female OG were preferred only by 13% to 23%, while in another Saudi study [24] the percentage was 47%. Despite the fact that both studies were conducted in Jeddah, this variation might be due to the demographic variation in the target population. In the other study [22] the sample included a majority of women who were unemployed and were from low to middle socioeconomic status. While in this study, data were collected from women in the population (shopping malls) in different geographical areas who are likely to be more reasonably distributed according to family income and employment. However, a more representative study is needed to generalize such finding.

This study result indicated that around ten percent of the participants reported they would not undergo surgery and treatment in problematic surgical delivery cases if they did not find the gender, they prefer but rather seek an alternative option (Figure 1). Surprisingly, this was applied more in participants who prefer male OG. This has two implications. First, refusing to do surgery in critical cases might be fatal to the mother or child, therefore this attitude could have serious consequences. It is important to respect patient desire and culture but without jeopardizing lives. So, it is recommended that the number of female OG specialist be increased to meet the demand and patient

preferences, and also to educate the female population to be more aware of the consequences of their decision. These findings are especially salient when Islam teaches about the importance of saving a person's life and making it as a priority in such Caesarean es. The second implication is that a large percentage of those females preferred male OG, which seems not based on religious teachings or cultural desire, but rather because they believe male OG is more reliable in emergency situations. This can be inferred by the answers of participants who ranked the ability of OG to deal with emergency cases with confidence as the most important factor in preferring OG gender over another. This might lead us to recommend more efforts be directed to foster the reputation and capability in female OG, to address the beliefs of the women during pregnancy.

This study result in comparison to a previous study shows a variation in the level of importance toward factors leading females to prefer specific OG gender. The highest rated factors that influenced the participant choices in her doctor's gender are how the doctor handles the emergency situations steadily, OG knowledge about women health and OG sympathy. Comparing to the previous studies, in King Abdulaziz University Hospital the highest factors were comfort with a female practitioner, husband preference for a female OG and physician's knowledge respectively [24]. In a study conducted in Iraq, the most important factors were embarrassment, religion and culture [15]. In Emirates [9, 14] and in Nigeria [16] the religion and the traditions were the main influences on the participant choice. Lastly, in Turkey the factors influencing their choice were good communication of the doctor, embarrassment and feeling comfortable with the female OG respectively [17]. This variation could be attributed to the variation cross-culturally. However, it is interesting that in this study, religion was not the most important factor compared with reports in the previous studies.

Despite the fact that OG gender preference is an important topic, this is only the second study conducted in Saudi Arabia. Authors of this study argue that the sample from Jeddah city was less representative of the general population of pregnant women than in the previous study [24]. However, this study is still not scientifically representative, and might have reporting bias due to the self-reported questionnaire. Also, the questionnaire was not validated. Thus, it is recommended to conduct a further multicentered study with participants from different cities, using a clustered random sample to have more generalized data, as it is believed that different cities would have a different opinion toward OG gender preference.

5. Conclusions

OG gender is an important issue among females in Saudi Arabia, which can end up with serious consequences. This study concluded that the majority of females prefer female OG in general. However, females tend to prefer male OG in cesarean section and critical emergency cases. Around one out of ten of the participants chose not to undergo surgery in problematic surgical delivery cases, if they did not find the gender they prefer and would rather seek an alternative option. The most important factor in selecting OG gender preference was the ability to handle emergency cases with confidence. It is recommended to increase the number of female OG in Saudi Arabia to meet patients' demand, and to improve the confidence image of the ability of female OG to deal with critical cases.

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- **Conflict of interest**

The authors declare that there is no conflict of interest.

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Prevalence and related potential risk factors of *Toxoplasma gondii* infection among slaughterhouse workers and animals in Makkah

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ABSTRACT

Toxoplasma gondii is a protozoan parasite that causes a parasitic disease known as toxoplasmosis. The disease transmits from animals to humans via the eating raw or insufficient cook meat or by contact with raw meat. The main aim of the present study was to define the prevalence and the associated risk factors of the disease in workers and animals in slaughterhouses in Makkah.

The study was based on a cross-sectional study in humans and animals including, sheep, goats, cattle, and camels. Data were collected using a short questionnaire. Approximately 108 blood samples were collected from workers included the butchers and the workers and 160 blood samples of the slaughterhouse animals. Serum samples were kept in -80º C till examined by using ELISA IgM and IgG tests. The data analyzed by using SPSS version 25.

The result showed that the prevalence in workers was 0.9% and 25% using IgM and IgG, respectively. In animals, all the results were negative using ELISA IgM and IgG tests. The result showed that there was a correlation between chronic infection and contact with a cat (p<0.015).

The study focussed on the *T. gondii* in slaughterhouse workers which found that both acute and chronic toxoplasmosis. The results also showed no infection was registered in different species of animals in both slaughterhouses.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a protozoan parasite that causes a parasitic disease known as toxoplasmosis. The causative agent of toxoplasmosis, the parasite called *T. gondii*, is a sporozoan belongs to coccidian. Infection caused by this parasite is intricately secured to its complex life cycle. Several mammals are hosts to infection and act as intermediate hosts, although domestic cats and other felines are the only definitive host [1]. Toxoplasmosis is not transmitted from person-to-person, except congenitally or vertically from mother-to-child, so that a woman who is recently infected with *T. gondii* can pass the infection to her unborn child through the placenta or during a blood transfusion or organ transplantation [2-3]. Horizontally, the intermediate hosts in nature can be infected by ingesting oocysts in water, plant material, or soil [4]. Humans can get the infection by different routes, including cutting meat or eating undercooked meat of animals harbouring bradyzoite [5]. The prevalence of infection by *T. gondii* in a different population of animals is varied. It was found that the parasite infected a varied range of animals existing in different environments [6]. Similar to most protozoa causing disease, the prevalence of toxoplasmosis is higher in developing areas. However, the parasite *T. gondii* is one of the protozoa parasites that have maintained a significant prevalence in developed countries such as the USA [4]. This high level of prevalence in humans may be due to the fact that people can get the infection with *T. gondii* by several routes. Subsequently, the level of heat used in cooking interrupts bradyzoites, but infections can occur via ingesting undercooked meat. Also, another factor of concern is that animals used in the food chain differ in their bradyzoite resistance and, therefore, their capability to infect people could be different. The current reason gives an answer to the question of why the consumption of meat from some species, such as bovines, which are relatively resistant to *T. gondii*, is a less common source of transmission as compared to the ingestion of meat from other animal food sources such as ovine or porcine species [7]. While the effects of fetal infection can be devastating, active fetal infection is a relatively rare event in addition to the fact that *T. gondii* prevalence increased with age [8, 9]. Occupational risks related to augmented *T. gondii* contact, including working with animals and gardening were studied [10]. In Saudi Arabia, there were numerous studies done to estimate seroprevalence, and all studies were conducted among pregnant women. Immunosorbent Assay (ELISA) was used to determine the prevalence rate among pregnant women in Jazan and found that the overall prevalence was 24.1 % [11]. In Najran, hemagglutination was used and it was estimated that 20.8 % were positive [12]. In Riyadh, the seroprevalence of hemagglutination test indicated that 38% of pregnant women were positive to *T. gondii* [13]. Another study done in south western Saudi Arabia among pregnant women used Enzyme Linked Immunosorbent Assay (ELISA) and found that about 38 % of women were positive to *T. gondii* [14]. In the Eastern region, the estimated seropositive test of *T. gondii* gave a prevalence of 25 % by using ELISA IgG and 5% by using ELISA IgM [15]. In Dhahran they found that the overall prevalence of *T. gondii* was 28.5% by using ELISA IgG and 3 % by detection IgM [16]. In the Aseer region, they used a polymerase chain reaction (PCR) and found that 41% was positive to *T. gondii* [17]. The study aimed to determine the prevalence of the disease in workers and animals in slaughterhouses in Makkah.

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2. Material and Methods

2.1. Study area

The study was located in Makkah lying in the west of Saudi Arabia, north west of Taif, east of Jeddah and south of Madinah. Makkah is location of the Kaaba which is visited annually by more than 20 million visitors, including pilgrims and tourists. The total population of the Makkah region is 8,557,766, of whom 4,516,577 are Saudi nationals, and 4,041,189 are non-Saudi residents, representing 47% of the total number of residents of the region.

2.2. Study population

Approximately 43 (39.8%) of the study population were butchers, and 65 (60.2%) were workers. The criteria of selection was included all people working inside the slaughterhouse and in real contact with meat during the time of the study. The criteria excluded the employees and the housekeepers. Their age ranged between 22 - 61 years with a mean of 40.49 years. Samples were collected from Al Ka'akya 97 (89.8%) and Al_Muasim 11 (10.2%). Their duration of working in slaughterhouses ranged between 1 to 45 years with a mean of 14.72 years. The distribution among nationality was found to be 92 (85.2%) from Bangladesh, 9 (8.3%) from Egypt, 4(3.7%) Pakistan and 3 (2.8%) were African.

2.3. Study design

A cross-sectional based study was done in people working in meat processing butchers or workers holding, cutting, and disposing of waste. The study was conducted during the period between March and April 2019.

2.4. Sample size

Data and blood samples were collected from 109 human workers who agreed to participate in the study. Also, 160 samples from different kinds of animals were collected from the slaughterhouse in Al_Kaa'kyah and Al_Muaysim in the Makkah area.

2.5. Data collection

Consent Forms: Approval was obtained from the Ethical Committee in the Faculty of Public Health and Health Informatics, Umm Al Qura University. The purpose and procedures of this study were explained to all participants, and written informed consent was obtained from all participants.

A questionnaire was completed by all participants that included demographical questions, occupation, duration of the profession, diet (consumption of undercooked meat, standard water, raw milk), daily activity (contact with cat, contact with soil), and questions about previous blood transfusions, previous screening for toxoplasmosis and use of gloves when slicing meat.

2.6. Samples Collection

Human Sample Collection: A sample of up to 3 ml of blood was obtained from each participant using a 5 ml syringe into a plain tube. The serum was separated from the blood by centrifuging and transferred to Eppendorf tubes to measure IgM and IgG. The serum was stored in a freezer at -80°C until used. The serum samples were screened for *T. gondii* using ELISA IgG and IgM.

Animal Sample Collection: Using a plain vacutainer tube, approximately 5 ml of blood was collected from the jugular vein of each animal, including sheep, goats, cattle, and camels after slaughtering. The samples were taken overnight in 4ºC and serum separated by centrifugation. The samples were separated in plain tubes and stored at – 80ºC until used.

2.7. Laboratory Investigation

IgM and IgG was done by enzyme-linked immunosorbent assay (ELISA) (ARCHITECT plus analyzer, Abbott, Abbott Park, Illinois, U.S.A) according to manufacturing procedures for all samples from humans and animals.

2.8. Data Analysis

Data were analyzed using SPSS software (version 25). The chi-square test was used to compare the serology result frequencies among the different taking a *p*-value of less than 0.05 as the level of significance.

3. Results

3.1. In Human

The present study including a total of 108 workers in the slaughterhouses in the Makkah area. Table (1) shows the total number of slaughterhouses tested for anti-*T. gondii* IgG and IgM by using ELISA. The prevalence of acute toxoplasmosis was 1 (1.5%) in workers and 0 (0.0%) in butchers while the prevalence of chronic toxoplasmosis was 13 (20%) in workers and 14 (32.6%) in butchers with overall prevalence 27(25.0%).

3.2. In Animals

The current study included 160 animal samples; these samples were collected from the slaughterhouses in the Makkah area in Al-Ka'akya and Al-Muasim. The results obtained confirmed that all the samples from camels, cattle, sheep, and goats had no reaction and showed negative results for IgM and IgG.

3.3. Risk Factors

Socio-demographical Factors: As shown in table (2), the age of participants, nationality, occupation, location and working duration played no role in the risk of acquiring infection ($p<0.05$).

Attitude, Behavior Risk Factors: In table (3), contact with cats has a significant relationship with acquired chronic infection ($p>0.05$). The other risk factors, such as blood transfusion and using gloves do not correlate with getting an infection ($p<0.05$). Some of the expected risk factors, such as eating raw or undercooked meat, drinking water, and drinking unboiled milk showed no differences because all the answers were no.

Table 1: Prevalence of toxoplasmosis in workers & butchers using ELISA IgM & IgG.

Variable	Target Group		Chi^2	*P*-value
	IgG Positive	IgG Negative		
Occupation				
Butchers	14(32.6%)	29(67.4%)	2.177	0.140
Workers	13 (20%)	52(80%)		
Age group				
20-29	0 (0.0%)	9(100%)	4.792	.0188
30-39	12(32.4%)	25(67.6%)		
40-49	13(26.5%)	36(73.5%)		
50>	2(15.4%)	11(84.6%)		
Location				
Al_Ka'akya	25(25.8%)	72(74.2%)	0.304	0.582
Al_Muasim	2(18.2%)	9(81.8%)		
Duration group				
1-10	4(25%)	12(75%)	5.587	0.232
11-20	19(22.6%)	65(77.4%)		
21-30	3(50%)	3(50%)		
30>	1(100%)	0(0.0%)		
Nationality				
Bangladesh	22(23.9%)	70(76.1%)	6.873	0.076
Egypt	5(55.6%)	4(44.4%)		
Pakistan	0(0.0%)	4(100%)		
African	0(0.0%)	3(100%)		

Table 2: The influence of socio-demographic factors

Target group	ELISA IgM		ELISA IgG	
	Positive	Negative	Positive	Negative
Workers	1(1.5%)	64(98.5%)	13(20%)	52(80%)
Butchers	0(0.0%)	43(100%)	14(32.6%)	29(67.4%)
Total	1(0.93%)	107(99.07%)	27(25%)	81(75%)

Table 3: The influence of Attitude and Behavior risk factors.

Variable	Target Group		Chi^2	*p*-value
	IgG Positive	IgG Negative		
Contact cat				
Yes	4(66.7%)	2(33.3%)	5.882	0.015
No	23(22.5%)	79(77.5%)		
Blood transfusion				
Yes	1(10%)	9(90%)	1.322	0.250
No	26(26.5%)	72(73.5%)		
Raw meat				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)		
Undercooked meat				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)		
Drinking water				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)		
Unboiled milk				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)		
Use gloves				
Yes	1(100%)	0(0.0%)	3.028	0.082
No	26(24.3%)	81(75.7%)		
Diagnosis				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)		

4. Discussion

The present study was comparable with a study in Mexico done by Alvarado-Esquivel et al., (2011). They were investigating the seropositivity of anti- *T. gondii* IgG and IgM antibodies among workers occupationally exposed to raw meat [18]. One hundred and twenty-four workers occupationally exposed to raw meat and 248 control subjects were examined. Eight (7%) of the butchers and 22 (9%) of the controls were positive for anti-*T. gondii* IgG antibodies. *T. gondii* IgM antibodies were found in five (4%) of the butchers and four (2%) of the controls. This study was also comparable with a study in Iran by Mardani et al., (2015). Their results showed that the seroprevalence of *Toxoplasma* IgG was 41.8% (56 cases) among the group of butchers in Khuzestan and 28.8% in the control group. The seroprevalence of *Toxoplasma* IgM was one case (2.2%) among butchers [19]. The prevalence of human *Toxoplasma* infection varies in different parts of the world, and has been reported with rates up to 75%. The variation of the rate of prevalence may be due to different locations and different weather.

Through the current study and other studies by Alvarado-Esquivel et al., (2011) and Mardani et al., (2015), exposure to raw meat is not related to infection with *T. gondii*. The result of our study suggests the seropositivity with *T. gondii* on workers who had contact with a cat was a statistically significant risk (p<0.015). The results indicated that contact with a cat was the main source of acquiring the infection in workers in the current study and similar to other studies done by Mardani et al., (2015). There was a study in Iraq done by Farah, (2008) that has been compared to the current study. Their study was seroprevalences of *Toxoplasma gondii* antibodies among butchers and slaughterhouse workers in Nenavah Governorate. They determined the specific toxoplasmosis antibodies for 27 serum samples had been collected from butchers and 52 sera from slaughterhouse workers in Nenavah governorate by using the latex agglutination test (LAT) and IgM enzyme immunosorbent assay test (IgM ELISA) [20]. Their result indicated that the rate of *T. gondii* specific antibodies by using latex agglutination test (LAT) in butchers was 59.3% in the slaughterhouses workers. The possible reason for the impact of age on the prevalence of *T. gondii* was that older adults accumulated exposure to different risk factors increased likelihood of the infection with *T. gondii*. In the present study, age showed no correlation with the infection. The results appeared that the main source of infection to the workers in slaughterhouses in Makkah may be in contact with the cat. In fact the actual source of infection may be due to previous handling of meat from infected animals at previous times. Some of the risks, such as blood transfusion, raw meat, drinking water, unboiled milk, working in the garden have shown no significant association with *T. gondii*.

5. Conclusions

The study focussed on *T. gondii* in slaughterhouse workers and reported the presence of chronic toxoplasmosis. The results also showed no infection was registered in different species of animals in both slaughterhouses.

- **No Conflict of Interest**

6. References

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Prevalence of Cytomegalovirus in First Trimesteric Spontaneous Abortion: A Prospective Serological and Molecular Case–control Study

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ABSTRACT

Aim: To measure the prevalence and assess the risk of cytomegalovirus (CMV) infection in women diagnosed with first-trimester spontaneous abortion. Comparison

Methods: This was a prospective case-control study; a total of 551 women were recruited from two Maternity and Children Hospitals (Makkah and Jeddah cities) in the Western province of the kingdom between March 2013 and June 2016

Serum and products of conceptions were collected prospectively from 201 women diagnosed with first-trimester spontaneous abortion. Another set of serum samples and placental tissues were collected from 350 healthy pregnant women at the time of normal vaginal delivery. CMV IgM and IgG antibodies were measured serologically by ELISA while viral DNA was detected in tissue specimens by PCR. Socio-economic data were analysed to identify potential risk factors of abortion and/or CMV infection.

Results: The overall prevalence was 88.4%, 13.6% and 10.1%, for IgG+, IgM+ and PCR+, respectively. IgG+ frequency was comparable between cases (89%) and controls (88%). Both exclusively IgM+ (7.9% vs. 0.6%) and dual IgM+/IgG+ (22.4% vs. 3.4%) antibodies were significantly more prevalent in abortion ($P < 0.01$). PCR+ was also significantly higher ($P < 0.01$) in abortion (25.4% vs. 1.4%). Women with exclusive IgM+ (OR 17.1; 95%CI [3.38 – 86.68]; $P = 0.001$) and those who were positive for all tests (OR 29.3; 95%CI [6.52–131.71]; $P < 0.001$) had a significantly increased risk for abortion.

Conclusions: The majority of the study sample of pregnant women in Saudi Arabia were previously exposed to CMV. First trimester CMV infection appears to be an independent risk for spontaneous abortion and viral spread to the intrauterine cavity was associated with the highest risk for miscarriage. More studies are required to measure the precise prevalence of CMV and its associated sequelae during pregnancy in the kingdom.

1. Introduction

Spontaneous miscarriage/abortion is a common form of early pregnancy loss [1, 2] and the World health Organization (WHO) defines it as a natural termination of a pregnancy before 20 weeks gestational age and it is believed that 10-20% of pregnancies are naturally lost during the first trimester [3]. Several risk factors associated with early pregnancy loss have been identified, among which maternal upper genital tract infection has been shown to have a major impact on the incidence of spontaneous abortions [4-6]. Cytomegalovirus (CMV) could be transmitted sexually and several studies have linked the virus with poor reproductive outcomes [7, 8]. Infection with CMV during human pregnancy is also common, usually latent, persistent and the majority of patients are asymptomatic [7, 9]. Additionally, the virus could result in spontaneous abortion as well as severe neonatal neurological manifestations following vertical transmission during pregnancy [10, 11].

Screening for CMV infection during pregnancy is mainly achieved by serological detection of IgM and IgG antibodies against the virus and the differentiation between primary and reactivation/re-infection status is established by performing IgG avidity test [12-15]. However, substantial numbers of those patients positive for both IgM and IgG antibodies will show a moderate IgG avidity and, hence, limiting the dating of infection and mandating further serial serological testing [14, 15]. Nucleic acid amplification tests (e.g. PCR) are more sensitive and specific methods that can complement/confirm the serological results [15-17].

Similar to many other countries [18, 19], screening for CMV infection is not routinely performed during clinical antenatal work-up in Saudi Arabia, except for selected cases, and therefore little is known regarding whether CMV infection could represent a risk factor for spontaneous abortion in the kingdom. The aim of this study was to measure the prevalence of IgM and IgG antibodies against CMV in serum as well as viral DNA by PCR in products of conception specimens obtained from women diagnosed with first trimesteric spontaneous miscarriage and the results were compared with those obtained from normal pregnancy following vaginal delivery. A better understanding about the role of CMV infection in the risk of spontaneous abortion could possibly lead to the development of appropriate policies regarding screening and/or treatment of CMV during pregnancy in the kingdom.

2. Subjects and methods

2.1. Ethical approval

Ethical approval was obtained from the Faculty of Applied Medical Sciences Ethics Committee (AMSEC 10-15-9-2013) and all serum and tissue samples were collected following obtaining informed written consent from all the participants.

2.2. Study design

This was a prospective case-control study; a total of 551 women were recruited from two Maternity and Children Hospitals (Makkah and Jeddah cities) in the Western province of the kingdom between March 2013 and June 2016; for whom TORCH screening was not performed during their routine clinical examination.

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Serum samples and fresh products of conception were collected from 201 pregnant women (cases) who attended the Emergency Department with complaints of vaginal bleeding or lower abdominal pain, had positive pregnancy test following natural conception and were diagnosed with spontaneous miscarriage during the first trimester (≤ 13 weeks gestation). None of the cases had a previous bad obstetric history, underwent infertility treatment or had a previous history of sexually transmitted diseases and/or pelvic inflammatory disease. Cases with pregnancy loss following accident or induced abortion were excluded. Treatment and management were performed according to the clinical background. The average estimated gestational age was calculated by subtracting the date of last menstrual period from the date of admission. The mean (SD) gestational age was 10.4 ± 2.3 weeks. The mean (SD) of serum β-human chorionic gonadotropin level was 3,751 ± 1,988 IU/L.

Since it is not ethically possible to collect placental tissue samples from first trimesteric healthy pregnant women to measure CMV by PCR, another set of serum samples and placental specimens were obtained from 350 healthy singleton pregnant women (controls) following normal vaginal delivery of a healthy new-born. All women in the control group were multiparous with livebirths, had no history of chronic diseases (e.g. diabetes, hypertension, etc.), previous abortion, sexually transmitted infections, pelvic inflammatory disease, ectopic pregnancy, uterine diseases (e.g. fibroid), bad obstetrics history or a family history of genetic diseases.

The study measured the frequency of IgM and IgG antibodies against CMV in serum together with viral DNA by TaqMan real-time PCR in all tissue specimens and the results were compared between the case and control groups. Socio-economic characteristics were also collected from all participants to measure and identify any potential associations with spontaneous miscarriage and/or CMV infection.

2.3. Enzyme linked immunosorbant assay (ELISA)

ELISA was used for the qualitative detection of IgG and IgM antibodies against CMV; all the used kits (Human Diagnostics, Germany) in the present study are IVD CE certified and from the same batch. All samples were processed in duplicate using a fully automated ELISA system (Human Diagnostics) according to the manufacturer's instructions including the internal controls provided within each kit for quality control. The sensitivity, specificity, inter and intra-assay coefficient of variation for each kit as reported by the manufacturer were 100%, 99.3%, 3.4% and 3.6% for the CMV IgG kit and, 93.75%, 99.2%, 5.6% and 7.8% for the CMV IgM kit, respectively. The threshold indices were calculated, and the cut-off value was 0.71 and 0.51 for CMV (IgG and IgM) antibodies, respectively. The samples were considered positive if the value of the sample was > (cut-off + 10% cut-off value) as recommended by the manufacturer. The validation criteria set by the manufacturer for each kit was checked and passed prior to the interpretation of the results.

2.4. Real-time TaqMan PCR for the detection of CMV in tubal samples

The extraction of DNA from the collected products of conception and placental tissue specimens was carried-out using MagMAX™ DNA Multi-Sample Kit (Thermo Fisher Scientific, Warrington, UK) according to the manufacturer's instructions and following homogenisation using a tissue raptor and sterile plastic tubes containing beads (Omni International, GA, USA). The quality and quantity of extracted DNA were measured on the BioSpec-nano (Shimadzu Corporation, Tokyo, Japan) and typically had an A260/A280 ratio of 1.7 to 1.9. Extracted DNA samples were stored at -20°C until used. Single plex TaqMan PCR was performed by using the IVD CE certified FTD Cytomegalovirus kit (Fast-track diagnostics, Junglinster, Luxembourg) on ABI® 7500 platform (Thermo Fisher Scientific) and according to the manufacturer's protocol. The detection limit of the kit was 10^3 copies/ml as reported by the manufacturer.

The internal control provided with the kit consisted of murine CMV, which was added at the lysis buffer stage with each tissue specimen during the DNA extraction process. Confirmation of proper isolation of nucleic acid was confirmed by the successful amplification of the internal control, which also assured the absence of PCR inhibitors. Furthermore, human genomic DNA was also detected by conventional PCR using primers specific for the human β-globin gene (PCO3 and PCO6) to also verify the absence of PCR inhibitors [20]. Extra in-house validation protocol was also performed for all negative samples as previously described [20].

The PCR reaction per 'well' specimens consisted of 12.5 µl mastermix, 1 µl enzyme, 1.5 µl of primers and 10 µl DNA. The amplification was performed as instructed by the manufacturer. The validation of the results was performed according to the manufacturer's guidelines and by using the provided positive and negative controls within the kit as previously described [7, 9].

2.5. Statistical analysis

Statistical analysis of the results was performed with SPSS version 16 (SPSS Inc., Chicago, USA). Categorical variables were compared by Chi square (χ^2) or Fisher's tests following cross-tabulation and crude odds ratio (COR) with 95% confidence interval (CI) were calculated. Binary regression was used to measure odds ratio (OR) and 95% CI of the potential risk factors that could be associated with CMV infection and/or spontaneous abortion. All tests performed were two-sided and significance was considered if $P < 0.05$.

3. Results

3.1. Subject characteristics

A total of 551 pregnant women were recruited from two maternity hospitals in the Western province of Saudi Arabia between March 2013 and June 2016. The participants were 201 cases (36.5%) diagnosed with first-trimester spontaneous miscarriage and 350 (63.5%) age-matched healthy ... multiparous women in the immediate period following normal vaginal delivery. For the case group, 57.2% (n = 115) of women were recruited from Makkah city and the remained were from Jeddah. The control subjects were equally distributed between both cities. Details of the numbers of recruited patients from each study centre are summarised in Table 1. Additionally, there were no significant difference (P = 0.6) in age between the case (Range 20-40 years; mean± SD 34.6 ± 5.3 years) and control (Range 20-40 years; mean ± SD 33.9 ± 5.6 years) groups.

Table 1: Details of recruitment of the 551 study participants according to study groups and study centres during the 3 years period of the study.

	Maternity Hospital-Makkah				Maternity Hospital-Jeddah			
	Year 1	Year 2	Year 3	Total	Year 1	Year 2	Year 3	Total
Abortion group (n = 201)	40	36	39	115	26	33	27	86
Control group (n = 350)	58	63	54	175	61	55	59	175
Total (n = 135)	98	99	93	290	87	88	86	261

The socio-economics characteristics of the study population are summarised in table 2. In general, all women from both groups were multiparous (≥ 1) with livebirths except for 9.9% of the case group (n = 20/201) who were primigravida. Regarding educational levels among the study participants, there was no significant difference between both groups and 6.9% (n = 38) had primary or lower education, 13.6% (n = 74) attended high school and 79.5% (n = 439) with college or higher education (Table 2).

Separating the study population according to profession showed that 22.8% (n = 126) were housewives, 14.7% (n = 81) were working in a day-care/nursery, 11.3% (n = 62) were healthcare professionals and the remaining (51.2%) were in assorted professions. The frequencies of housewives (33.8% vs. 16.6%; P = 0.0001) and working in a child day-care/nursery (22.4% vs. 10.3%; P = 0.0003) were significantly more prevalent in the case compared with control group. Based on the data of household income per month, 5.2% (n = 29) earned < 3000 Saudi riyals (SR), 18.9% (n = 104) were ≥ 3000 and < 5000 SR, 43.4% (n = 239) were ≥ 5000 and < 10000 SR and the remainder (32.5%) were ≥ 10000 SR (Table 2).

Additionally, the rates of having a child enrolled in a day-care/nursery was significantly (P = 0.01) more frequent in the case group (43.3%) compared with control (31.7%) and was associated with a significantly 1.5 times higher risk of having abortion (COR 1.65; 95% CI: 1.13–2.4; P = 0.01). Similarly, 28.4% of the case group were resident in a rural area and this prevalence was significantly higher ($P = 0.01 \times 10^{-5}$) than the control group (13.2%) and was associated with six times greater risk of developing abortion (COR

6.77; 95% CI: 3.73–12.29; P = 0.01 X 10^{-5}). Not sure about p values some further in text 10^{-17}?

Table 2: Maternal Socio-economic characteristics and their distribution in the study control and case groups.

Maternal Characteristics		Control Group % (n = 350)	Abortion group % (n = 201)	P Value	Total % (n = 551)
Age groups (years)	*20 – 25 years*	16.6% (58)	16.4% (33)	0.6	16.5 (91)
	25 – 30 years	28.3% (99)	29.8% (60)	0.8	28.8 (159)
	30 – 35 years	28.6% (100)	24.9% (50)	0.4	27.2 (150)
	> 35	26.6% (93)	28.8% (58)	0.6	27.4 (151)
Parity	*≤ 1*	32% (112)	33.3% (67)	0.3	32.5 (179)
	2	29.1% (102)	34.3% (69)	0.2	31 (171)
	≥ 3	38.8% (136)	32.3% (65)	0.1	36.5 (201)
Educational level	*Primary or below*	6% (21)	8.4% (17)	0.4	6.9 (38)
	High school	8.3% (29)	22.4% (45)	0.06	13.6 (74)
	College and above	81.1% (284)	69.2% (155)	0.03	79.5 (439)
Monthly Household income	*≤ 3000 SR*	4.8% (17)	5.8% (12)	0.2	5.2 (29)
	> 3000 & ≤ 5000 SR	17.1% (60)	21.9% (44)	0.2	18.9 (104)
	> 5000 & ≤ 10000 SR	44.3% (155)	41.8% (84)	0.05	43.4 (239)
	> 10000 SR	33.7% (118)	30.3% (61)	0.001	32.5 (179)
Profession	*Housewife*	16.6% (58)	33.8% (68)	0.0001	22.8 (126)
	Child day-care/nursery	10.3% (36)	22.4% (45)	0.0003	14.7 (81)
	Healthcare	10.8% (38)	11.9% (24)	0.1	11.3 (62)
	Other	62.3% (218)	31.8% (64)	0.01 X 10^{-12}	51.2 282)
Having a child in a day-care/nursery	*No*	68.3% (239)	56.7% (114)	0.01	64.1 (353)
	Yes	31.7% (111)	43.3% (87)		35.9 (198)
Residency	*Urban*	86.8% (304)	71.6% (144)	0.01 X 10^{-5}	81.3 (448)
	Rural	13.2% (46)	28.4% (57)		18.7 (103)

3.2. Seroprevalence of CMV IgM and IgG antibodies

The overall seroprevalence of IgG and IgM antibodies against CMV in the study participants were 88.4% (cases) and 13.6% (controls), respectively. The frequency of exclusively positive cases for IgM antibodies was 3.2% while the rate of concurrently positive cases for both antibodies was 10.3% (Table 3). None of the tested samples showed equivocal reaction against IgG antibodies but 15 women (2.7%) had equivocal results against CMV IgM antibodies.

By further analysis, there was no significant difference (P = 0.7) in the total rates of detecting CMV IgG antibodies between the control (88%) and case (89.1%) groups. However, a significant increase (P = 0.06 X 10^{-18}) in the overall frequency of IgM against CMV was detected in the case (30.3%) compared with control (4%) and the detection of CMV IgM was associated with a significantly greater risk of having early spontaneous abortion (COR 10.4; 95% CI: 5.66–19.31; P = 0.03 X 10^{-16}). Additionally, the prevalence of dual seropositivity for both IgM and IgG was significantly higher (P = 0.01 X 10^{-10}) in the case group (22.4%) compared with controls (3.4%) and was associated with a significantly increased risk of developing miscarriage (COR 8.1; 95% CI: 4.18–15.79; P = 0.06 X 10^{-10}). Similarly, the sole detection of IgM antibodies was significantly more prevalent (P = 0.02 X 10^{-11}) in the case (8%) compared with controls (0.6%) and was associated with a significantly higher risk of abortion (COR 15.04; 95% CI: 3.42–66.15; P = 0.0004). The age-adjusted odds ratio with 95% CI for the detection of CMV infection by the different methods are summarised in table 3.

Table 3: Distribution of CMV serological and PCR results in the study groups with age adjusted odds ratio (A-OR) and 95% confidence intervals (95% CI) for developing spontaneous abortion.

CMV Serology		N (%)	Control (%)	Abortion (%)	A-OR [95% CI]	P value
Overall IgG positive	*No*	64 (11.6)	42 (12)	22 (10.9)	1.09 [0.63 – 1.89]	0.7
	Yes	487 (88.4)	308 (88)	179 (89.1)		
	Total N	551 (100)	350	201		
Overall IgM positive	*No*	461 (83.7)	332 (94.9)	129 (64.2)	6.82 [4.12 – 11.28]	0.7 X 10^{-13}
	Equi-vocal	15 (2.7)	4 (1.1)	11 (5.5)		
	Yes	75 (13.6)	14 (4)	61 (30.3)*		
	Total N	551 (100)	350 (100)	201 (100)		
Overall PCR positive	*No*	495 (89.8)	345 (98.6)	150 (74.6)	24.24 [9.45 – 62.16]	0.3 X 10^{-10}
	Yes	56 (10.2)	5 (1.4)	51 (25.4)*		
	Total N	551 (100)	350 (100)	201 (100)		
Sole IgG positive	*No*	127 (23.1)	51 (14.6)	76 (37.8)	0.27 [0.17 – 0.41]	0.8 X 10^{-9}
	Yes	424 (76.9)	299 (85.4)	125 (61.2)*		
	Total N	551 (100)	350 (100)	201 (100)		
Sole IgM positive	*No*	533 (96.7)	348 (99.4)	185 (92)	16.03 [3.62 – 70.99]	0.02 X 10^{-11}
	Yes	18 (3.3)	2 (0.6)	16 (8)*		
	Total N	551 (100)	350 (100)	201 (100)		
Dual IgM & IgG positive	*No*	494 (89.7)	338 (96.6)	156 (77.6)	8.29 [4.25 – 16.17]	0.5 X 10^{-9}
	Yes	57 (10.3)	12 (3.4)	45 (22.4)*		
	Total N	551 (100)	350 (100)	201 (100)		
PCR & sole IgM positive	*No*	537 (97.5)	349 (99.7)	188 (93.5)	25.42 [3.28 – 197.12]	0.0002
	Yes	14 (2.5)	1 (0.3)	13 (6.5)*		
	Total N	551 (100)	350 (100)	201 (100)		
PCR with dual IgM & IgG positive	*No*	517 (93.8)	348 (99.4)	169 (84.1)	34.1 [8.05 – 144.38]	0.1 X 10^{-5}
	Yes	34 (6.2)	2 (0.6)	32 (15.9)*		
	Total N	551 (100)	350 (100)	201 (100)		

* = P < 0.01 compared with control by Chi-square analysis.

3.3. Rates of CMV infection by PCR in tissues collected from the study groups

All standards that are included within the analysis kit as positive control measures showed an overall positive signal with the detection of human *β-globin* gene in all extracted samples (data not shown). Additionally, there was no signal detected in the negative control wells, reassuring the observed results of this study.

In general, CMV DNA was detected in 10.2% of tissue samples obtained from the 551 women of both study groups (Table 3). There were 51 (25.4%) positive samples from the products of conception and the rate was significantly higher (P = 0.03 x10^{-17}) than those detected in placental tissues collected from controls (1.4%). The detection of CMV by PCR in the products of conception was also associated with a 24 fold greater risk of developing spontaneous abortion (Table 3).

Among those cases with positive IgM serology, 64% were also positive by PCR. Interestingly, 8 cases (53.3%) of those participants with equivocal IgM results (n = 15) were also positive with PCR. Additionally, 14 subjects (77.8%) from both study groups were exclusively positive for IgM antibodies and also had positive PCR results for CMV in the collected tissue specimens. The overall rate of detecting both CMV antibodies (IgG & IgM) and PCR was 6.2%. None of the women with sole positive IgG antibodies was positive by PCR in both study groups. The distribution of CMV PCR findings according to study groups, serological results and maternal socio-economic characteristics is summarised in Table 4.

Table 4: Distribution of PCR CMV positive tissue samples according to study groups, serological results and maternal socio-economic characteristics.

			N	PCR with sole positive CMV IgM	PCR with dual positive CMV IgG & IgM
				N positive cases (%)	N positive cases (%)
Age groups (years)	> 20 & ≤ 25	C G	58	ND (0)	ND (0)
		A G	33	6 (18.2)[b]	5 (15.1)[b]
	> 25 & ≤ 30	C G	99	ND (0)	1 (1)
		A G	60	3 (5)	14 (23.3)[b,**]
	> 30 & ≤ 35	C G	100	1 (1)	1 (1)
		A G	50	2 (4)	8 (16)[b]
	> 35	C G	93	ND (0)	ND (0)
		A G	58	2 (3.4)	5 (8.6)[b]
Parity	≤ 1	C G	112	ND (0)	1 (0.9)
		A G	67	5 (7.4)[b]	5 (7.4)[a]
	2	C G	102	ND (0%)	ND (0)
		A G	69	4 (5.8)[a]	11 (15.9)[b,**]
	≥ 3	C G	136	1 (0.75)	1 (0.75)
		A G	65	4 (6.1)[a]	16 (24.6)[b,**]
Educational level	Primary or below	C G	21	1 (4.7)	1 (12.5)
		A G	17	4 (23.5)	10 (42.8)
	High school	C G	45	ND (0)	1 (3.8)
		A G	29	9 (31)[b]	12 (44)[b]
	College and above	C G	284	ND (0)	ND (0)
		A G	155	ND (0)	10 (10.2)[b,*]
Monthly Household income	≤ 3000 SR	C G	17	1 (5.9)	1 (5.9)
		A G	12	4 (33.3)[a]	5 (41.6)[a]
	> 3000 & ≤ 5000SR	C G	60	ND (0)	1 (1.6)
		A G	44	8 (18.2)[b]	13 (29.5)[b,*]
	> 5000 & ≤ 10000 SR	C G	155	ND (0)	ND (0)
		A G	84	1 (1.2)	10 (11.9)[b,**]
	> 10000 SR	C G	118	ND (0)	ND (0)
		A G	61	ND (0)	4 (6.5)[b,*]
Profession	Housewife	C G	58	ND (0)	2 (3.4)
		A G	68	5 (7.3)[b]	17 (25)[b,**]
	Child day-care/nursery	C G	36	1 (2.7)	ND (0)
		A G	45	8 (17.8)[b]	8 (17.8)[b]
	Healthcare	C G	38	ND (0)	ND (0)
		A G	24	ND (0)	3 (12.5)[a]
	Other	C G	218	ND (0)	ND (0)
		A G	64	ND (0)	4 (6.2)[b,*]
Having a child in a daycare / nursery	No	C G	239	ND (0)	ND (0)
		A G	114	3 (2.6)[b]	9 (7.9)[b,**]
	Yes	C G	111	1 (0.9)	2 (1.8)
		A G	87	10 (11.5)[b]	23 (26.4)[b,**]
Residency	Urban	C G	304	ND (0)	ND (0)
		A G	144	7 (4.6)[b]	13 (9)[b,**]
	Rural	C G	46	1 (2.2)	2 (4.4)
		A G	57	6 (10.5)[b]	19 (33.3)[b,**]

C G: Control Group A G: Abortion Group

(N/A = not applicable, ND = not detected, a = P < 0.05 compared with control, b = P < 0.01 compared with control, * = P < 0.05 compared with IgM only positive cases and ** = P < 0.01 compared with IgM only positive cases).

3.4. Factors associated with spontaneous abortion and/or CMV genital tract infection among the study population

Logistic regression model that included the socio-economic characteristics, CMV serology and PCR results showed that the odds ratio of developing spontaneous abortion was significantly higher in housewives, above 35 years of age, ≤ 1 parity, primary or lower education level and living in a rural area (Table 5). Additionally, the exclusive detection of IgM antibodies against CMV with or without positive PCR significantly increased the risk of spontaneous abortion by more than 15 folds (Table 5). The highest significant odds ratio was observed for those women with positive IgM, IgG and PCR results and was associated with a 27 fold increase in the risk of spontaneous abortion. Interestingly, the sole detection of CMV IgG antibodies significantly decreased the risk of abortion by 3 fold (Table 5).

Table 5: Maternal socio-economic characteristics, CMV serology and PCR results and odds ratio (OR) with 95% confidence intervals [95% CI] for developing spontaneous abortion.

	Variable Categories	N	Control (%)	Abortion (%)	Logistic Regression	
					OR [95% CI]	P value
Age groups (years)	20 - 25 years	91	58 (63.8)	33 (36.2)	Ref.	-
	26 - 30 years	159	99 (62.7)	60 (37.3)	1.23 [0.63 – 2.4]	0.5
	31 - 35 years	150	100 (66.7)	50 (33.3)	1.17 [0.57 – 2.41]	0.6
	> 35 years	151	93 (61.6)	58 (38.4)	2.64 [1.18 – 5.91]	0.01
Parity	≤ 1	179	112 (62.6)	67 (37.4)	Ref.	-
	2	171	102 (61.5)	69 (38.5)	1.01 [0.58 – 1.76]	0.9
	≥ 3	201	136 (67.7)	65 (32.3)	0.40 [0.20 – 0.78]	0.007
Educational level	Primary or below	38	21 (56.3)	17 (44.7)	Ref.	-
	High school	74	45 (60.8)	29 (39.2)	0.10 [0.039 – 0.28]	0.0001
	College and above	439	284 (64.7)	155 (35.3)	0.046 [0.010 – 0.22]	0.0001
Monthly Household income	≤ 3000 SR	29	17 (58.6)	12 (41.4)	Ref.	-
	> 3000 & ≤ 5000SR	104	60 (57.7)	44 (42.3)	0.90 [0.56 – 1.45]	0.7
	> 5000 & ≤ 10000 SR	239	155 (64.9)	84 (35.1)	0.31 [0.14 – 1.62]	0. 1
	> 10000 SR	179	118 (65.9)	61 (34.1)	0.43 [0.22 – 0.86]	0.01
Profession	Housewife	126	58 (46.9)	68 (53.1)	Ref.	-
	Child day-care/nursery	81	36 (44.5)	45 (55.5)	0.48 [0.23 – 0.99]	0.04
	Healthcare	62	38 (62.5)	24 (37.5)	0.31 [0.14 – 0.67]	0.003
	Other	282	218 (77.3)	64 (22.7)	0.15 [0.08 – 0.29]	0.2 X10^{-8}
Having a child in a day-care/nursery	No	353	239 (67.7)	114 (32.3)	Ref.	-
	Yes	198	111 (56.1)	87 (43.9)	1.29 [0.82 – 2.02]	0.2
Residency	Urban	448	304 (67.9)	144 (32.1)	Ref.	-
	Rural	103	46 (44.7)	57 (55.3)	1.73 [0.92 – 3.26]	0.08
Sole IgG positive	No	127	51 (40.2)	76 (59.8)	Ref.	-
	Yes	424	299 (70.5)	125 (29.5)	0.27 [0.16 – 0.46]	0.1 X10^{-4}
Sole IgM positive	No	533	348 (65.3)	185 (34.7)	Ref.	-
	Yes	18	2 (21.1)	16 (88.9)	17.1 [3.38 – 86.68]	0.001
Dual IgM & IgG positive	No	494	338 (68.4)	156 (31.6)	Ref.	-
	Yes	57	12 (21.6)	45 (78.4)	8.39 [3.81 – 18.43]	0.1 X10^{-6}
PCR & sole IgM positive	No	537	349 (65)	188 (35)	Ref.	-
	Yes	14	1 (7.2)	13 (92.8)	27.99 [3.17 – 246.52]	0.003
PCR with IgM & IgG positive	No	517	348 (67.3)	169 (32.7)	Ref.	-
	Yes	34	2 (5.9)	32 (94.1)	29.3 [6.52 – 131.71]	0.1 X10^{-5}

Ref. = indicates reference group to which the other group(s) were compared

Furthermore, regression analysis also revealed that age < 30 years, multiparous (≥ 2), primary or lower education, resident of rural areas and working or having a child in a nursery significantly increased the

risk of CMV reproductive tract infection that can be detected by PCR (Table 6).

Table 6: Maternal socio-economic characteristics and adjusted odds ratio (AOR) with 95% confidence intervals (95% CI) for reproductive tract infection with CMV as indicated by PCR results.

	Variable Categories	N	Reproductive tract CMV infection		Logistic Regression	
			Yes (%)	No (%)	AOR [95% CI]	P value
Age groups (years)	20 – 25 years	91	12 (13.2)	79 (86.8)	Ref.	-
	25 – 30 years	159	20 (12.6)	139 (87.4)	0.61 [0.18 – 1.99]	0.4
	30 – 35 years	150	14 (9.3)	136 (90.7)	0.29 [0.075 – 1.2]	0.08
	> 35 years	151	9 (5.9)	142 (94.1)	0.10 [0.02 – 0.47]	0.004
Parity	≤ 1	179	11 (6.1)	168 (93.9)	Ref.	-
	2	171	18 (10.5)	153 (89.5)	2.76 [1.1 – 8.48]	0.03
	≥ 3	201	26 (12.9)	175 (87.1)	8.25 [2.31 – 29.44]	0.001
Educational level	Primary or below	38	16 (42.1)	22 (57.9)	Ref.	-
	High school	74	22 (29.7)	52 (70.3)	0.78 [0.24 – 2.51]	0.6
	College and above	437	17 (3.9)	422 (96.1)	0.27 [0.07 – 0.86]	0.03
Monthly Household income	≤ 3000 SR	29	11 (37.9)	18 (62.1)	Ref.	-
	> 3000 & ≤ 5000SR	104	22 (21.1)	82 (78.9)	0.69 [0.18 – 2.57]	0.6
	> 5000 & ≤ 10000 SR	239	15 (6.3)	224 (93.7)	0.41 [0.10 – 1.59]	0.2
	> 10000 SR	179	7 (3.9)	172 (96.1)	0.31 [0.08 – 0.78]	0.02
Profession	Housewife	126	25 (19.8)	101 (80.2)	Ref.	-
	Child day-care/nursery	81	21 (25.9)	60 (74.1)	1.7 (0.80 – 4.77)	0.03
	Healthcare	62	4 (6.4)	58 (93.6)	0.61 (0.15 – 2.5)	0.4
	Other	282	5 (1.8)	277 (98.2)	0.14 (0.04 – 0.50)	0.002
Having a child in a day-care/nursery	No	353	15 (4.2)	338 (95.8)	Ref.	-
	Yes	198	40 (20.2)	158 (79.8)	5.13 (2.30 – 11.43)	0.6 $X10^{-5}$
Residency	Urban	448	21 (4.7)	427 (95.3)	Ref.	-
	Rural	103	34 (32.7)	69 (67.3)	5.52 (2.41 – 12.64)	0.5 $X10^{-5}$

Ref. = indicates reference group to which the other group(s) were compared

4. Discussion

Herein, the prevalence of CMV was measured by serology and PCR in samples obtained from women diagnosed with first trimesteric spontaneous abortion and the results were compared with those samples collected at the time of normal vaginal delivery from age-matched healthy post-delivery women. The results showed a high seroprevalence rate of IgG antibodies in the study case population with no significant difference in the frequency between both study groups. However, the seroprevalence of CMV IgM antibodies was significantly higher, with or without co-detection of IgG antibodies, in the spontaneous abortion group. Furthermore, the viral DNA was more frequently detected by PCR in the products of conception from the case group compared with those placental specimens obtained from controls. These observations are in parallel with the findings of several previous studies that showed high rates of CMV immunity among their populations and support the notion that this viral infection is more commonly detected in women with early pregnancy loss [21-26].

Additionally, this is the first study to report that, among several factors, CMV infection during early pregnancy independently and significantly increased the risk of developing spontaneous abortion in Saudi Arabia. The present study also showed that the detection of CMV IgM exclusively or together with IgG antibodies significantly increased the risk of abortion by 17 and 8 fold, respectively. These observations suggest that acute CMV infection in early pregnancy, as indicated by sole IgM positivity, could have a worse pregnancy outcome compared with viral re-activation/re-infection in early pregnant women. Interestingly, the detection of CMV in tissues by PCR was associated with 28- and 29-fold increase in spontaneous abortion when simultaneously positive with either sole IgM or both IgM and IgG antibodies, respectively. The current findings, therefore, suggest that intrauterine CMV presence during early pregnancy, either in the form of acute infection or viral re-activation/re-infection/persistence, could be a major risk factor for early pregnancy loss in the kingdom.

CMV has been estimated to infect 0.15–2% of pregnant women and the virus can later be transmitted in utero leading to several pre- and postnatal complications [6, 18]. This viral infection is usually asymptomatic, latent and the rates increase dramatically during pregnancy, mainly due to the associated suppression of maternal immune system [27]. The acquisition of CMV during pregnancy could occur either following primary exposure, viral reactivation or reinfection with a different viral strain [28]. Additionally, the rates of recurrent CMV is more common than primary infection during pregnancy [29], suggesting that a prior exposure to the virus does not provide full immunity against intrauterine transmission [21].

The present findings correlate and provide further support to the aforementioned observations since they demonstrated an overall seroprevalence of 88.4% for IgG antibodies and 13.6% for IgM antibodies. Currently, little is known about the frequency of CMV during pregnancy in Saudi Arabia and only a single study has reported a seroprevalence of 92.1% for CMV IgG antibodies in 926 first trimester healthy pregnant women, which is in agreement with the current findings [30]. Furthermore, similar seroprevalence of CMV has also been reported in pregnant women from several other regional [21, 23] and international countries [24-26]. The current observations advocate that the majority of women of childbearing age in the Western province of Saudi Arabia were previously exposed to CMV infection and have acquired natural immunity against the virus prior to pregnancy. Therefore, these women could, theoretically, be at higher risk of acquiring CMV infection during subsequent pregnancies following viral reactivation and/or re-infection with a different strain [28, 31]. This hypothesis could further be supported by the observed significant higher rate of simultaneously positive IgM and IgG antibodies (10.3%) than the sole detection of IgM against the virus (3.2%) in our study population.

Nevertheless, the results of IgM should be cautiously interpreted since the majority of available IgM serological assays are associated with high false-positive rate [12, 13]. Additionally, IgM antibodies could persist for months following a primary exposure to the virus and/or could be produced against other viruses such Epstein-Barr virus [15]. Hence, serological results cannot accurately differentiate between primary and reinfection/reactivation of CMV infection [12-15]. The IgG avidity test is an alternate method that is currently applied for more precise diagnosis of primary infection serologically in clinical settings. Nevertheless, the performance of this diagnostic assay is limited due to the high rate of intermediate avidity reaction and lack of appropriate cut-off for low avidity [14, 15]. Therefore, the present study used an IVD CE singleplex PCR kit to confirm the serological findings since the detection of CMV DNA by PCR is another feasible approach for the diagnosis of current infection and is associated with a much higher specificity ranging between 97-100% and positive predictive value of almost 100% [15, 17].

The results showed an overall prevalence of intrauterine CMV infection by PCR in 10.2% of the study participants and the frequency was significantly higher in the spontaneous abortion group. The present findings also revealed that, 77.8% of tissue specimens obtained from those patients who were exclusively positive for IgM antibodies were also positive by PCR, suggesting a primary CMV infection that could have had spread to the intrauterine cavity. Interestingly, the viral DNA was also detected by PCR in more than 50% of those cases with equivocal reaction for CMV IgM antibodies, supporting the notion that PCR is a more sensitive and specific tool for the diagnosis of CMV infection than serological tests [15, 17]. Additionally, 59.6% of those patients with dual IgM & IgG positive serology were also positive by PCR, advocating possible reactivation, reinfection or persistent intrauterine viral infection.

Although the majority of the previous studies focused mainly on the neonatal neurological manifestations following in utero CMV infection, the sequelae of CMV infection during pregnancy is far more complex and could result in other complications including pregnancy

loss [13]. CMV-induced pregnancy adverse outcomes are trimester-dependent and the earlier the infection the more severe the complications [27]. In this regards, the acquisition of CMV during peri-conceptional or early weeks of pregnancy has been associated with miscarriage [29]. Spano et al. have also shown that 75% of archived placental tissues collected from first trimesteric abortion were positive for CMV by immunofluorescence [32]. Another study from Japan has also demonstrated a rate of 7.1% of CMV infection by using Real-Time PCR and vaginal swab samples obtained from 848 healthy pregnant women and CMV infection was associated with 7 folds greater relative risk for spontaneous abortion [10]. A later study has also shown significantly greater prevalence of CMV DNA by PCR in placental tissues obtained from 62 cases of foetal deaths compared with 35 controls [33]. Similar results have also been reported by more recent serological studies in which the rates of CMV IgM seropositivity were significantly higher than controls and increased the risk of abortion [11, 34].

The findings of the present study correlate with those previously reported from a variety of populations since they showed significantly higher rates of CMV DNA and IgM antibodies in the case group compared with control. Additionally, these observations provide further support to the notion that, CMV infection during pregnancy is associated with higher risks of spontaneous abortion as shown by the results of logistic regression. The mechanisms by which CMV is disseminated into the intrauterine cavity are still largely illusive [22, 29]. However, it is believed that in utero CMV infection is initiated in the endometrium before the foetus and could result in impaired placentation and subsequently abortion and/or foetal death [29]. In this context, CMV induced villitis in infected placental tissues in vivo [35, 36] and inhibited the invasion activities of cytotrophoblasts in vitro, together with increased trophoblastic cell damage lead to the production of inflammatory cytokines [22, 37-39]. Hence, it could be postulated that, intrauterine dissemination of CMV during the peri-conceptional time and/or early weeks of pregnancy increase the risk of spontaneous abortion by inducing trophoblastic damage and compromising the process of placentation. However, further studies are needed to explore the effects of CMV infection on placental tissue integrity and invasiveness during early pregnancy.

A variety of risk factors associated with the acquisition of CMV infection during pregnancy have been identified and they include lower income [15], low level of education [28], maternal age [40], increased parity [41] and close contact with young children [40], which are in agreement with the findings of the present study. Therefore, those women at high risk could benefit from a screening program to avoid/prevent CMV associated morbidity and mortality during pregnancy. Nevertheless, the implementation of CMV screening programs is debatable and not recommended in many developed and undeveloped countries [18, 19]. Hence, large prospective cross-sectional multi-centres studies are still required to investigate the precise CMV infection rates among pregnant women and neonates in Saudi Arabia. Such research could provide strong evidence on the magnitude of this viral infection and its related adverse events during pregnancy. In the meanwhile, the introduction of preventive programs (e.g. counselling-based interventions) for those women at high risk of acquiring CMV during their pregnancy could result in lowering the rates of CMV-induced pre- and postnatal sequelae [1, 5, 14, 21, 42].

5. Conclusion

The majority of pregnant women in the Western province of Saudi Arabia were previously exposed to CMV and could be at risk of viral reactivation during subsequent pregnancies. CMV infection during the first trimester with the possibility of viral dissemination into the intrauterine cavity, also appears to be an independent risk for the development of spontaneous abortion. The initiation of preventive programs during antenatal consultations, especially for those women at high risk, could be beneficial in controlling the associated adverse events during pregnancy. Further studies are needed to measure the actual prevalence of CMV and its associated sequelae during pregnancy in the different regions of the kingdom.

- ***Conflict of Interest***

The authors declare that they have no competing interests

- ***Ethics approval***

Ethical approval was agreed upon by the Faculty of Applied Medical Sciences Ethics Committee (AMSEC 10-15-9-2013). All procedures performed in studies involving human participants were in accordance with the ethical standards of Umm Al-Qura University research committee and with the 1964 Helsinki declaration and its later amendments.

- ***Informed consent***

Informed consent was also obtained from all participants included in the study.

- ***Availability of data and materials***

All data generated or analysed during this study are included in this published article.

- ***Acknowledgment***

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The Role of CYP 450 Isozymes in Drug-Drug Interaction

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ABSTRACT

The main focus of the pharmacokinetic process is hepatic metabolism, which is responsible for biotransformation and elimination of xenobiotics such as drugs. This step depends primarily on the CYP450 system, which plays a role in drug metabolism and changes their therapeutic responses. CYP450 enzyme system includes many notable points such as major families, their functions and also the factors that can alter their activity and cause significant interaction. According to previous studies, the most important factor affecting this enzyme system is the medications that can enhance or inhibit its activity.

The aim of this article is to discuss the detailed mechanism of the interaction between CYP450 enzyme and other drugs. This mechanism is very important when prescribing various medications to avoid the problem of drug-drug interaction**.**

We must remember that many drugs undergo metabolism through more than just isozyme interactions or may pass through dual metabolism pathways. Examples of such drugs are anticonvulsants, antidepressant, anti-infectives, immunosuppressant, and cardiovascular drugs.

1. Introduction

The adverse reaction of drug-drug interaction in the therapeutic system is of utmost importance. This can be a result of the change in metabolic enzymes.

Drugs pass through many processes to enhance their excretion from the body. One of these processes is metabolism which is done by several types of reaction such as reduction, oxidation, hydration, hydrolysis, isomerization, condensation, or conjugation [6]. While the rate of metabolism is affected by many factors such as chronic liver disease, genetic polymorphisms, or drug interactions [7], the metabolic processes result in the generation of inactive metabolites, or sometimes produce an active metabolite called a pro-drug, and of course, enzymes play a major role in this mechanism [8].

These enzymes are concentrated in the liver more than other tissues (extra-hepatic tissues) and are called the hepatic cytochrome P450 system. This system includes a group of enzymes that divide into families and subfamilies [9].

The enzyme system controls the main pharmacokinetic interactions of various medications. But some of the specific drugs can act as enzyme inhibitors or inducers when co-administrating with other drugs [10].

So the conception of this mechanism between enzyme and drug is very important when prescribing various medications. Finally, more research is needed to detect the alteration in individual enzymes to avoid or reduce the incidence of drug interactions and therefore decrease adverse effects [11].

2. Purpose

The main purpose of this research is to enhance our knowledge about the common role of CYP 450 isozymes in drug interactions.

3. Method

This review depends on research in reliable and popular medical databases which are PubMed, Saudi Digital Library, Google Scholar and Web of Science (ISI). The terms used to search the published research are isozymes, cytochrome P450 system, liver enzyme, drug metabolism, drug-drug interactions, enzyme inhibitors, and an enzyme inducer. The studies identified were published in 51 references which are distributed between the years 2008 to 2017.

3.1. Cytochrome P450 system

The P450 cytochrome system is controlled via the human genome 57 [12], which regulates more than twelve enzymes in three families using a coding system consisting of numbers and letters related to the system [13].

We can divide their functions into three portions. The first participates in the biosynthesis of signaling molecules and regulatory factors or sterols. The second is responsible for the metabolism and detoxify of the xenobiotics. But the role of the last is still unclear [14].

By focusing more, on the most important function of the system, which is biotransformation, that affects 70-80% of drugs. Where the drug undergoes the first-pass metabolism before entering to plasma this causes variation of the individual pharmacokinetics between people.

This function also helps the clearance of other products like tobacco, alcohol, and methamphetamine [15].

Generally, the CYP450 system involves several enzymes regulated in CYP1, 2, and 3 groups, each of which plays a role in metabolism for roughly two-thirds of discovered drugs. Usually metabolism results in the release of reactive oxygen species called prodrug. Toxic metabolites are also sometimes released [16]. Among them, CYP1A2, 3A4, 2C8, 2C9 and 2E1 which are present in high concentrations in the liver whereas CYP2A6, 3A5, 2B6, 2C19, and 2D6 exist in low concentration, but CYP1A1, 1B1, 2J2,2S1 and 2W1 are extrahepatic enzymes that appear in certain tissues like hypoxic or tumor tissues [17]. Factors that can alter the activity of enzymes must be taken into consideration e.g. gene polymorphisms for CYP450 which effect the action of CYPs 2A6, 3A5, 2B6,2C9, 2C19 and 2D6 [18]. The other notable factors are received xenobiotics like specific medication or food, disease condition, age, sex, hormones and cytokines regulation [16].

More precisely, these factors may enhance the activity of enzymes (called hyper or extensive) and change their reactions resulting in

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reduced bioavailability and plasma concentration and so reduced efficacy of the drugs.

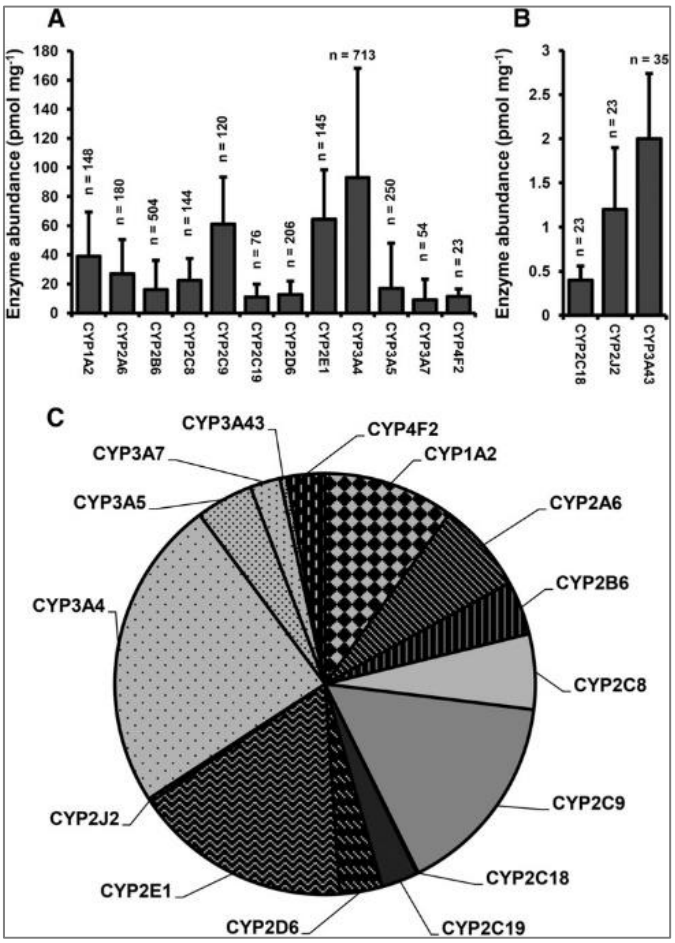

Fig 1: Types of cytochrome P450 enzymes [5]

But the positive thing is decreased toxicity and reduced access to the brain. On the other hand, they may reduce the activity of individual enzymes and cause the opposite action to the above [19].

Table 1: Specific cytochrome P450 enzymes and drug substrates with its inhibitors and inducer [3]

CYP450 Enzyme	Common drug substrates	Inhibitors	Inducers
CYP1A2	Clozapine, Estrogen, Clomipramine, Fluvoxamine, Haloperidol, Theophylline, Tacrine	Amiodarone, cimetidine, fluoroquinolones, ticlopidine	Olycyclic aromatic hydrocarbon, Omeprazole, Ritonavir, Phenobarbital
CYP3A	Diltiazem, Nifedipine, Felodipine, Verapamil, Tacrolimus, Sildenafil, Cyclosporin, Alprazolam, Triazolam, Midazolam, Ritonavir, Atorvastatin, Clarithromycin, Losartan, Erythromycin, Indinavir.	Troleandomycin, Itraconazole, Verapamil, Ketoconazole, Mifepristone, Clarithromycin, Nefazodone, Erythromycin, Diltiazem, Delavirdine, Indinavir, Ritonavir, Saquinavir, Grapefruit juice.	Rifapentine, Rifampin, Rifabutin, St John's Wort, Carbamazepine, Phenytoin, Phenobarbital, Nevirapine, Efavirenz, Topiramate.
CYP2C9	Phenytoin, Tolbutamide, Warfarin, Glipizide.	Amiodarone, Miconazole, Fluconazole, Phenylbutazone, Sulphinpyrazone.	Phenobarbital, Rifampin, Carbamazepine, St John's Wort.
CYP2E1	Paracetamol, Ethanol, Benzene, Theophylline, Isoflurane.	Diethyl-dithiocarbamate, Disulfiram.	Isoniazid, Ethanol.
CYP2D6	Alprenolol, Carvedilol, Bufuralol, Metoprolol, Timolol, Propranolol, Amitriptyline, Desipramine, Clomipramine, Imipramine, Flecainide, Nortriptyline, Mexiletine, Fluoxetine, Propafenone, Haloperidol, Perphenazine, Paroxetine, Codeine, Venlafaxine, Dextromethorphan.	Clomipramine, Fluoxetine, Quinidine, Paroxetine, Haloperidol.	Not inducible.

3.2. Main Mechanism of Cytochrome P450 system

Cytochromes P450, which was discovered in 1958, is a family of cysteine thiolate-ligated heme that containing monooxygenase enzyme [20]. It catalyzes the movement of the oxygen atom from the oxygen molecule creating broad species of substrates as part of the oxidation reaction. It also reduces the second O_2 by 2 electrons to create water molecules that causes an interaction of NADPH [21]. The reaction can be explained in this simple equation:

(1) RH + (1) NAD(P)H + (1) O_2 + H^+ → (1) ROH + (1) $NAD(P)^+$ + (1) H_2O (2)

Now, we focus on the mechanism of these enzymes in metabolism. First, they can add O_2 to N-H or C-H bond to produce hydroxyl derivative and the usual terminal product is alcohol (22), but the hydroxylation in C-H bond that is attached to the heteroatom produces fragments which are carbonyl groups with others like hydroxyl, thiol or amine groups, and this is the major method of P450 in catalyzing prodrug generation [23].

Secondly, the enzymes can accelerate the epoxidation of olefinic bonds and also aromatic π-bonds, and finally, they can bond O_2 with sulfur or nitrogen atoms (in electron pair) to produce S-oxide or N-oxide molecules [24].

In summary, the most oxidation reactions are hydroxylation, heteroatom oxidation, epoxidation, and heteroatom dealkylation [25].

4. Significant drug interactions affected by CYP system

The widely known major therapeutic metabolic enzyme is CYP3A4. In addition to its known actions, it can metabolism various narcotics like cocaine and methamphetamine, whilst the metabolic enzymes for nicotine, alcohol, and polyaromatic-hydrocarbon are CYP2A6, CYP2E1, and CYP1A1/1B1 [26].

We must remember that several drugs undergo metabolizism through more than just one isozyme, or in other words they pass through dual metabolism pathways [16].

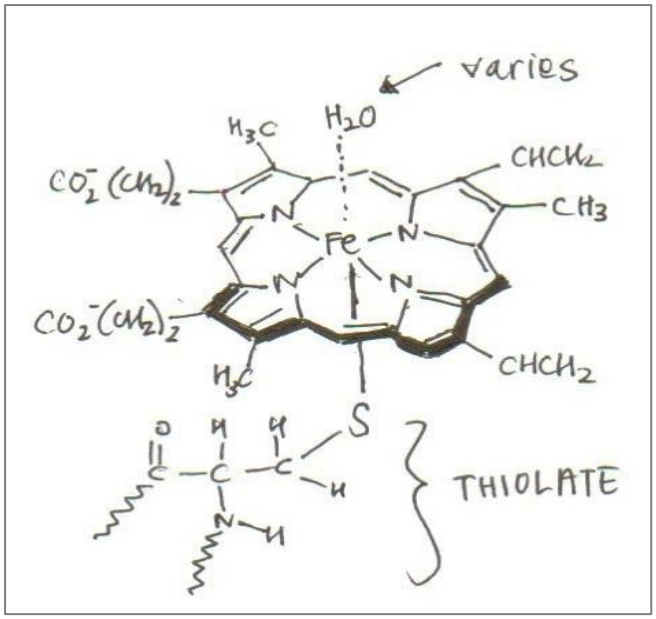

Fig 2: Chemical structure of cytochrome P450 [2].

4.1. Anticonvulsants Drugs

CYP3A4 is induced by phenytoin, oxcarbazepine, and carbamazepine that stimulates metabolism and causes a decrease in the concentration of oral contraceptives that can lead to unplanned pregnancies [27].

So, many clinical effects of critical drugs like cyclosporin or warfarin, that have low therapeutic index, are changed and desired effects are reduced when taken at the same time with these drugs [28].

But if inhibitor drugs like verapamil are taken, they can decrease the rate of metabolism which can lead to the raising of carbamazepine concentration and the result is an increase in toxicity [29].

4.2. Antidepressant Drug

Most psychiatrists have a perception about certain parts of antidepressants' structure which can generate critical drug interactions due to inhibition of a CYP2D6 enzyme [30]. These can be enumerated as follows: escitalopram, citalopram, paroxetine, fluoxetine, fluvoxamine, mirtazapine, venlafaxine, desvenlafaxine, and vilazodone [31]. The FDA advises that if possible, genetic testing is required before starting treatment with certain SSRIs like paroxetine, fluoxetine, and fluvoxamine, to determine the individual activity of CYP2D6 [32].

The effect of fluvoxamine, which is a potent inhibitor, appeared when received with codeine, which inhibits it, to convert it to morphine by inhibiting CYP2D6 enzyme resulting in the loss of painkiller effect [33].

On the other hand, the different effect of inhibition of CYP2D6 enzyme is shown in plasma concentration, in which clozapine and carvedilol levels are increased by 30–42%, whilst galantamine, metoprolol, risperidone, and donepezil levels are increased by 2 to 5 fold [34]. The effects of warfarin can be increased when combined with antidepressants, due to an inhibition of metabolism that leads to an increased risk of bleeding [35]. Harvard medical school reported, that the combination of antidepressants and tamoxifen can cause interference with the benefit of breast cancer treatment due to inhibition conversion of tamoxifen to endoxifen as active form [36].

Antiepileptic Drugs	Major Hepatic Enzymes
Carbamazepine	CYP3A4; CYP1A2; CYP2C8
Ethosuximide	CYP3A4
Felbamate	CYP3A4; CYP2E1; other
Gabapentin	None
Lacosamide	CYP2C19
Lamotrigine	GT
Levetiracetam	None (undergoes non-hepatic hydrolysis)
Oxcarbazepine (MHD is active oxcarbazepine metabolite)	Cytosolic system
Phenobarbital	CYP2C9; other
Phenytoin	CYP2C9; CYP2C19
Pregabalin	None
Rufinamide	Hydrolysis
Tiagabine	CYP3A4
Topiramate	Not known
Valproate	GT; β-oxidation
Vigabatrin	None
Zonisamide	CYP3A4

Fig 3: Major cytochrome P450 effect antiepileptic drugs [1].

4.3. Anti-infectives Drugs

In general, ketoconazole, miconazole and itraconazole are famous antifungals which inhibit all CYP450 enzymes and cause numerous critical interactions [37]. They can increase the concentrations of other medications specially drugs with low therapeutic index like cyclosporin, digoxin, erythromycin and also warfarin for which they increase INR level and cause an increased risk of bleeding [38].

On the contrary, rifampicin is an example of an enzyme inducer that decreases the concentration of other drugs by stimulating metabolism and enhancing clearance. So, it must be noted when prescribed with other drugs like digoxin, aprepitant, ranolazine, tacrolimus, sulfasalazine, and theophylline [39].

4.4. Cardiovascular Drugs

From the many antihypertensive drugs, the calcium channel blocker (CCB) is a familiar prescription for several conditions other than hypertension like angina, heart failure, coronary artery and arrhythmia disease.[40]

Felodipine is one of the CCB and its concentration is affected via CYP3A4 enzyme inhibitors such as ritonavir, itraconazole, and erythromycin that lead to increased concentration and may cause rapid hypotension in some patients [41], while in epileptic patients receiving carbamazepine, which is a CYP3A4 enzyme inducer, it is necessary to take felodipine with high dose to achieve its effect [42].

Other examples of CCB are verapamil and diltiazem, which are considered as moderate CYP3A4 enzyme inhibitors, and they can increase concentrations and effects of other drugs especially low therapeutic index drugs, which, when taken at the same time like warfarin increases the risk of hemorrhage [43].

Pimozide or cisapride can produce QT prolongation and ventricular arrhythmias when taken with diltiazem or verapamil. This occurs due to inhibition of metabolism and accumulated drugs that lead to excessive side effects [44]. Also, fentanyl is the other drug that has significant drug interaction with verapamil, which leads to an increase in blood levels of the drug and therefore a rise in its effects [45].

The other group of therapy is statin drugs like atorvastatin, simvastatin, and lovastatin that are used to lower hyperlipidemia, but they must be taken with caution if prescribed with CYP3A4 enzyme inhibitors such as erythromycin, ritonavir, nefazodone and grapefruit juice to avoid incidence of rhabdomyolysis which is a serious side effect due to an increase in the concentration of statins [46].

One of the famous drugs with critical drug interactions is warfarin because it has a complex pathway in CYP450 metabolism, so it needs frequent monitoring for INR to confirm the steady state of plasma concentration [47]. Clinical studies investigating the combination of warfarin with amiodarone, which is the antiarrhythmic drug, showed a significant drug interaction. Warfarin blood levels were increased by between 6 and 65% as a result of the resultant decrease metabolism of warfarin via amiodarone, leading to increased risk of bleeding [48]. However, other cases of warfarin drug interaction occur when co-administred with primidone. Primidone has a broad spectrum as an enzyme inducer and can affect the activity of many subfamilies such as CYP3A4, CYP2C9, CYP1A2, and CYP2C19. So, the result of the interaction is to decrease the INR level and therefore the anticoagulant activity of warfarin through increasing its clearance [28].

4.5. Immunosuppressants Drugs

According to clinical studies, the combination of immunosuppressant with some enzyme inhibitors can benefit the patient and help to give him a low dose of immunosuppressant drugs. For example, the physician can add ketoconazole to the therapeutic plan contain cyclosporin because it has the highly active CYP3A4 enzyme inhibitor and can therefore decrease the dose of cyclosporin [49]. Furthermore, if the plan combines cyclosporin and St John's wort, which is a CYP3A4 enzyme inducer, the result generates a sequence of organ loss by rejection [50]. The clinical observation reported about the co-administration of CYP3A4 enzyme inhibitors like diltiazem with tacrolimus, can increase the concentration and raise toxicity risk of tacrolimus [51]. Conversely, if the tacrolimus combines with CYP3A4 enzyme inducers like carbamazepine, the concentration of tacrolimus will decrease and the toxicity risk will reduce [52].

4.6. Grapefruit juice:

It is important to note, and usually the physician will warn patients to avoid drinking grapefruit juice before medication due to its characteristic inhibitory action on CYP3A that causes a rise in the concentration, bioavailability and also side effects of many drugs [53]. According to research at the University of California, scientists compared the potency between grapefruit and cannabinoid (CBD) and it showed that CBD is as highly a potent inhibitor as grapefruit [54].

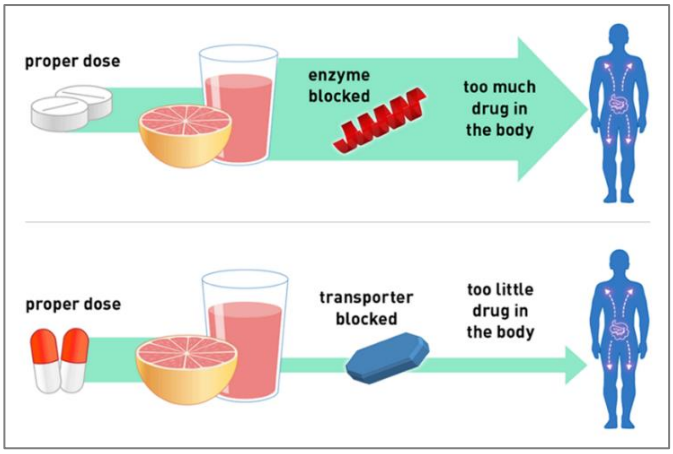

Fig 4: Effect of grapefruit in metabolism of drugs [4].

5. Conclusions

Metabolic enzymes are the main player in drug-drug interactions that cause individual variation of drug response. The major hepatic enzyme family is the CYP system, which has more than twelve enzymes

regulated in three groups and they are responsible for biotransformation and elimination of 70-80% of marketed drugs.

Each enzyme has a specific inducer and inhibitor drug but the critical enzyme is CYP3A4 which is then followed by CYP2D6, CYP1A2, CYP2C9, and CYP2E1.

The safest method to define metabolic interactions of drugs is by understanding the rules controlling interactions. Drugs which inhibit CYP450 can decrease metabolism rate and therefore increase the activity of other drugs and occasionally raise toxicity when taken at same time.

But, the enzyme inducer drugs that are stimulators of metabolism can cause a decrease in concentration and activity of other drugs.

So, special attention should be given to drugs that have a low therapeutic index or have severe adverse effects like antibiotic, immunosuppressant, anticonvulsants or cardiovascular drugs.

However, researchers are investigating the positive applications of some of the drug-drug interactions in the therapeutic field, to take advantages of actions like a reduction in drug dose or minimizing the occurrence of serious side effects for prescribed drugs.

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