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The Correlation Between Breastfeeding and Obesity Among Children

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Abstract

1. **Background:** breastfeeding play as the brilliant standard in accomplishing the proper development factors and nutrition requirements for children. Likewise correlating with numerous positive prolonged wellbeing outcomes such as, diminished risks of obesity and diabetes during childhood and puberty. To identify the correlation between breastfeeding and obesity we applied this study among children aged (8-14 years).
2. **Subject and method:** cross sectional design was chosen to fulfill the aim of this study conducted in Kut city at Wasit governorate, data collected from schools (governmental primary and intermediate schools). Over 2 months period starting from 1st October until end of September 2014. An appropriate sample (simple random sample) consist of 162 children both male and female was collected by use special structured questionnaire. The questionnaire prepared to include some features of children as (age, gender, socioeconomic status, mother education, height and weight). The weight estimation has been in kilogram by utilizing advanced digital scale and height measured in centimeters by using appropriate tape meter scale. .Body Mass Index (BMI) calculated according to sex& age specific body mass index percentile chart.
3. **Result:** The study showed highly statistical association between child feeding and body mass index at P- value (0.000). The major percentage of obese children was found in those who feeding on formula milk which represent (80%) while obesity among breastfeeding children was represent (20%), on other hand overweight formed (66.67, 33.33) among formula feeding and breastfeeding respectively.
4. **Conclusion:** From the current study, we conclude breastfeeding has effective role in prevent childhood obesity.

Key words: breastfeeding, formula feeding and obesity

العلاقة بين الرضاعة الطبيعية والبدانة بين الأطفال

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الخلاصة

1. **المقدمة:** تلعب الرضاعة الطبيعية كمعيار بارز في تحقيق عوامل التنمية السليمة ومتطلبات التغذية للأطفال. وبالمثل ترتبط مع العديد من النتائج الإيجابية طويلة الأمد الرفاهية مثل، تقلص مخاطر السمنة والسكري خلال مرحلة الطفولة والبلوغ. ولتحديد العلاقة بين الرضاعة الطبيعية والبدانة، طبقنا هذه الدراسة بين الأطفال الذين تتراوح أعمارهم بين 8–14 سنة.

2. **المنهجية:** تم اختيار تصميم المقطع العرضي لتحقيق الهدف من هذه الدراسة التي أجريت في مدينة الكوت بمحافظة واسط، تم جمع البيانات من المدارس (المدارس الحكومية الابتدائية والمتوسطة) لمدة شهرين تبدأ من 1 تشرين الاول حتى نهاية كانون الاول 2014. تم اختيار عينة مناسبة (عينة عشوائية بسيطة) تتكون من 162 طفلا تم جمع كل من الذكور والإناث باستخدام استبيان منظم خاص. وقد أعد الاستبيان ليشمل بعض خصائص الأطفال (العمر والجنس والحالة الاجتماعية والاقتصادية وتعليم الأم والطول والوزن). وقد تم تقدير الوزن بالكيلوغرام عن طريق استخدام مقياس رقمي متطور وقياس الارتفاع بالسنتمترات باستخدام مقياس قياس الشريط المناسب. مؤشر كتلة الجسم المحسوبة وفقا لمؤشر كتلة الجسم والجنس محددة العمر المخطط المئوي.
3. **النتيجة:** أظهرت الدراسة ارتباطا إحصائيا بين تغذية الطفل ومؤشر كتلة الجسم عند القيمة .(0.000) P ووجدت نسبة كبيرة من الأطفال البدناء في أولئك الذين يتغذون تغذية اصطناعية التي تمثل (80٪) في حين كانت السمنة بين الأطفال الرضاعة الطبيعية تمثل (20٪)، من ناحية أخرى شكلت زيادة الوزن (66.67، 33.33) بين التغذية الاصطناعية والرضاعة الطبيعية على التوالي.
4. **الاستنتاج:** من الدراسة الحالية، نستنتج أن الرضاعة الطبيعية لها دور فعال في الوقاية من السمنة في مرحلة الطفولة

Introduction:

Breast milk (BF) is the ideal food that provide adequate physiological and psychological needs for baby (Maheswari *et. al.*, 2010). It consist of all fundamental nutrients including carbohydrates, basic fats, proteins, minerals, and immunological agents (Sarita *et. al.*, 2017). Exclusive breastfeeding is the most efficient kind of baby nourishing for the initial a half year of life (Janet *et. al.*, 2014). It has evident benefits for children's health lasting only a short time, especially the decreasing of morbidity and mortality because lessened occurrence of infection as gastro-intestinal infections, diarrheal diseases, and types of extra-intestinal infections and hypersensitive response (Geni *et. al.*, 2014; Bernardo, 2013; Sandra, 1999). It has been established that 1.3 million deaths may be prevented annually when baby solely breastfed during first six months (Lucen, 2012). Worldwide, lesser than 40% of babies are exclusively breastfed (Tarek, 2014).

Some points such as socio-economic status, work, and mother education influence the maternal choice about breastfeeding (Monika, 2017). Exclusive breastfeeding play as the brilliant standard in accomplishing the proper development factors and nutrition requirements for children (Jenny and Julia, 2011). Lactation is likewise correlating with numerous positive prolonged wellbeing outcomes such as, diminished risks of obesity and diabetes, and raised achievement in intelligence tests during childhood and puberty (Zhang *et. al.*, 2015). It is related with a 13–22% lessened chances of overweight or obesity in adulthood (Kramer, 2001).

Subjects and Methods

Study design: cross sectional design was applied to fulfill the aim of this study.

Setting: this study was conducted in Kut city at Wasit governorate, data collected from schools (governmental primary and intermediate schools).

Study period: The study data was collected over 2 months period starting from 1st October until end of December 2014.

Study population: An appropriate sample (simple random sample) consist of 162 children both male and female was selected from the chosen schools to form study population.

Inclusion criteria: both sex healthy children who aged 8-14 years, only breast feeding or artificial feeding during first 6 month of life and who accepted to participate.

Exclusion criteria: children who were under 8 years and above 14years, sick children as children with (thalassemia, sickle cell disease and diabetes children), mixed feeding and who didn't know their feeding history.

Data collection: Data collection done by use special questionnaire structured by the investigators. The questionnaire prepared to include some features of children as (age, gender, mother education, socioeconomic status scales (SESS) it was scored as high level, middle level and low level (Tiwari, 2005), height and weight). Some of the information was taken directly from the child and others from the parents. A questionnaire was distributed to the children and filled by parents such as the socioeconomic situation as well as the nutritional history of the child and the educational level of the parents. The weight estimation has been in kilogram by utilizing advanced digital scale and height measured in centimeters by using appropriate tape meter scale. BMI calculated by the researcher according to sex& age specific body mass index (BMI) percentile chart and used standard formula:

$$\textbf{BMI = weight (kg)/ height (m}^2\textbf{)}$$

Then children were classified into subgroups according to their BMI (Dietary Guidelines for Americans, 2010; Jaber, 2017)

Weight Status Category	Percentile Range	BMI(kg/m²)
Underweight	Less than the 5th percentile	Below 18.5
Healthy weight	5th percentile to less than the 85th percentile	18.5 - 24.9
Overweight	85th to less than the 95th percentile	25.0 - 29.9
Obese	Equal to or greater than the 95th percentile	30 and above

Statistical analysis: all the statistical analysis was done by using Minitab program (Version-18) percentage and Excel application:

1- Descriptive statistic: statistical tables with contingency tables (frequencies and percentage).
2- Inferential statistics: independency test of contingency tables, through using Chi-squared test. P- Value <0.05 considered significant.

Result

Table (1): Represent descriptive characteristic of study sample according to age and gender where it was the higher percentage of males at the age of $< 12^{th}$ where represent (44.23%) while the highest percentage of female was at age $\geq 12^{th}$, which formed (57.27%).

Table (1) The distribution of study sample according to age& gender.

Variable	Groups	Number and Percent	Age groups		Total	P-value
			<12	≥12		
Gender	Male	Number	23	47	70	0.857
		%Groups	44.23	42.73	43.21	
	Female	Number	29	63	92	
		%Groups	55.77	57.27	56.79	
	Total	Number	52	110	162	
		%Groups	100.00	100.00	100.00	

Pearson chi-Square= 0.033
DF=1

The current study showed highly statistical association between child feeding and body mass index at P- value (0.000). The major percentage of obese children was found in those who feeding on formula milk which represent (80%) while obesity among breastfeeding children was represent (20%), on other hand overweight formed (66.67, 33.33) among formula feeding and breastfeeding respectively.

Table (2) Correlation between child feeding and body mass index.

Variable	Groups	Number and Percent	BMI			Total	P-value
			Normal	Overweight	Obesity		
Feeding	Breastfeeding	Number	80	17	4	101	0.000
		%Groups	87.91	33.33	20.00	62.35	
	Formula feeding	Number	11	34	16	61	
		%Groups	12.09	66.67	80.00	37.65	
	Total	Number	91	51	20	162	
		%Groups	100.00	100.00	100.00	100.00	

Pearson chi-Square = 58.900
DF=2

The table below reveal that higher percentage of illiterate mothers feed their baby breastfeeding that formed (70%) but the higher percentage formula feed (39.39%) was used by literate mothers to feeding their baby. There is no statistical association found.

Table (3) association between child feeding and mother education.

Variable	Groups	Number and Percent	mothers' education level		Total	P-value
			Illiterate	Literate		
Feeding	Breastfeeding	Number	21	80	101	0.338
		%Groups	70.00	60.61	62.35	
	Formula feeding	Number	9	52	61	
		%Groups	30.00	39.39	37.65	
	Total	Number	30	132	162	
		%Groups	100.00	100.00	100.00	

Pearson chi-Square = 0.919
DF=1

Table (4) display the relationship between type of child feeding and socioeconomic status, higher percentage of breastfeeding children (75.41) were in low socioeconomic scale and lower percentage fail in moderate SES scale on the contrary the higher percentage of formula feeding children (46.77) were in moderate scale of SES while the lower percentage was in low SES scale that represent (24.59 %). There was a significant statistical association between SES and type of feeding (P-value =0.001).

Table (4) Relationship between type of child feeding and socioeconomic status.

Variable	Groups	Number and Percent	SES			Total	P-value
			Low	Moderate	High		
Feeding	Breastfeeding	**Number**	46	33	22	101	0.027
		%Groups	75.41	53.23	56.41	62.35	
	Formula feeding	**Number**	15	29	17	61	
		%Groups	24.59	46.77	43.59	37.65	
	Total	**Number**	61	62	39	162	
		%Groups	100.00	100.00	100.00	100.00	

Pearson chi-Square = 7.217
DF=2

Discussion:

Breastfeeding is idealistic nourishment for baby, obesity form serious health problems in children life that may be extend to adulthood therefore, understanding the correlation between breastfeeding and obesity is important.

Statistical association between child feeding and body mass index at P- value (0.000) was reveal in current study. The major percentage of obese children was found in those who feeding on formula milk which represent (80%) while obesity among breastfeeding children was represent (20%), on other hand overweight formed (66.67) among formula feeding children and (33.33) among those have breastfeeding history. This because breastfed newborn children might be better in self-regulating their intake the reason that breast milk contain hormones, as leptin, adiponectin and ghrelin, which may facilitate define baby appetite, also are more probable than artificial-fed children to attempt and adapt with new nourishments, Breastfeeding has different effects than formula feeding on baby' digestion and hormones (Elizabeth, 2006; Dewey, 2003; Matthew, 2006). Moreover, breast milk have lesser amount of protein than formula milk [(17)]. The present study results similar to the results that obtained from several studies such as that was done by Jessica, et al. (2011) in Netherlands and Renata (2007) in Brazil.

Also this study displayed that higher percentage of breastfeeding children (75.41) were in low socioeconomic scale and lower percentage fail in moderate SES scale on the contrary the higher percentage of formula feeding children (46.77) were in moderate scale of SES while the lower percentage was in low SES scale that represent (24.59 %). There was a significant statistical association between SES and type of feeding (P-value =0.001). This result match with Crory and Richard (2012) in Ireland who found the rates of overweight/obesity and the probability that a child will have been breastfed as an infant are strongly associated with socio-economic characteristics of the household, and with parental weight status. From our point of

view, poor families can not buy artificial milk so they depend on breastfeeding for their children

Regarding education of mothers the study shows that higher percentage of illiterate mothers feed their baby breastfeeding that formed (70%) but the higher percentage formula feed (39.39%) was used by literate mothers to feeding their baby. This may be due that, educated mothers have an employment and don't have enough time to breastfeeding so they prefer formula feeding. This agree with Zhang et. al. (2015) in China who find out 16% choose bottle feeding because return to work.

Conclusion

From the current study, we conclude breastfeeding has effective role in prevent childhood obesity, because we found highly statistical association between child feeding and obesity. In addition to that, there was a significant statistical association between SES and type of feeding.

Recommendation:

1- Increase awareness of community about importance of breastfeeding for baby and mother, its effect on childhood obesity and health problems related to obesity.
2- Health education of female at child bearing age and pregnant women about practice of breastfeeding, importance of exclusive breastfeeding during first six months of baby life.

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Early Detection of Ovary Cancer by Tumour Marker CA125, CEA and β-HCG

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Abstract

Ovary Cancer is the more common disease and second causing of death in women according to WHO reports, the purpose of the present study was to evaluate the ability of the tumour marker cancer antigen 125 in combination with carcinoembryonic antigen (CEA) and β-HCG to differentiate malignant ovarian and the determinate the levels of cancer antigen and some of trace element in fifty women, aged were (25 – 75) year they divided into two groups included ovarian cancer patients and control groups. This study showed significant increasing ($P<0.05$) in the level of cancer antigen 125, CEA and β-HCG concentration in ovary patients and the sensitive ratio of these tumour markerto detect disease in patients were 87.5% , 81.25% ,85% and showed significant difference in ($P<0.05$) serum trace element concentration.these result comparative with healthy group.

KeyWords: Ovary Cancer, CA125, CEA, B-HCG and Trace Element.

الكشف المبكر لسرطان المبيض بواسطة الدالات الورمية CA125 و CEA و β-HCG

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الخلاصة

سرطان المبيض هو أكثر أنواع السرطان شيوعا والسبب الرئيسي الثاني لوفيات السرطان لدى النساء، أن الغرض من هذا البحث هو تحديد مستوى الدالات الورمية CA125 و CEA و β-HCG. في هذه الدراسة تم التحقيق عن مستويات المستضدات السرطانية وبعض العناصر النزرة في مئة أمراه، تتراوح أعمارهم كانت (25–75) سنة قسموا إلى مجموعتين شملت مرضى سرطان المبيض ومجموعة السيطرة، وأظهرت هذه الدراسة زيادة معنوية ($P <0.05$) في مستوى تركيز CA125 وCEA وβ-HCG لمرضى سرطان المبيض. وكانت حساسية الفحص للدالات الورمية لكشف المرض 87.5% و81.25% و 85 % على التوالي ولوحظ اختلاف كبير في تركيز العناصر النزرة عند ($P <0.05$) في مصل المرضى. هذه النتائج قورنت مع مجموعة الأصحاء.

الكلمات المفتاحية: سرطان المبيض، CA125، CEA، β-HCG والعناصر النزرة .

Introduction

Ovarian cancer accounts for 4% of all cancers in women and around 31% of cancers of the genital tract in developed countries (the most common cancer of the female gynecological tract worldwide is cervical cancer) however, ovarian cancer has been increase incidence to death ratio of all the gynecological malignancies, in both well and under resourced parts of the world (Parkin *et. al.,* 2005). The results for women with ovarian cancer is generally few, with an overall five year ago rate of less than 35% (Jacobs *et. al.,* 1990). This is because most women, who have ovarian cancer, present with advanced disease.

The stage of the disease is the most important factor affecting result. The woman's general health at the time of presentation is also important because it affects what treatments can be used, Most women have had symptoms for months before presentation, and there are often delays between presentation and specialist referral (Meyer and Rustin, 2000). There is a need for greater awareness of the disease and also for initial investiga-tions in primary and secondary care that enable earlier referral and optimum treatment reports having any of the following symptoms on a persistent or frequent basis particularly more than 12 times per month, persistent abdominal distension (women often refer to this as bloating), feeling full (early satiety) and/or loss of appetite and pelvic or abdominal pain increased urinary urgency and/or frequency.

Numerous tumour markers have been tested to improve the sensitivity and specificity of preoperative tests in patients suspected of having ovarian cancer (Hogdall *et. al.,* 2007). In many countries cancer antigen 125 (CA-125) is used routinely as part of the RMI in which ultrasound, menopausal status and serum CA-125 are integrated into one scoring system that helps predict whether an ovarian tumor was benign or malignant (Jacobs *et. al.,* 1989).

Recently, many research works indicated to development multimarker assay for early detection of ovarian cancer, suggesting a panel of CA-125, human epididymis protein (HE_4), carcinoembryonic antigen (CEA) and vascular cell adhesion molecule1 (VCAM-1) in postmenopausal normal risk women as an initial step in a screening strategy for epithelial ovarian cancer similar to the RMI. The previous research concluded that the new findings required additional validation.

Elevated CA-125 levels are found in 82% of patients with ovarian cancer, 28% of patients with non-gynecological cancers , 6% of patients with benign gynecological diseases or other medical conditions and, furthermore, in 1% of the normal popul-ation (Høgdall *et. al.,* 2007). For malignant epithelial ovarian tumours, the CA-125 level is related to both the histological subtype and the stage of disease. CA-125 is more often elevated in serous than in mucinous ovarian tumours, and while only 50% of ovarian cancers in stage I and II are associated with elevated CA-125, this is found in 90% of patients at stage III or IV (Jacobs *et. al.,*1989, Yamashita and Watanabe, 2009).

CEA is a glycoprotein that is synthesized in fetal tissues and in some carcinomas, Serum concentrations exc-eeding 5ng/ml are often found in patients with gastrointestinal carcin-omas, breast cancer, lung cancer and some types of gynecological tumours. Furthermore, elevated CEA is correl-ated with infection, pancreatitis, hepatic cirrhosis and certain benign tumours.

However, CEA may still have clinical importance in colorectal cancer, as values above 20 ng/ml are associated with metastatic disease (Hogdall *et. al.,* 2008). The marker is now used routinely for monitoring of patients after surgery for colorectal cancer, where a rise in CEA suggests progression. Serum CEA is elevated in approximately 35% of all ovarian cancer patients and occurs more often in mucinous tumours (88%) than in serous tumours (19%) (De Sanctis et. al., 2011, Nossov *et. al.,* 2008, Gibson and Bast, 2001).

Some researchers advise that in patients with an undiagnosed tumor in the pelvis, the CA-125/CEA ratio may be used to preoperatively identify a substantial fraction of patients with ovarian and nonovarian malignancies (Nossov *et. al.,* 2008). The Human Chorionic Gonadotrophin (HCG) is an hormone normally synthesized in pregnancy by the syncytiotrophoblast the Products of human chorionic gonadotrophin (HCG) have a prognostic association with many tumours of the female reproductive trace and free beta-subunit and degradation products of the beta core fragment can be found in serum or urine of patients with ovarian, endometrial or cervical cancers (56% to 84%, 51%, and 46%, respectively), They fail to detect ovarian cancer at early stages (Kinugasa et. al., 1995).

Amongst all ovarian cancers, the germ cell tumors with chorionic component are the only one to produce high levels of HCG (Thomas *et. al.,* 1997). Properties or allosteric configuration. Zn and Cu are the prosthetic groups of some metalloenzymes containing superoxide dismutase (SOD), which is an important antioxidant enzyme for cellular protection from reactive oxygen species (ROS) (Gibson et. al., 2001).

The repair of oxidative DNA base modifications is disturbed by Ni and Cd (Mehri *et. al.,* 2011). One reason for repair inhibition appears to be the displacement of Zn and Mg. Zn essential elements that are cofactors for DNA polymerase are effective protectors against carcinogenesis in vivo (Yaman *et. al.,* 2007). The capacity of trace elements (such as Se and/or Zn) to reduce oxidative damage or enhance repair capacity relies on capacity to act as essential Co-factors for antioxidant enzymes such as Cu, Zn-Superoxide Dismutase, Catalase (Cu, Fe), and the different types of glutathione peroxi-dase (Se) (Zowczak *et. al.,* 2001).

These enzymes are crucial to limit oxidation of lipids, nucleic acids or proteins occurring in chronic diseases (such as cancers and cardiovascular disorders) and in ageing. additionally, Zinc is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumour suppressor protein P53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival upon various forms of cellular stress (Ong *et. al.,* 2012).

The aim of the present study was directed to evaluate the levels of tumor marker CA125, CEA and β-HCG and determine the trace elements including (Se, Cu, Zn, Mg, Cd, Pb, and Ca) in ovary cancer patients.

Material & Methods

1. Samples of one hundred healthy women and patients aged (25 – 75) years divided into two groups each groups formed 50 women Control and ovarian cancer group.
2. Determination of CA125, β-HCG and Carcinoembryonic antigen (CEA) by used vadas kit, (Minivadas, Biometrax, France) according to manufact-ured company.
3. Determination of trace elements in Sera was achieved by utiliz-ing atomic absorption spectroph-otometer. Standard solutions in the ranges (0.1 – 1) µg/ml for (Se, Cu, Zn, Mg, Cd, Pb and Ca) were prepared, the sera were precipitated by using equal volume of 1.2N (TCA) after centrifugation, diluted (1: 10) for element measurements by the flameless atomic absorption spectrophotometer (6800) Shimadzu, Japan .

Statistical Analysis of Data

Other data were analyzed by SPSS11.0 software and reported as mean ± standard deviation using one-way ANOVA. Student's T-Test was used for comparison between groups. P values of 0.05 or less are considered statistically significant.

Results

Through these findings observed in Table1, a significant increase of cancer Antigen125, CEA, β-HCG, and sensitive test for tumour marker. From Table 2 that

represented the rang of tumour marker, it can be noticed the increase of range of CA125, CEA, and β-HCG in ovary patients compared with healthy women.

In Table 3, it can be observed the significant decrease in the concentration of selenium, magnesium, and zinc beside a significant increase in the concentration level of copper and calcium in serum patients with ovary cancer and the absence of change in the concentration of, cadmium and lead in the serum of patients with ovary cancer` when compared the results with the control group which showed close results to the limits of the natural level elements in serum.

Table (1) Level of cancer Antigen 125, Carcinoembryonic Antigen and Beta Human Chorionic Gonadotropin in serum ovary cancer patients, Mean±SE.

Tumour Marker	Age Mean±SE	Control Group Mean±SE	Ovary Cancer Patients Mean±SE	Sensitivity test	P – value
CA125(U/mL)	49.94±1.582	10.9737±1.27	262.2±40.289	87. 5%	P<0.05
CEA(ng/mL)	49.94±1.582	0.81±0.140	71.83±17.238	81.25%	P<0.05
β-HCG(U/mL)	49.94±1.582	1.015±0.139	77.44±11.868	85%	P<0.05

Table (2) the data acted range, skewness and kurtosis for cancer antigen for ovary cancer patients.

Tumour Marker	Range		Skewness Mean±SE	Kurtosis Mean±SE
	Control	Patient		
CA125(U/ml)	21.80	573.00	0.660±0.414	-1.345±0.809
CEA(ng/ml)	1.60	189.79	3.492±0.597	12.552±1.154
β-HCG(U/ml)	1.80	148.00	0.883±0.597	-0.640±1.154

Table (3) Level of some trace element in serum ovary cancer patient, M±SE.

Trace element	Control group Mean±SE (ppm.)	ovary cancer patients Mean±SE (ppm.)	P – value
Se	0.0692±0.003201	0.0351±0.0110	P<0.05
Zn	1.203±0.034566	0.633±0.04158	P<0.05
Cu	1.24±0.033433	1.860±0.6109	P<0.05
Ca	89.20±11.63497	195.9±12.7532	P<0.05
Mg	14.221±0.75614	11.212±0.8274	P<0.05
Cd	0.0108±0.001272	0.015±0.00143	NS
Pb	0.0024±0.000652	0.0023±0.00163	NS

NS = Non Significant

Discussion

Ovarian cancer was previously known as the ‘silent killer’ but studies have shown that it is associated with symptoms in 93% of cases before diagnosis, In this study showed incre-ased of CA125, CEA and β-HCG concentration in patients

Sufficient evidence has been noted in there currency of the disease by rise in serum CA125 concentration earlier than any clinical or radiological investigation.

Therefore, they suggest that patients with ovarian carcinoma shou-ld get serial estimation of serum CA 125 during the development of cancer cell (Yamashita and Watanabe, 2009) Cupric ions are reported to inhibit the production of singlet oxygen; this is of particular significance because of the latter's ability to cross the cell membrane and its high reactivity towards various biomolecules.

In this research, a increase in CEA concentration in ovary cancer patient in all of age. Carcinoemb-ryonic antigen (CEA) is a glycopro-tein that is synthesized in fetal tissues and in some carcinomas. Serum concentrations exceeding 5 ng/mL are often found in patients with gastro-intestinal carcinomas, breast cancer, lung cancer and some types of gynaecological tumours. Furthermore, elevated CEA is correlated with infection, pancreatitis, hepatic cirrho-sis and certain benign tumours.

In patients with colorectal cancer, the presence of elevated CEA depends on the stage of the disease (Yamashita and Watanabe, 2009). The elevated of CEA in serum of ovary cancer patient may be indicator to present cancer cell in serum. In this study, elevated serum human chorion-ic Gonadotropin (HCG) levels in pati-ents with malignant ovarian tumors. HCG and its subunits can be measure-ed at low dose in the serum of most men and women (Alfthan et. al., 1992)

Its values differ according to the level of Gonadotropin releasing hormone. HCG production can be suspected to have an influence on Gonadotropin receptor expression in cancer tissue. The fact that we obser-ved a positive correlation of HCG to LH-receptor expression supports this assumption. (Guo et. al., 2011) in the present study we showed difference in concentration of trace element zinc, selenium, calcium and magnesium.

Many studies indicated to trace element play role in immune system Zinc prevents the growth of experimentally induced cancers. Zinc deficiency depresses killer cell active-ity. Zinc deficiency promotes cancer by inhibiting normal vitamin A and lipid metabolism and DNA repair. Selenium's antioxidant properties, inhibition of tumor growth and inver-se epidemiological correlations with cancer (Mundy and Edwards, 2008). The inhibitory effect of selenium on growth of tumors has again been documented. Some researchers incri-minate zinc in cancer because it reduces the symptoms of selenium toxicity, perhaps implying that zinc may be a selenium antagonist.

In addition, some scientists have reported the accumulation of zinc in various tumors (Zowczak *et al.,* 2001). In our study showed decre-ase in concentration of Zinc, Magnes-ium and selenium which may be development of ovary cancer in this study. Selenium has been hypothesi-zed to play a role in preventing cancers. Epidemiologic studies have demonstrated an inverse association between circulating selenium levels and cancers of the prostate, lung and colorectal (Ong *et. al.,* 2012, Helena *et. al.,* 1984).

This observation was confir-med In contrast, with respect to skin cancer hypothesis, recent analyses suggest that selenium supplementa-tion actually increases the risk of nonmelanoma skin cancer, particul-arly squamous cell carcinoma, among participants with higher baseline serum selenium levels (Mundy and Edwards, 2008).

In the present study decrease of level the selenium concentration in serum ovary cancer may be defected the body against cancer cell The mechanisms by which selenium may decrease cancer risk (Young *et. al.,* 1994). The mechanisms included among others the role of selenium in cell cycle arrest, decreasing cells proliferation, inducing apoptosis, facilitating DNA repair by activation of P53, disruption of androgen receptor signaling, and being a key component of selenoenzymes the changed of concentration of trace element was very important, increase of levels of serum copper may be chronic disease (Piura, 2008).

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Micro Spectrophotometric Determination and Cloud Point Extraction of Cefixime in Pure form and Pharmaceutical Preparation

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Abstract

We suggested two methods simple, rapid and useful spectrophotometric determination of Cefixime (CFX) with and without using cloud point extraction technique in pure form and pharmaceutical preparation. The first method based without cloud point on diazotization of the Cefixime drug by sodium nitrite at 5Cº followed by coupling with Ortho nitro phenol in basic medium to form orange colour. The product was stabilized and measured at 400 nm. Beer's law is obeyed in the concentration range of (10-160) $\mu g \cdot ml^{-1}$. Sandell's sensitivity was 0.0888 $\mu g \cdot cm^{-1}$, the detection limit was 0.07896 $\mu g \cdot ml^{-1}$, and the limit of Quantitation was 0.085389 $\mu g \cdot ml^{-1}$.The second method is cloud point extraction (CPE) with used Trtion X-114 as surfactant. Beer's law is obeyed in the concentration range of (10-160) $\mu g \cdot ml^{-1.}$ Sandell's sensitivity was 0.1470 $\mu g \cdot cm^{-1}$, the detection limit was 0.06680 $\mu g \cdot ml^{-1}$, and the limit of quantitation was 0.07293 $\mu g \cdot ml^{-1.}$ All variables including the reagent concentration, reaction time, colour stability period, and mole ratio were studied in order to optimize the reaction conditions. The composition of product (1:1). The methods were effectively useful to the determination of Cefixime in pharmaceutical dose form, and the attained results were in good agreement with the official and other methods in literature .No interference was observed from the commonly encountered additives and excipients.

Keyword: Cloud Point Extraction, orthonitrophenol and Triton X-114, Cefixime.

التقدير الطيفي والمايكروي والاستخلاص بنقطة الغيمة للسفكسيم في المواد النقية والمستحضرات الصيدلانية

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الخلاصة

اقترحنا طريقتين بسيطة وسريعة ومفيدة لتقدير السفكسيم مع وبدون استخدام تقنية استخراج نقطة سحابة في شكل نقي والمستحضرات الصيدلانية. تعتمد الطريقة الأولى بدون نقطة سحابة على ازوتة الدواء بواسطة نتريت الصوديوم في 5Cº متبوعًا بالاقتران مع اورثونايتروفينول لتشكيل اللون البرتقالي. تم تثبيت المنتج وقياسه عند 400 نانومتر. يطبق قانون البيرة في نطاق التركيز (10–160) ميكروغرام/مل. كانت حساسية ساندیل هي 0.0888 ملغم/مل، وكان حد الاكتشاف 0.07896 ملغم/مل، وكان حد الكمي 0.085389 ملغم /مل. الطريقة الثانية هي استخراج نقطة السحب (CPE) مع استخدام ترايتون X-114. والتي تخضع لقانون بيرلامبرت في نطاق التركيز (10–160) ميكروغرام/مل. كانت حساسية ساندیل هي 0.1470 ملغم/مل، وكان حد الاكتشاف 0.06680 ملغم/مل، وكان الحد الكمي 0.07293 ملغم/مل. تمت دراسة جميع المتغيرات بما في ذلك تركيز الكاشف، زمن التفاعل، فترة استقرار اللون من أجل تحسين ظروف التفاعل. تكوين المنتج (1: 1). كانت الطرق مفيدة بشكل فعال لتقدير السفكسيم في شكل جرعة صيدلانية، وكانت النتائج التي تم التوصل إليها متوافقة بشكل جيد مع الطرق الرسمية وغيرها في الأدب. لم يلاحظ أي تداخل من الإضافات بشكل شائع.

الكلمة المفتاحية: استخراج نقطة السحب، اورثونايتروفينول، ترايتون X-114 و السفكسيم.

Introduction

sulfa drugs were the first antibiotics used regularly and paved the way for the revolution of antibiotics in medicine. The first sulfonamide, named Prontosil (red dye), was discovered in 1932 by Gerhard Dumagk (Gonzalez, 2011).

Cefixime : (6R, 7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2- (carboxymethoxy) imino] acetyl] amino] 3-ethenyl-8-oxo-5-yhia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid trihydrates .Is an antibiotic that belongs to the third generation of cephalosporin and is taken orally to treat bacterial infections, including pharyngitis, middle ear, sore throat and urinary tract infection. Approved for medical use in 1989. Over-all characterizes of Cefixime in table (1) (Nayon, 2013).

Table (1) General properties of Cefixime (CFX) .

Chemical structure	
Nomenclature	(6R, 7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2- (carboxymethoxy) imino] acetyl] amino] 3-ethenyl-8-oxo-5-yhia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid trihydrates
Formula	$C_{16}H_{15}N_5O_7S_2$
Molecular Weight	453.452

Instrumentation and Apparatus

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV1800 made in Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm.,Water Bath : A thermostat water bath, Memmert, made in Germany, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Triup International corp, TRIU 800 Centrifuge, made in Korea & PH meter: HANNA, PH meter, HI 83141.

General procedure for Azo coupling

The prepared Azo Coupling product are added in volumetric flask (10ml) in ice bath, 1ml of Cefixime (CFX) (1000 µg ml^{-1}), 1ml for hydrochloric acid, 1ml for sodium nitrate (1%), 1ml for sulphamic acid (1%), 1ml for Orthonitrophenol (1000µg ml^{-1}), at last added 1ml for sodium hydroxide and complete the volume by distilled water .Then absorbance is measured by UV-VIS. And the maximum wave length show in figure: (1).

General procedure for CPE

A typical experiment of cloud point include the following steps: taking the volumetric flask (10ml) and added the optimum condition of azo coupling and added 1ml for surfactant (10%) and complete the volume by distilled water. The contain of volumetric flask transfer to centrifuge test tube then added the mixture in water bath 60 C^0 at 20 min and separated by centrifugation 4000 rpm at 20 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated . The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS. And the maximum wave length show in fig:(1).

RESULT AND DISCUSSION

First methods: Spectrophotometric determination of sulphadimidine sodium (SDMS) by oxidation coupling reactions.

Optimization Parameters for Reaction.

All of the factors that affect to the absorbance of formation of azo dye product are optimized to improve the sensitivity and detection limit for the determination of the drugs. All optimization work under wavelength at 400 nm.

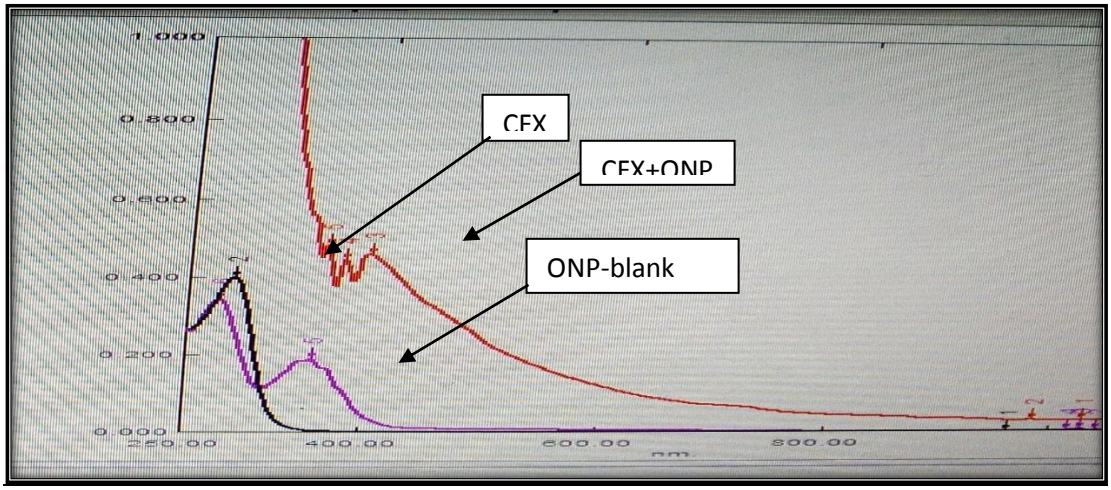


Figure (1) Absorbance spectra of the Resulting Dye CFX

Effect of Acid Type

In this study, using 1ml of (0.5M) from different acids [HCl, H_2SO_4, HNO_3, H_3PO_4 and CH_3COOH] and added [1ml of CFX, 1 ml of each acid , 1ml of $NaNO_2$, 1ml of H_3NSO_3, 1ml ONP and 1ml of NaOH] in 10 ml of volumetric flask and complete the volume by distilled water to formation diazonium salt. Then the absorbance is measured at 400 nm, the absorbance result show in table (2).

Table (2) Data of Absorbance of Effect of Acid Type.

0.5M different acids	HCl	H_2SO_4	HNO_3	H_3PO_4	CH_3COOH
Absorbance at	0.496	0.265	0.439	0.267	0.149

It is clear from this study that the hydrochloric acid gives higher absorbance, this acid is a few of use in subsequent experiments show in the table (1-2) .

Effect of Optimum Volume of 0.5M of acid.

The same addition for CFX[1ml CFX, with varying volumes of 0.5M HCl from (0.1-1) ml, 1 ml $NaNO_2$, 1ml H_3NSO_3, 1ml ONP and 1ml of NaOH] in 10 ml volumetric flask and complete the volume by distill water. Then measured the absorbance and the optimum volume for higher absorbance that fixed for sequence experiment, the result absorbance show in table (3).

Table (3) Data of Absorbance to Optimum Volume of 0.5M

Volume HCl	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Abs.	0.264	0.287	0.318	0.337	0.406	0.451	0.464	0.504	0.494	0.437

It is obvious that absorbance increase with increase the volume of acid, suddenly the absorbance decrease because the primary amine becomes inactive (Younis, 2009). The optimum volume for higher absorbance fixed in subsequent experiments (for HCl with CFX) which affects the composition of diazonium salt.

Effect of Base Type.

In this experiment using different basic [NaOH, KOH, K_2CO_3, Na_2CO_3, NH_4OH, $NaHCO_3$] and that follow the addition [1ml CFX, 0.8ml HCl, 1ml $NaNO_2$, 1ml H_3NSO_3, 1ml ONP and 1ml of each base]in volumetric flask10 ml and complete the mark by distill water. The absorbance is measured the absorbance results are shown in table (4).

Table (4) Data of Absorbance to Base Type.

Volume of 0.5 base	NaOH	KOH	NH_4OH	Na_2CO_3	K_2CO_3	$NaHCO_3$
Abs.	0.510	0.274	0.043	0.358	0.377	0.217

Scheming of the absorbance values against the volume of Different bases type displayed in figure (2).

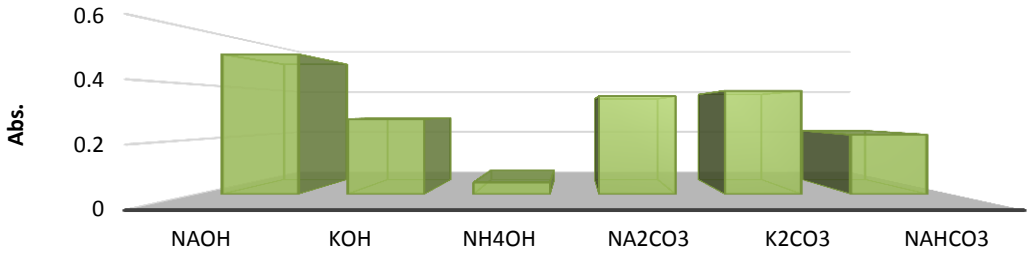


Figure (2) Data of absorbance for different bases with CFX.

It is clear the Sodium hydroxide give the higher absorbance, this base it is fixed in subsequent (Solomons, 1980) show in table (1-4)& figure (1-2).

Effect of Optimum Volume of 0.5M [NaOH].

The same addition for for CFX[1ml CFX , **0.8** ml HCl, 1 ml NaOH , 1ml H_3NSO_3, 1ml ONP and varying volume of o.5 M NaOH (0.1-1) ml] in 10 ml volumetric flask and complete the volume by distill water. Then measured the absorbance and the optimum volume for higher absorbance are fixed for sequence experiment. The absorbance result show in table (5).

Table (5) Data of Absorbance to different volume of 0.5M [KOH, Na_2CO_3, NaOH].

Volume of 0.5M bases	Abs	Volume of 0.5M bases	Abs
0.1	0.205	0.8	0.476
0.2	0.269	0.9	0.497
0.3	0.293	1	0.532
0.4	0.340	1.1	0.492
0.5	0.399	1.2	0.482
0.6	0.413	1.3	0.411
0.7	0.428		

It is evident that absorbance increase with increase the volume of NaOH, but suddenly decrease the absorbance because the decomposition happen when increase basicity and formation diazotate ions may coupling and agreement with previous studies (Saadiyah, 2012) The optimum value of **1** ml for NaOH with CFX.

Effect of Optimum Volume of 1% Sodium Nitrite.

The same additions are [1ml for CFX, 0.8 ml HCl, with varying volume of 1% $NaNO_2$ from (0.1-1) ml, 1ml H_3NSO_3, 1ml ONP and **1** ml NaOH] in volumetric flask 10 ml and complete the mark by distill water. Then the higher absorbance of optimum volume are fixed for sequence experiment show in table (6).

Table (6) Data of Absorbance to Optimum Volume of 1% $NaNO_2$.

Volume of 1% Sodium Nitrite	Absorbance	Volume of 1% Sodium Nitrite	Absorbance
0.1	0.069	0.6	0.432
0.2	0.179	0.7	0.509
0.3	0.243	0.8	0.556
0.4	0.285	0.9	0.497
0.5	0.375	1	0.475

It is clear in table (1-6) and figure (1-6) the absorbance increase with increase the volume of $NaNO_2$,but the signals decrease because the nitrate toxic may because a high rate of pollutants affecting on diazonium salt (Saadiyah, 2013). The optimum value of Sodium Nitrate 0.8 ml for CFX.

Effect of Optimum Volume of 1% Sulphamic Acid.

The additions for experimental are [1ml for CFX,**0.8** ml HCl, **0.8** ml 1% Na_2NO_2 with varying volume of 1% H_3NSO_3 from (0.1-1) ml,1ml ONP and **1** ml NaOH] in volumetric flask 10 ml and complete the volume by distill water. Then the higher absorbance of optimum volume are fixed for sequence experiment. Table (7) show the data of the absorbance.

Table (17): Data of the Absorbance od Optimum Volume of 1% H_3NSO_3.

Volume	0.07	0.08	0.09	0.1	0.2	0.3	0.4	0.5
Abs.	0.398	0.456	0.498	0.525	0.557	0.531	0.451	0.420

In this graph is clear the absorbance increase with increase the volume of Sulphamic acid, but the signals decrease suddenly because this volume remove nitrite and escape of nitrogen gas (Saadiyah, 2011). The optimum volume of Sulphamic acid are 0.2 ml.

Effect of Optimum Volume of (100 µg ml^{-1}) Reagent.

The same additions are [1ml for CFX, 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, with varying volume of (100 µg mL^{-1}) ONP from (0.1-1) ml and **1** ml NaOH] in 10 ml of volumetric flask and complete the volume by distill water. Then the higher absorbance of optimum volume at maximum wavelength are fixed for sequence experiment show in table (8).

Table (8) Data of Absorbance of Optimum Volume of (100 µg ml^{-1}) Reagent.

Volume of reagent	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Abs.	0.298	0.368	0.442	0.501	0.567	0.533	0.478	0.447

The absorbance increase when increase the volume of reagent but, suddenly decrease because this is required volume to coupling with drug. The optimum volume of reagent [0.5 ml O-Nitro Phenol with CFX].

Effect of Reaction Time on Stability Colour Product.

The optimum volumes of parameters are complete [1ml for CFX, 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP and **1** ml NaOH]. The time on stability colour of product one of the important factors to cloud point and diazotization, as well as, we needed to study time (0-60) min., then absorbance is measured and fixed the higher absorbance at maximum wavelength show in table (9).

Table (9) Data of Absorbance of Reaction Time on Stability Colour Product.

Time	Absorbance	Time	Absorbance
0	0.198	35	0.502
5	0.237	40	0.531
10	0.289	45	0.575
15	0.319	50	0.566
20	0.391	55	0.543
25	0.420	60	0.531
30	0.479	65	0.509

This clear the time of product remain stable for CFX is 45 min display in table (9).

Effect of Order Addition.

It has been taken for the sequence of addition with optimum volume but different order. The effect of order addition shown in table (10).

Table (10) Effect of Order Addition.

No	Addition	Absorbance
1	**R+H+N+S+D+B**	0.218
2	**D+H+N+S+R+B**	0.589
3	**D+H+N+B+R+S**	0.416
4	**D+B+R+N+H+S**	0.231
5	**R+B+D+H+N+S**	0.389
6	**R+H+N+B+D+S**	0.077

D= Drug (CFX) , H= acid (HCl) ,N= $NaNO_2$, S=H_3NSO_3 , B = Base (NaOH),
R= Reagent (O-Nitro Phenol).

At the maximum wavelength the absorbance is measured, and fixed the higher absorbance for the best order addition display in table (10).

Effect of Solvents.

All additions of diazotization and coupling reaction are added for CFX with optimum condition. Then followed diluted by different polar solvent [water, ethanol, methanol, 1-propanol, acetonitrile & acetone] in volumetric flask 10 ml, at maximum wavelength for each drug the absorbance are measured and recorded for the best solvent. The effect of absorbance shown in table (11).

Table (11) Data of Absorbance to Solvents.

Solvent	Water	Ethanol	Methanol	Acetonitril	1-Propanol	Acetone
Abs.	0.539	0.620	0.521	0.157	0.104	0.283

In this study show the best of solvent is water to SDMS & SMX but Ethanol is the best solvent for CFX. The water & ethanol is sensitive, cheap, economically and nontoxic show in table (11).

Effect Temperature in the Formation of Colour Product and Stabilization.

The conclusion of different temperature on colour product has been studied from (5-60) C^0. And the rest of adding are optimal settings then dilution with distill water except the CFX dilute by ethanol in volumetric flask 10 ml. Then absorbance are measured at the maximum wavelength.

Table (12) Data of Absorbance to Temperature in the Formation of Colour Product and Stabilization.

Time	5	15	20	30	40	50	60
Abs	0.350	0.482	0.519	0.493	0.484	0.439	0.419

It's perfect that at temperature ($15C^0$ & 20 C^0) is the greatest absorbency for all drugs, on the other hand when temperature rises the absorbency starts lessening suggestion dissociation of product and can be notice from strength of color. The results are in arrangement with literatures (Saadiyah, 2012), and this temperature is stable in later experiment.

Stoichiometric Determination of Product.

Continuous Variation Method (Asha, 2014).

The calculation of the conformation of the azo dyes product is supported by using the slope analysis method. In this method, the absorbance is planned against [reagent] / [reagent]+[drug]. This test is complete by taking a series of volumetric flasks 10 ml having varying volumes of Drug (0.1-0.9 ml) with concentration [$6X10^{-4}$] M and varying volumes of Reagent (0.9 -0.1 ml) with concentration ($6x10^{-4}$) M and the rest addition is optimum condition then complete the volume by ethanol. That followed the absorbance is measured at the maximum wavelength λmax =400nm for CFX. The stoichiometric ratio between drug with reagent 1:1 results are displayed in the Table (13).

Table (13) Data of Absorbance for Continuous Variation Method Result.

Volume of Drug/ml	Volume of Reagent/ ml	Abs of CFX
0.1	0.9	0.002
0.2	0.8	0.005
0.3	0.7	0.041
0.4	0.6	0.117
0.5	0.5	0.173
0.6	0.4	0.101
0.7	0.3	0.042
0.8	0.2	0.019
0.9	0.1	0.007

Scheming the value of absorbance against the [R]/[R]+[D] displayed in figure (3), when [R]=Reagent and [D]=Drug.

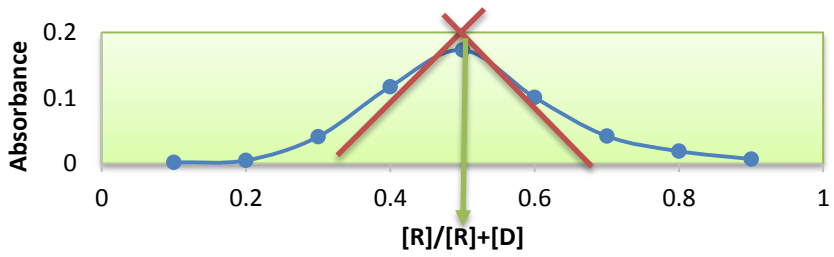


Figure (3) Continuous Variation Method Plot for CFX .

Mole Ratio Method.

Mole Ratio Method is useful to study the wildlife of coloured product (العبايجي, 1983) .In this method the volume of drug is constant in 1 ml with concentration ($6x10^{-4}$M) for CFX and concertation of ONP is [$6X10^{-4}$] M in volumetric flask 10 ml , the rest of addition was optimum conditions and complete the volume by ethanol . That followed the absorbance is measured at the maximum λmax =400 nm for CFX . The stoichiometric ratio between drug with reagent 1:1 results are displayed in the Table (14).

Table (14) Data of Absorbance for Mole Ratio Method Results for CFX:ONP.

Volume	0.5	1	1.5	2	2.5	3	3.5	4	4.5
Abs.	0.108	0.187	0.201	0.211	0.222	0.234	0.244	0.251	0.259

Scheming the value of absorbance against the [R]/[R] displayed in figure (4), figure when [R]=Reagent and [D]=Drug.

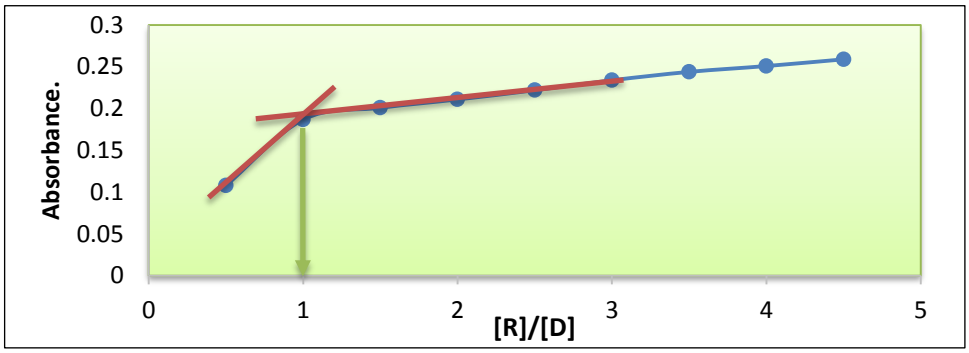
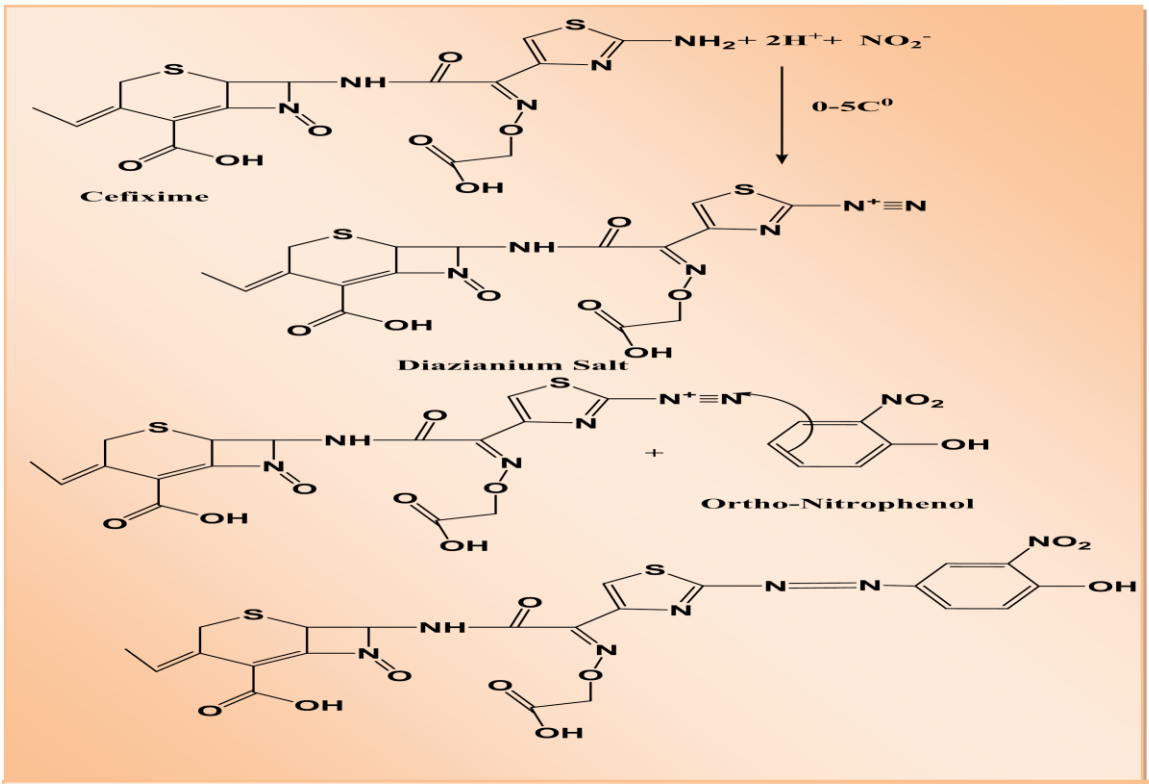


Figure (4) Mole Ratio Plot for CFX

Calibration Curve for CFX-ONP:

Aliquots of 10 ml solution is prepared ,having growing concentration of CFX taking [varying volume of SMX(0.1-1.6 ml) with concentration (10-160 µg ml^{-1}), 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP and 1 ml NaOH]. In 10 ml volumetric flask and complete the volume by ethanol, then measured the absorbance at maximum wavelength against a blank solution able under alike condition without drug. Linear calibration graph is founded by scheming absorbance against concentration of CFX in figure (5) the Concentration (10-160) µg ml^{-1} obeys the Bear law, the molar absorption coefficient of product equals ($4.904 x10^3 L.mol^{-1}.cm^{-1}$) and Sandall's sensitivity (0.0888 µg.cm^{-2}).

The probably of product formation display in scheme (1).



(6R, 7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2- (carboxymethoxy) imino] acetyl] amino] 3-ethenyl-8-oxo-5-yhia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid trihydrates- 2-nitro phenol

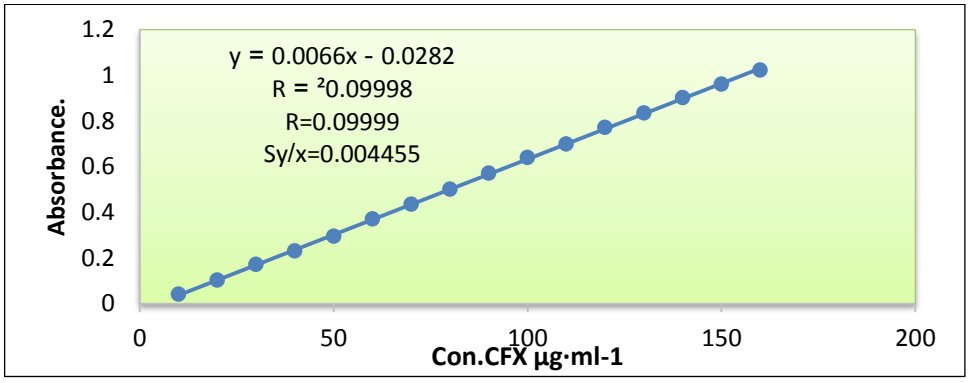


Figure (5) Calibration Graph of CFX.

Effect of Interference.

The effect of interference ordinary present in each drug [SDMS,SMZ,CFX] to know method fussiness under learning by addition 1ml (1000 ppm) from each interference [Lactose, Starch, Arabic Gum, Glucose, Talc, $Ca_3(PO_4)_2$, $CaCl_3$, $FeCl_3$, $CoCl_2$, $CaCl_2$, $NiCl_2$, Tri methyprine] with 1ml (1000 ppm) from CFX 1ml (1000 ppm). And the rest of adding are optimal settings then dilution with ethanol in 10 ml volumetric flask. Then absorbance are measured at400 nm. The interference experimentation is made to estimation the systematic error affected by other materials that may be found in the specimen being analysed. It necessity be the size of interference is lesser for a sample to limit the dilution of sample and

use the maximum concentration probable in the sample (Valcarcel, 2000). The results are shown in Table (15).

Table (15) Data of Absorbance of interference for CFX.

NO.	100ppm interference	Abs.	Recovery %	E_{rel}%
1	Lactose	0.619	98.0606	-1.939
2	Starch	0.622	98.636	-1.363
3	Arabic Gum	0.589	93.515	-6.484
4	Glucose	0.614	97.454	-2.545
5	Talc	0.566	90.181	-9.818
6	$Ca_3(PO_4)_2$	0.569	90.484	-9.515
7	$CaCl_3$	0.611	96.848	-3.151
8	$FeCl_3$	0.572	91.090	-8.909
9	$CoCl_2$	0.602	95.484	-4.515
10	$CaCl_2$	0.599	95.030	-4.969
11	$NiCl_2$	0.594	94.424	-5.575
12	Tri methyprine	0.596	94.727	-5.272
13	Without interference	0.630	99.72	-0.28

The results in this tables displayed and expected there is no interference to present with drug in pharmaceuticals (Abed, 2012).

The Stability Constant of Coloured Product.

Dependent on two conducts, mole ratio and continuous variations methods revealed former, the composite product is [D: R] [drug: reagent] in the result is 1:1 as in the following equation.

$$K= [DR] / [D][R] \longrightarrow K= 1- \alpha/ \alpha^2 c$$

K= the stability constant of the dye (Liter.mol^{-1})
C=Final concentration of the drug interacting with the reagent.
α =Degree of dissociation which can be determined from the following equation
α= (Am-As)/Am
As= Absorbance of the solution containing stoichiometric amounts of the drug and reagent.
Am = Absorbance of the solution containing excess amounts of the reagent. The stability constant K (Jihan, 2012). It is clear the stability constant is high, so the dye formed is very stable display in table (16).

Table (16) Data of The Stability Constant of Colour Product of CFX.

Volume of 4x10^{-4}M of SDMS / ml	Final con. SDMS /M	As*	Am*	α	K (L.Mol^{-1})	Mean of K (L.Mol^{-1})
0.3	1.2×10^{-3}	0.178	0.176	0.01123	3.574×10^{3}	3.0918×10^{3}
0.5	2×10^{-3}	0.299	0.295	0.0133	3.566×10^{3}	
0.7	2.8×10^{-3}	0.435	0.433	4.5977×10^{-3}	2.1355×10^{4}	

[*]= Average of three determinations

It is clear the stability constant is high, so the dye formed is very stable.

Accuracy and Precision Test.

The tables (17) display effects accuracy and precision of CFX correspondingly. These tests are calculated using four different concentrations (3, 6, 9, 12) µg ml^{-1} from drug for five repetitions then application of the offered method at optimum conditions (Yamamoto, 1967). It is pure the results from this method has good accuracy and precision, so the value of

recovery rate is 100.294% for CFX. The relative standard deviation percent (RSD%) can be calculated by the relationship.

$$\mathbf{RSD\ \% = (S / \bar{x}) \times 100}$$

Table (17) Data of Accuracy and Precision to Determination of CFX.

Amount of CFX / µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.9090	100.7575	100.2945	0.7575	0.2945	0.1633
90	90.606	100.6733		0.6733		0.4163
60	60.1515	100.2525		0.2525		0.4506
30	29.8484	99.4947		-0..5053		0.7530

[*]= Average of five determinations

Applications of the Proposed Method on Pharmaceuticals.

The suggested method has been applied on pharmaceutical for CFX. The similar method is applied on Syrup Cefixime, the manufacture company is [Pharma International Co. Amman. Jorden] that contains (200mg) in 100 ml and the sample is prepared in accordance with the method described in paragraph (2.4.5.3). The results are good and of great dependability in the analysis of samples in the pharmaceutical preparation. The results are shown in the table (18) for CFX.

Table (18) Data of Determination CFX in the Pharmaceutical Preparation.

Amount of SMX / µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.5448	100.454	100.0315	0.454	-.0.0595	0.2282
90	90.424	100.4711		0.471		0.4030
60	60.2116	100.3526		0.352		0.0542
30	29.5454	98.8485		-1.515		4.2043

[*]= Average of five determinations

Second methods :Cloud Point Extraction of Cefixime in Aqueous Solution.

Effect Type of Surfactant with Drug CFX.

The kind of surfactant shows identical important part in cloud point extraction method wherever each surface keeps ghostly depend on practical centre of Micelles. Aliquots of 10 ml of a solution inclosing [1ml for CFX, 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP and 1 ml NaOH] in 10 mlvolumetric flask and changed surfactant is used for drug [Tween 20, Tween 80, Triton X-114, Triton X-100, CTAP, SDS], at 60 C^0 for 20 min then centrifuge 4000 rpm for 20 min .The surfactant amusing part is parted, dissolved in 1ml ethanol, at maximum wave length the absorbance are measured for CFX at 400 nm. The results shown in table (19).

Table (19) Data of Absorbance to Type of Surfactant with CFX.

Addition	Tween 20	Triton X-100	Tween 80	SDS	Triton X-114	CTAP
Abs.	0.061	0.122	0.057	0.090	0.275	0.119

It is clear in this result the surfactant Triton X-114 increase the Absorbance and efficiently of cloud point extraction (Wifky, 2010).

Effect of Triton X-114 Volume.

Sum of 10 ml solution is primed [1ml for CFX, 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP and 1 ml NaOH] in 10 ml volumetric flask and custom changing volumes of 10% (v/v) Triton X-114 (0.2-2) ml for all drug, then whole the volume by ethanol, are heated at 60 C^0 for 20 min to practice cloud point then centrifugation at 4000 rpm for 20 min. The surfactant – opulent phase softened by 1ml ethanol then at maximum wavelength at λmax = 400 nm the absorbance are measured and the best is recorded. This results displayed in table (20).

Table (20) Data of Absorbance to Triton X-114 Volume with CFX.

Volume of Triton X-114	Abs of CFX λmax = 400 nm
0.2	0.095
0.4	0.112
0.6	0.165
0.8	0.189
1	0.224
1.2	0.265
1.4	0.278
1.6	0.266
1.8	0.247
2	0.201

It is notice from the result that the absorbance rises with the upturn volume of Triton X-114 but unexpectedly drops at higher amount. Conclusion the amount of surfactant on the effectiveness of extraction and develop the enrichment factor (Al-Abachi, 2014). These characterize the optimal Volume of Triton X-114/ml that provide highest competence with lesser size and greater density in cloud layer. The drop in absorbance under the optimum volume is unpaid to lacking micelles to catch the hydrophobic product quantitatively. Consequently the best volume of Triton X-114 (1.4 ml) for CFX individually stable in following experimentations to complete high extraction efficiency.

Effect of Equilibrium Temperature.

In a chains of solution are set [1ml for CFX, 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP, 1 ml NaOH and 1.4 ml 10% (v/v) Triton X-114] in volumetric flask 10 ml and complete the volume by ethanol, the temperature is varied from (35-70) C^0 and the incubation time from (5-35) min for all drug. At the maximum wavelength the absorbance are measured and recorded. This result displayed in table (21).

Table (21) Data of Absorbance to Temperature / ^{0}C with CFX.

Temperature	Abs of CFX
35	0.265
40	0.271
45	0.278
50	0.281
55	0.280
60	0.278
65	0.276

The effect of this factor is measured of the greatest central step in CPE, in order to certify the effective phase separation, which returns certainly the degree of extraction efficiency of the aim analyte (Shariati, 2015). Figure (2-3) shows the difference on the absorption pointer through changing the temperature between 35 to 65ºC at 20 min. It shown that the maximum absorption pointer of target analyte is completed at (50) ºC for the azo dye product because of great number of micelles designed in cloud point layer important the total transfer of the azo dye product into surfactant-rich phase that make the most of the sensitivity (NCCLS, 1986). An important drop of the absorbance comeback has been detected thereafter, maybe due to the instability or separation of the azo dye product at upper temperature than best. Thus (50 °C) are certain from CFX used as best in the common CPE procedures of this drugs (Lal, 2015).

Effect of the Incubation Time.

In a chains of solution are set [1ml for CFX**,** 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP, 1 ml NaOH and 1.4 ml 10%(v/v) Triton X-114] in 10 ml volumetric flask and complete the volume by ethanol, the temperature is 50 ^{0}C for CFX and the incubation time from (5-35) min for all drug. At the maximum wavelength 400 nm, the absorbance are measured and recorded the result shown in table (22).

Table (22) Data of Absorbance for the Incubation Time with CFX.

Time /min	5	10	15	20	25	30	35
Abs	0.381	0.392	0.402	0.409	0.400	0.399	0.393

CPE needs enough time to become balance between aqueous phase and surfactant- rich phase by more accumulation the micelles. This time signifies the amount of high temperature accumulated in the solution that lets Micelles drop water molecules in order to give small size hydrophobic with high viscosity easily entrap the product in it (Saadiyah, 2015). It is perfect that the best incubation time is (20) min for CFX. The rate and time of centrifugation is inspected and effected too ,as well as , the absorbance was not good at 3500 rpm for 15 min, however at 4000 rpm for 20 min is carefully chosen to provide high extraction efficiency and no increases detected for longer time (Saadiyah, 2014).

Preparation of Calibration Curve in CPE.

In a chains of solution are [changing volume of CFX (0.1-1.6ml) with concentration (1-16 µg ml^{-1}), 0.8 ml HCl, 0.8 ml 1% $NaNO_2$, 0.2 ml H_3NSO_3, 0.5 ml ONP, 1 ml NaOH and 1.4 ml 10%(v/v) Triton X-114] in 10 ml volumetric flask and full the volume by ethanol, at the best temperature and incubation time are heated in water bath to configuration cloud point and separated by centrifuge at 4000 rpm for 20 min. The rich phase liquefied by 1ml ethanol, then at maximum wavelength at 400 nm, the absorbance are measured beside the blank that set below the similar adding deprived of drug. Scheming the absorbance values of the cloud point as against the concentration of CFX is displayed in Figure (6).

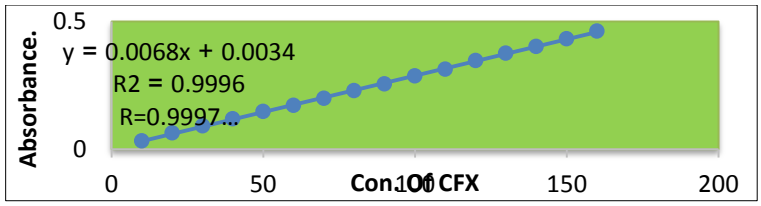


Figure (6) (CFX+CPE) Calibration Curve.

Accuracy and Precision Test.

The result of Accuracy and Precision Test displayed in table (23) for CFX. I n these experiment used four different concentration (12, 9, 6, 3) µg ml^{-1} from drugs for five repetition, then applicate of Cloud Point method with finest condition. It is perfect from these results that the technique has good accuracy and precision as a significance of recovery rate is 100.8182 % for CFX.

Table (23) Data for Accuracy and Precision of the CPE to Determination of CFX.

Amount of CFX / µg ml^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.3571	100.2975	100.8182	0.297	0.818	0.3981
90	90.0714	101.190		1.1904		1.0196
60	60.2148	100.357		0.357		0.5136
30	30.4285	101.4283		1.428		1.0735

[*]= Average of five determinations

Applications of the Cloud Point Extraction on Pharmaceticals:

Cefixime (CFX)

The similar method is applied on Syrup Cefixime, the manufacture company is [Pharma International Co.Amman. Jorden] that contains (200mg) in 100 ml and the sample is prepared in accordance with the method described in paragraph (2.4.5.3). The results are good and of great dependability in the analysis of samples in the pharmaceutical preparation. The results are shown in the table (24) for CFX.

Table (24) Data of Determination CFX in the Pharmaceutical Preparation.

Amount of SMX / µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.1428	100.119	100.5194	0.119	0.5182	0.3875
90	90.2856	100.3173		0.317		0.9020
60	60.5566	100.9276		0.927		2.3892
30	30.2142	100.714		0.71		4.4067

Average of five determinations.

Conclusion

Cloud point extraction is demeans, calm, safe and useful pre-concentration technique to determine Cefixime by UV/VIS. In planned method is a kindliness, selectivity and gave a good RSD and low limit of detection.

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Postpartum Depression Among Mothers Attending Primary Health Care Centers, Iraq 2017-2018

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Abstract
Background: Pregnancy is a significant event in a woman's life and is associated with psychological and biological changes. Antenatal and postnatal care has traditionally neglects the emotional and psychological health and focuses on the physical health and consequently depression after pregnancy is overlooked and underdiagnosed. The study aims to identify the prevalence and the underlying determinants of Postpartum Depression (PPD) among newly delivered mothers.
Methods: A cross-sectional study conducted in convenient sample of 52 PHCCs belonging to 13 health directorates (Kirkuk, Salahadin, Diyala, Anbar, Baghdad/ Karkh, Baghdad/ Rusafa, Babel, Karbala, Najaf, Wasit, Diwaniyah, Muthanna and Dhi Qar). The (1608) mothers within the first six weeks after giving birth who attended to PHCCs were included. Basic socio-demographic variables were compiled using a form that was filled through direct interview. PPD was assessed using Edinburg Postnatal Depression Scale with cutoff point ≥12. Data was collected during the period from 1 October to 30 November of 2017.
Results: The prevalence of PPD was 37.4%. The depressed mothers were significantly associated with; Age of mother and her husband, insufficient income family, neonatal complications, history of infertility, pregnancy complications, unplanned pregnancy, inadequate family care, family discord or domestic violence, history of depression or anxiety symptoms before or during pregnancy, and family history of mental disorders. The determinants of PPD were (inadequate family support, pregnancy complications, neonatal complications, family discord or domestic violence, psychological symptoms before or during the pregnancy and family history of mental disorders).
Conclusions: PPD is a common illness and associated with many physical, social and psychological factors, it is not highlighted by society and health service providers, which requires spreading community awareness of the mental health concepts and increases the ability of health services provider to assess the mental health of pregnant mothers and manage mental disorders accompanying them.
KeyWords: Postpartum Depression, prevalence, determinants and PHCCs.

اكتئاب ما بعد الولادة بين الأمهات المراجعات لمراكز الرعاية الصحية الاولية/ العراق 2017- 2018

رياض الرديني
المدير الوطني للرعاية النفسية الاولية/ قسم الوقاية والسيطرة على الامراض غير الانتقالية/ دائرة الصحة العامة/ وزارة الصحة
بغداد_العراق

الخلاصة
المقدمة: يعد الحمل حدثًا مهمًا في حياة المرأة ويرتبط الحالة الصحية للمراة بالتغيرات النفسية والبيولوجية التي تحدث اثناءه. عادة ما تهمل الرعاية الصحية النفسية والعاطفية اثناء الحمل وما بعد الولادة وتركز الرعاية على الصحة الجسدية

وبالتالي يتم التغاضي عن الاكتئاب بعد الحمل وعدم تشخيصه محليًا وعالميًا. تهدف الدراسة إلى تحديد انتشار اكتئاب بعد الولادة بين الأمهات حديثات الولادة والعوامل الكامنة وراءه.

طريقة العمل: أجريت دراسة مقطعية مستعرضة لعينة ملائمة من المراكز الصحية الاولية (52 مركز صحس) في 13 من دوائر الصحة المختلفة (كركوك، صلاح الدين، ديالى، الأنبار، بغداد/ الكرخ، بغداد/ الرصافة، بابل، كربلاء، النجف، واسط، الديوانية، المثنى، وذي قار) وشملت 1608 من امهات حديثات الولادة (خلال ست اسابيع الاولى من الولادة) والذين حضروا مراكز الرعاية الصحية الأولية اثناء فترة الدراسة، تم تجميع المتغيرات الاجتماعية والديموغرافية الأساسية باستخدام نموذج عد من قبل شعبة الصحة النفسية/ دائرة الصحة العامة يتم ملؤه من خلال المقابلة المباشرة كما وتم تقييم الاكتئاب مابعد الولادة باستخدام مقياس إدينبورغ بالنقطة الفاصلة ≤12. تم جمع البيانات من المراكز في الفترة من 1 تشرين الاول الى 30 تشرين الثاني من عام 2017.

النتائج: كان معدل انتشار الاكتئاب ما بعد الولادة في عينة الدراسة 37.4 ٪. وارتبطت الأمهات المكتئبات بشكل كبير مع؛ عمر الأم وزوجها، عدم كفاية دخل الاسرة، المضاعفات الصحية لحديثي الولادة، العقم ومضاعفات الحمل، الحمل غير المخطط له اوغير المرغوب به، الرعاية الأسرية غير الكافية، الخلافات الأسرية أو العنف الاسري، أعراض الاضطراب النفسي السابق، والتاريخ العائلي للاضطرابات النفسية.

تم من خلال تحليل الانحدار اللوجستي تحديد والعوامل الكامنة وراء انتشار الاكتئاب ما بعد الولادة.بالتالية: عدم كفاية دخل الاسرة، المضاعفات الصحية اثناء الحمل، المضاعفات الصحية لحديثي الولادة، الخلافات الأسرية أو العنف الاسري، أعراض للاضطراب النفسي السابق، التاريخ العائلي للاضطراب النفسي.

الاستنتاجات: الاكتئاب ما بعد الولادة مرض شائع ويرتبط بالعديد من العوامل الجسدية والاجتماعية والنفسية، لكنه لا يعطى الاهمية من قبل المجتمع ولا من مقدمي الخدمات الصحية، مما يتطلب نشر الوعي المجتمعي بمفاهيم الصحة النفسية وزيادة قدرة مقدمي الخدمات الصحية على تقييم الصحة النفسية للامهات اثناء الحمل ومعالجة الاضطرابات النفسية المصاحبة لها.

الكلمات الرئيسية: اكتئاب مابعد الولادة، سعة الانتشار، العوامل المحددة، مراكز الرعاية الصحية الأولية.

Introduction

Depression is a common mental disorder, affects about 121 million people worldwide. It is an important risk factor for suicide, particularly affecting adolescents and women during reproductive age (Emro.who.int. 2018). Pregnancy is a significant event in woman's life and is associated with psychological and biological changes (Waqas, 2014). Antenatal and postnatal care traditionally neglects the emotional and psychological health and focuses on the physical health (Abdollahi, 2011).

Postpartum depression (PPD) is defined as a mood disorder that affects a woman after giving birth (Kettunen, 2014). It is diagnosis was based on a person's symptoms. Most of the women experience a brief period of unhappiness or worry after childbirth, but PPD should be suspected when a woman complains from severe symptoms of extreme sadness, anxiety, crying episodes, low energy, irritability, and changes in sleeping or eating patterns. These symptoms last over two weeks and typically onset between one week and one month from delivery (Pearlstein, 2009).

The salient features of PPD include:

- PPD is a serious under-recognized public health problem, making a substantial contribution to maternal and infant morbidity and mortality (Postpartum Depression Facts, 2017).

- One in five newborn's mother experiences significant mental health problems, the most common of which are depression and anxiety states (Postpartum Depression Facts, 2017).
- Newborn's Mothers with PPD are less able to care for themselves and their infants, whose survival, health and development could be then compromised (Tefera, 2015).
- PPD can affect the health and development of children; it may predict poor growth and high risk of diarrhea in infants, which may reduce child survival (WHO, 2008).
- Recognition of depression during the postnatal period can be done with simple, reliable, and affordable tools (WHO, 2008).
- PPD can be identified relatively easily, within the context of primary health care, so it is an important marker for high-risk infants (WHO, 2008).

The exact cause of PPD is unclear and believed to be a combination of emotional and physical factors (Postpartum Depression Facts, 2017). These may include factors such as hormonal changes, sleep deprivation, a prior episode of PPD, bipolar disorder, a family history of depression, psychological stress, complications of childbirth, lack of support, or a drug use disorder (Postpartum Depression Facts, 2017). The PPD represents a considerable public health problem that affects the whole family. It is effects on marital relationship and children as well as the mother make it an important condition to prevent, diagnose, and treat (Postpartum Depression Facts, 2017). Untreated PPD can have adverse long-term effects; for the mother, it may be the precursor of chronic recurrent depression and for her children, ongoing depression can contribute to emotional, behavioral, cognitive and interpersonal problems in later life (Rai, 2015).

Objectives

- To estimate the prevalence of PPD among a sample newly delivered mothers attending PHCCs in Iraq, 2018.
- To identify the determinants of PPD among those mothers.

Methods

A cross-sectional study with analytic component was conducted in convenient sample of 52 PHCCs belonging to 13 health directorates (Kirkuk, Salahadin, Diyala, Anbar, Baghdad / Karkh, Baghdad / Rusafa, Babel, Karbala, Najaf, Wasit, Diwaniyah, Muthanna and Dhi Qar). All mothers within the first six weeks after delivery who attended the selected PHCCs and meeting the eligibility criteria were informed about the purpose of the study and those who agreed to participate were given an informed consent and enrolled in the study.

- **Inclusion criteria:** All mothers after giving birth who attended the selected PHCCs and accepted to participate in this study
- **Exclusion criteria:** Any mother within the first sex weeks after delivery, who complained from any of following conditions were excluded from the study:
  - History of chronic disease such as (diabetes mellitus, hypertension, renal disease, liver disease, thyroid disease, malignancy).
  - A recent history of psychological trauma (DSM-IV; defines trauma as a direct personal experience of an event that involves actual or threatened death or serious injury) (Castillo 2007).
  - Diagnosed physical or learning disability.
  - Known cases of depression.

This study was conducted during the period 1st September 2017 – 30th April 2018, and the total number of mothers who participated in this study was 1608. A structured questionnaire was developed by Mental Health Section/ NCDs Department, General Directorate of Public Health, MoH after reviewing related studies, required data was collected during the period from 1 October to 30 November of 2017 through direct interview by trained healthcare workers in psychosocial units with assistance of healthcare worker in

immunization, maternal and child health units in selected PHCCs. The data collected to gather the following:

- Age of participant and age of her husband
- Educational level of participant and her husband is classified into illiterate or primary school, secondary school, and higher education
- Occupation is classified into employer and housewife.
- Family income was divided into three categories; insufficient, sufficient, and more than sufficient based on a subjective estimation of participants.
- Smoking (cigarettes and shisha) is classified into three types; current smoker, ex-smoker, and non-smoker.
  - Current smoker: individual who has smoked greater than 100 cigarettes in their lifetime and currently smokes at least monthly (Definitions of smoking status, 2018).
  - Ex-smoker: individual who has smoked greater than 100 cigarettes in their lifetime and does not smokes at last month (Definitions of smoking status 2018).
  - Non-smoker: individual who has smoked less than 100 cigarettes in their lifetime and does not currently smoke (Definitions of smoking status, 2018).
- Mode of delivery is divided into normal vaginal delivery and caesarian section.
- Neonatal complications: The participants asked about the following health problems of their last child (prematurity, low birth weight, asphyxia, congenital anomalies, septicemia, hypoglycemia, jaundice, and any acute infections).
- Antenatal care is classified into no visit, one to three visits, and four visits or more (Extranet.who.int., 2018).
- complications during the last pregnancy: the participants asked about the following health problems during the last pregnancy (gestational diabetes mellitus, heart diseases, hyperemesis gravidarum, antepartum hemorrhage, preeclampsia, eclampsia, preterm delivery)
- Infertility is divided into; primary infertility when a woman is unable to ever bear a child, either due to the inability to become pregnant or the inability to carry a pregnancy to a live birth, and secondary infertility when a woman is unable to bear a child, either due to the inability to become pregnant or the inability to carry a pregnancy to a live birth following either a previous pregnancy or a previous ability to carry a pregnancy to a live birth (Luettu, 2013)
- Unintended pregnancy or unwanted pregnancy is defined as pregnancy that is reported to have been either unwanted (that is, the pregnancy occurred when no children, or no more children, were desired) or mistimed (that is, the pregnancy occurred earlier than desired). It is a core concept that is used to better understand the fertility of populations and the unmet need for contraception (birth control) and family planning (USA: CDC., 2013).
- Family support after delivery: The participants asked about family support from their parents or husbands after delivery, and it described subjectively by participants as adequate and inadequate family support
- Domestic violence is defined as a pattern of abusive and threatening behaviors that may include physical, emotional, economic and sexual violence as well as intimidation, isolation and coercion. The purpose of it is to establish and exert power and control over another; men most often use it against their intimate partners (Thawley, 2018).
- Anxiety or depression symptoms before or during the last pregnancy: these symptoms included: persistent fear or tension, anger or persistent emotion, persistent sadness, fatigue without reason, worthless or suicide ideation, insomnia, and eating and sleeping disorders (Skarl, 2015).
- Family history of mental disorders: The participants were asked whether any of their family members had a diagnosed mental disorder.
- **Screening of PPD:** by using the Edinburgh Postnatal Depression Scale (EPDS), the instrument was designed to screen for PPD (Cox, 1987).

Edinburgh Postnatal Depression Scale (EPDS) is a 10-item scale that designed to screen PPD (Cox, 1987). It is the most widely used and validated screening questionnaire for PPD (Moraes, 2017). For each item, women are asked to select one of four responses that most closely describe how they have felt over the past 7 days. Each response has a value between 0 and 3; scores for the 10 items are summed to give a total score between 0 and 30. The cut-off point to indicate the presence of depression is ≥12. At this cut-off point, the sensitivity of this scale for identification of major depression has been found to be >95% with a specificity of > 95% and the consistency was at level of 0.83 (Ekeroma 2012).

Pilot study: The pilot study carried out in Al Salam PHCC of Baghdad/ Al-Karkh DoH, and involved 10 mothers within 6 weeks after delivery before starting data collection, to test the clarity and applicability of the study tool, the time needed for filling the questionnaire and to address the difficulties that may be faced during the study.

Data Management and Statistical Analysis:

- The data were coded and each questionnaire assigned with a serial identifying number when the data entered and analysis by the researcher using Statistical Package for Social Sciences (SPSS v.21).
- The data were presented as frequency table, pie and bar charts.
- Chi-square test applied to test the association between categorical data.
- Logistic regression analysis was applied using PPD as the dependent variable and the variables that showed significant association in the binary analysis as the independent variables.
- The level of significance was set at a P value of < 0.05.

Official and Ethical Consideration:

The official approval was granted from:

- NCDs Department, General Directorate of Public Health, MoH-Iraq
- Verbal consent was obtained from the participants.
- All personal information was kept anonymous and not be divulged except for the study purpose.

Results

The total number of mothers who participated in this study was 1608. They were distributed in 52 PHCCs in 13 different DoH in Iraq. The highest proportion of participants was found in age group 25 - 34 years (44.7%). Around 40% of participants were illiterate or finished the primary school. The highest proportions of participants (74.2%) were housewives and 73% had more than one child. Family income was subjectively considered insufficient in 22.2% of participants. The vast majority of mothers who included in this study were non-smokers (95.1%) and others either current or ex-smoker.

Regarding reproductive health; about 34% of participants had delivered their last child by cesarean section and about 6.6% of newborn child suffered from neonatal complications as (prematurity, asphyxia, congenital anomaly, septicemia, hypoglycemia, respiratory distress syndrome, jaundice, and severe infections) after delivery. The proportion of male newborn delivered by the participants was slightly more than that of female children (52.5%).

Mother without previous child was represented 16.7% of study sample, those with child aged 1 year and 2 years represented (23.9%, 27.3%) respectively and those of 3 years or more child represent 32.1%. The 20.8% of participants not received antenatal care and 31.9% of them not complete antenatal care visits (four visits) during the last pregnancy. About 17% of participants had history of infertility (primary or secondary infertility) before the last pregnancy and 65.4% suffered from complications as gestational diabetes mellitus, heart diseases, hyperemesis gravidarum, antepartum hemorrhage, preeclampsia, eclampsia, preterm

delivery…etc. during their last pregnancy and 35% of the study participants reported that the last pregnancy was unwanted.

About 17% of participants had inadequate family support from their parents or husbands after delivery, and 27% of participants had emotional problems or domestic violence with their husbands or other close relatives. Considering mental health state, bout 39% of participants suffered from symptoms of depression or anxiety before or during the last pregnancy, and 9.6% of them had family history of mental disorders.

Prevalence of PPD:

The prevalence of PPD among participants was 37.4% (33.8 – 38.9) 95% C.I. (Figure 1).

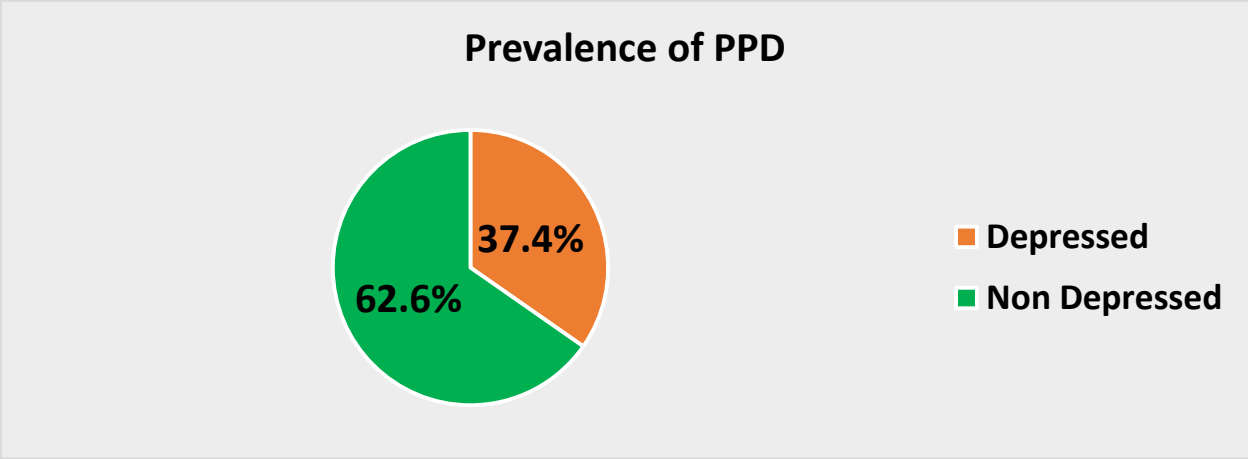

Figure (1) Prevalence of PPD among the study participants

Table 2 shows**;** the highest prevalence of PPD was found among participants aged ≥ 45 years (62.5%) with significant association (P=0.009). We noticed that there was a significant association (P=0.001) between prevalence of PPD and family income, about half of participants with PPD had insufficient income (48.5%). There was no significant association between prevalence of PPD and participants educational level, it is occupation, and their children number (P ≥ 0.05), and the highest prevalence of PPD was seen among current smoker's mothers (47.7%) with no significant association (P=0.166) between PPD and smoking.

We noticed that the prevalence of PPD increased with the increase age of participant's husbands and the highest prevalence of PPD was seen among ≥ 45 years age group (46.4%) with significant association (P=0.003) between aging of participants' husbands and prevalence of PPD. No significant association found between prevalence of PPD and the level of education of participants' husbands (P ≥ 0.05).

Table 2 shows**;** the highest prevalence of PPD (40.3%) was seen among participants who had delivered their last child by cesarean section with no significant association between mode of delivery and prevalence of PPD (P=0.083). We found that more than half of participants (58.5%) who had children suffered from neonatal complications were complaining from PPD with a significant association (P=0.001) between neonatal complications and prevalence of PPD.

The highest prevalence of PPD was seen among mothers who had a child of one to two year old (39%) with no significant association (P=0.604) between age of the last child and prevalence of PPD.

Regarding the association between PPD and antenatal care, we found that the highest prevalence of PPD was found among participants of irregular or not had antenatal care (37.8%, 37.3% respectively) with no significant association (P=0.967) between prevalence of PPD and antenatal care. Also the highest prevalence of PPD was seen in mothers with

previous history of infertility and mothers who suffered from complications during their last pregnancy (44.5% and 44% respectively) with a statistically significant association between history of infertility, pregnancy complications and increased prevalence of PPD (P=0.008, P= 0.001 respectively).

Table (1) Distribution of the study group by PPD and participant's characteristics.

Variable	PPD		Total (%) n=1608	P- Value
	Yes (%) n= 601	No (%) n= 1007		
Age group (Years)				
15 - 24	204 (33.7)	402 (66.3)	606 (37.7)	**0.009**
25 - 34	272 (37.9)	446 (62.1)	718 (44.7)	
35 - 44	115 (42.9)	153 (57.1)	268 (16.7)	
≥ 45	10 (62.5)	6 (37.5)	16 (1.0)	
Husband age group (Years)				
15 - 24	66 (29.6)	157 (70.4)	223 (13.9)	**0.003**
25 - 34	267 (35.8)	479 (64.2)	746 (46.4)	
35 - 44	190 (40.3)	281 (59.7)	471 (29.3)	
≥ 45	78 (46.4)	90 (53.6)	168 (10.4)	
Educational level				
Illiterate or Primary School	243 (38.2)	393 (61.8)	636 (39.6)	0.652
Secondary School	230 (36.0)	409 (64.0)	639 (39.7)	
Higher Education	128 (38.4)	205 (61.6)	333 (20.7)	
Husband Educational level				
Illiterate or Primary School	214 (40.7)	312 (59.3)	526 (32.7)	0.097
Secondary School	245 (37.0)	418 (63.0)	663 (41.2)	
Higher Education	142 (33.9)	277 (66.1)	419 (26.1)	
Occupation				
Employer	160 (38.6)	255 (61.4)	415 (25.8)	0.596
Housewife	441 (37.0)	752 (63.0)	1193 (74.2)	
Smoking				
Non smoker	564 (36.9)	966 (63.1)	1530 (95.1)	0.166
Current smoker	31 (47.7)	34 (52.3)	65 (4.0)	
Ex-smoker	6 (46.2)	7 (53.8)	13 (0.9)	
Number of Children				
One	148 (34.1)	286 (65.9)	434 (27.0)	0.104
More than one	453 (38.6)	721 (61.4)	1174 (73.0)	
Monthly income				
Insufficient	173 (48.5)	184 (51.5)	357 (22.2)	**0.001**
Sufficient	320 (33.5)	634 (66.5)	954 (59.3)	
More than sufficient	108 (36.4)	189 (63.5)	297 (18.5)	

We also noticed that 42.9% of participants who didn't want the last pregnancy complained from PPD with a significant association (P=0.001) between unwanted pregnancy and prevalence of PPD. There was no significant association found between prevalence of PPD and gender of the last child (P ≥ 0.05).

Table (2) Distribution of the study group by PPD and certain obstetrical factors.

Variable	PPD		Total (%) n=1608	P- Value
	Yes (%) n= 601	No (%) n= 1007		
Mode Of Delivery				
NVD	379 (35.9)	678 (64.1)	1057 (65.7)	0.083
C/S	222 (40.3)	329 (59.7)	551 (34.3)	
Child Gender				
Male	304 (36.0)	541 (64.0)	845 (52.5)	0.235
Female	297 (38.9)	466 (61.1)	763 (47.5)	
Neonatal Complications				
Yes	65 (58.5)	46 (41.5)	111 (6.9)	**0.001**
No	493 (32.9)	1004 (67.1)	1497 (93.1)	
Age of The Previous Child (Years)				
No Previous Child	91 (34.0)	177 (66.0)	268 (16.7)	0.604
< 1 Year	146 (37.9)	239 (62.1)	385 (23.9)	
1 - 2 Years	171 (39.0)	268 (61.0)	439 (27.3)	
≥ 3 Years	193 (37.4)	323 (62.6)	516 (32.1)	
Antenatal Care				
No Visit	125 (37.3)	210 (62.7)	335 (20.8)	0.967
1 - 3 Visits	194 (37.8)	319 (62.2)	219 (31.9)	
≥ 4 Visits	282 (37.1)	478 (62.9)	760 (47.3)	
History of infertility (primary or secondary)				
Yes	122 (44.5)	152 (55.5)	274 (17.0)	**0.008**
No	479 (35.9)	855 (64.1)	1334 (83.0)	
Complications During Pregnancy				
Yes	463 (44.0)	589 (56.0)	1052 (65.4)	**0.001**
No	138 (24.8)	418 (75.2)	556 (34.6)	
Unwanted Pregnancy				
Yes	244 (42.9)	325 (57.1)	569 (35.4)	**0.001**
No	357 (34.4)	682 (65.6)	1039 (64.6)	

Table 3 shows; regarding the association between prevalence of PPD and family support after delivery, 50.7% of participants who had inadequate family support from their parents or husbands after delivery were complaining from PPD with a statistically significant association (P=0.001) between family support and increased prevalence of PPD. We noticed that more than half proportion of participants who had emotional problems or domestic violence with their husbands or other close relatives suffered from PPD (51.4%) with a significant association (P=0.001) between emotional problems and increased prevalence of PPD.

Concerning the association between prevalence of PPD and previous depression or anxiety before or during the last pregnancy, we found that 58.5% of participants who had a history of depression or anxiety were suffered from PPD with a statistically significant association (P=0.001) between the previous history of depression and increased prevalence of PPD. The highest proportion of participants with PPD was seen in participants with a positive family

history of mental disorders (70.3%) with significant association (P=0.001) between positive family history of mental disorders and increased prevalence of PPD.

Table (3) The association between PPD and mental health status of participants.

Variable	PPD		Total (%) n= 1608	P- value
	YES (%) n= 601	NO (%) n= 1007		
Family Support After Delivery				
Yes	461 (34.6)	871 (65.4)	1332 (82.8)	**0.001**
No	140 (50.7)	136 (49.3)	276 (17.2)	
Emotional Problems or Domestic Violence				
Yes	223 (51.4)	211 (48.6)	434 (27.0)	**0.001**
No	378 (32.2)	796 (67.8)	1174 (73.0)	
Previous symptoms of Depression or Anxiety				
Yes	367 (58.5)	260 (41.5)	627 (39.0)	**0.001**
No	234 (23.9)	747 (76.1)	981 (60.0)	
Family History of Mental Disorders				
Yes	109 (70.3)	46 (29.7)	155 (9.6)	**0.001**
No	492 (33.9)	961 (66.1)	1453 (90.4)	

Table 4 shows; by using binary logistic regression analysis, six factors were found to be the determinants factors of PPD. These factors were participant's history of mental disorder, neonatal complications, family history of mental disorders, inadequate family support, pregnancy complications, emotional problems with husbands and important persons.

Table (4) Determinants of PPD by logistic regression analysis.

Factors	Odds ratio	95% C.I.		P - Value
		lower	upper	
History of mental disorder	3.485	2,767	4.390	0.001
Neonatal complications	3.144	1.520	6.503	0.001
Family history of mental disorders	3.020	2.038	4.476	0.001
Inadequate family support	2.288	1.392	3.759	0.001
Pregnancy complications	1.746	1.364	2.235	0.001
Domestic discord	1.324	1.022	1.716	0.034

Discussion

The objective of this study was to estimate the prevalence of PPD among 1608 newly delivered mothers attending 52 PHCCs in Iraq, 2017 and to identify the determinants of PPD among them. It illustrates that the prevalence of PPD was 37.4% by using EPDS with cutoff point 12 **(**Cox, 1987), which is regarded as a gold standard and the most commonly self-rated scale used (Moraes, 2017). Iranian study in 2017 **(**Sarah, 2017) and Saudi study in 2014 (Alharbi, 2014) revealed lower results (33.4% and 33.2% respectively). Nearly similar results were found in a study conducted in Bahrain 2012 (Al Dallal, 2012) and another one in the USA 2006 when the prevalence of PPD was (37.1% and 39% respectively) (McCoy, 2006).

Lower results observed in a number of studies as, a Chinese study 2014 (27.3%) (Deng, 2014), Also Canadian studies in 2005 where PDD was ranging between 10% and 20% (Zinga, 2005). An American study in 2006 reported a prevalence of PPD was 15.4% and the mean prevalence rate in the United Kingdom was 12.8% (Halbreich, 2006). These wide differences in the results obtained from different studies might be attributed to the tools and timing of the PPD survey in each study (might obtained in an earlier or late time) (Baker,

2005) and might be attributed to the possible contribution of socioeconomic differences between different populations.

Another significant determinant was the family income as about half of women with PPD had insufficient income (48.5%). This was agreed to a study in Japan (2011) which showed that income from employment status was associated with a reduced risk of PPD (Miyake 2011). Also another study conducted in Taiwan (2012) showed that low income is associated with the risk of PPD, which agreed to that noticed in the current study (Chien, 2012) and finally to American study in 2007 which revealed that women with high income ($70,000 annually) had four times lower risk of developing PPD than women with low incomes (Segre, 2007). This might be attributed to limited financial means for domestic's needs especially infant requirements provide a high amount of stress for the mother, which can lead to depression.

More than half of mothers (58.5%) complained from PPD had children suffered from neonatal complications; making neonatal complications as one of the significant determinant of PPD. This is consistent with studies in India (Upadhyaya, 2014) and Sweden (Nager, 2008). They found that neonatal complications like severe birth asphyxia, preterm birth, and perinatal death were significantly related to risk of PPD. These neonatal complications might create a considerable sense of fear in those mothers from losing the child that make her at risk for PDD development. This factor was a significant, independent and un-confounded risk factor for PPD.

Those mothers who had a history of infertility and pregnancy complications (44.5% and 44% respectively) were significantly associated with increased prevalence of PPD. Different results observed in a local study in Iraq (Ahmed 2015) in which 60% had history of infertility most of them had primary infertility and 8% had early pregnancy complications. Our results are in agreement with two Australian studies, one in 2012 (Matthey, 2012) and the other done in 2013 similar to a Canadian study in 2004 (Husain, 2013). The considerable sense of fear from losing the child might have explained the association with PPD.

Less than half of participants (42.9%) who didn't want the last pregnancy were significantly complained from PPD. This was similar to the finding noticed in an Iranian study in 2014 where they found a significant relationship between unplanned pregnancy and PPD (Mazaheri, 2014).

Similarly, findings were reported from study in USA (Nakku, 2007). Pregnancy and childbirth as pathological stress are known to human. But, if the pregnancy is unplanned, the problems get complicated as unwanted pregnancy can lead to a state of rejection of baby and followed by many psychological and physical problems to the mother.

PPD was found to be associated with family support. PPD in the current study noticed in 50.7% of participants who had inadequate family support (parents or husbands). Similar results were concluded by Canadian researchers in their study in 2010 who noticed that lack of postnatal family care is an important risk factor of PPD (Ri-Hua, 2010). Similar findings were reported in a study on Chinese women in Canada (Xie, 2010). This might emphasize the importance of the relationship with the husband and its role in postpartum disorders, especially depression. This factor was a significant, independent and un-confounded risk factor for PPD.

In addition, more than half of participants who had emotional problems or domestic violence with their husbands or other close relatives (51.4%) were significantly suffered from PPD. This is in agreement with two studies conducted in India (Upadhyaya 2014) and UK (Upadhyaya, 2012) which reveals that marital discord or intimate partner violence has negative impact on postpartum mental health. In Sweden (2005) they found that single mothers or those women not in contact with the father of the child are at increased risk of PPD (Nager, 2005). This factor was a significant independent and un-confounded risk factor for PPD.

Previous depression or anxiety was a significant determinant of PPD in this study as 58.5% of the participants who had a history of depression or anxiety before or during the pregnancy suffered from PPD. Previous history of depression or anxiety is among the factors that are associated with a higher risk of PPD as reported in study conducted in Canada 2011 where they found that a history of depression and anxiety predicted women to be at an increased risk of PPD development (Davey, 2011). Other study done in Iran 2009 also considered history of depression or anxiety as a powerful factor in PPD (Kheirabadi, 2015) The occurrence of mental disorders such as depression during pregnancy is a powerful factor in predicting PPD as stated by a study conducted in the USA (Lancaster, 2012).

Finally, positive family history of mental disorders was significantly associated with PPD, where 70.3% of those with positive family history developed PPD later. This is similar to a study done in Saudi Arabia in 2014 that found family history of depression as the strongest predictor of PPD (Alasoom, 2014). A study in India (2014) revealed a completely different result as considered family history of psychiatric disorder as a non-significant factor (Upadhyaya, 2014). This factor was a significant, independent and un-confounded risk factor for PPD.

Conclusions

1. More than one third of newly delivered mothers were complaining of PPD.
2. The most vulnerable mothers to PPD were those who had:
    a. Born children suffering from neonatal complications
    b. Symptoms of depression or anxiety before or during the pregnancy
    c. Inadequate family care
    d. Pregnancy complications
    e. Family history of mental disorders
    f. Family discord or domestic violence

Recommendations

- Spreading of community awareness for mental health concepts on a wider scale.
- Promotional and preventive interventions to reduce the prevalence of PPD through:
    - Emphasize the importance of the counseling before the marriage as well as before and during the pregnancy and educate the couples on the importance of family planning
    - Mental health assessment of mothers through the antenatal care program in PHCCs.
    - Early detection of mental disorders among the most vulnerable mothers and activating the referral system to Psycho-social Health Units in PHCCs to provide the necessary managements.
    - Family education about providing adequate health care to the mother and her child during and after the pregnancy.
- Integrating mental health care into maternal and child health programs at primary and secondary level of health care
- Integrating mental health services with governmental and non-governmental institutions to promote women's empowerment programs.
- Insure woman's rights and reduce all types of violence like physical, psychological, sexual, and economical.

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The Proportion of Vitamin D Deficiency in Reproductive Age Group Women in Baghdad/Iraq, and its Association with Menstrual Cycle Characteristics and Anthropometric Measurements

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Abstract

Background: Vitamin D deficiency is the most common medical condition worldwide. The prevalence especially in developing countries, varying widely by and within regions between 30–90%. Anti-Mullerian Hormone regulates follicular recruitment, which provides a mechanism for vitamin D to influence ovarian function and menstrual cycle regularity. Many conditions may influence the vitamin D status and excess body weight may be one of them. Body fat may act as a reservoir for storage of the fat soluble vitamin D, reducing its bioavailability**.**

Objectives: To determine the proportion of vitamin D deficiency and its association with menstrual cycle characteristics and anthropometric measurements in reproductive age group women in Baghdad/Iraq.

Methodology: A cross-sectional study, a total number of 99 women within the reproductive age, divided into two groups: 49 women, classified as having oligomenorrhea, and polymenorrhea, were selected for having irregular menstrual cycle. 50 women with regular cycle were included as a control group. Height, weight, and waist circumference were measured, and body mass index was calculated. Vitamin D status was determined by the measurement of serum 25 hydroxy vitamin D. Vitamin D status was defined as deficiency if 25 hydroxy vitamin D was <20 ng/mL, insufficiency if between 20-29 ng/mL, and sufficiency if ≥30 ng/mL).

Results: The proportion of vitamin D deficiency among participants was dramatically high (93%). There was no significant relationship between Vitamin D Status and menstrual cycle irregularity, although that irregular cycle group was more likely to be deficient than the regular cycle group (93.9% to 92.0%). There were no significant relationships between vitamin D status and age, age of menarche, marital status, dysmenorrhea, and body mass index. The relationship between vitamin D status and waist circumference was not significant, but the vitamin D deficient group had higher waist circumference (Mean=83.8 cm) compared to the vitamin D sufficient group (Mean=76.6 cm). The effect of vitamin D supplementation was obvious in improving the vitamin D status in the participants.

Conclusion: The proportion of vitamin D deficiency was very high among the studied group. Although that the prevalence of deficiency was higher among irregular cycle group, the relation was not significant. The relation of vitamin D status with other parameters was insignificant.

Key Words: Vitamin D deficiency, Proportion, Irregular menstrual cycle and Body mass index.

نسبة حدوث نقص فيتامين (د) لدى النساء في سن الانجاب في بغداد وعلاقته مع خصائص دورة الطمث والقياسات الفيزياوية للجسم البشري

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مختبر الصحة العامة المركزي / دائرة الصحة العامة / وزارة الصحة

بغداد_العراق

الخلاصة

المقدمة: نقص فيتامين دال هو أكثر الحالات الطبية شيوعًا في جميع أنحاء العالم .الانتشار خاصةً في البلدان النامية، حيث تتفاوت بشكل كبير بين المناطق وضمن المناطق بين 30–90٪ . توجد مستقبلات لفيتامين د في المبيض والمشيمة والرحم . يرتبط انخفاض فيتامين دال بأورام الرحم الليفية. هورمون (AMH)، ينظم التجنيد الجرابي، والذي يوفر آلية لفيتامين دال للتأثير على وظيفة المبيض وانتظام الدورة الشهرية. العديد من الحالات قد تؤثر على حالة فيتامين د وقد يكون الوزن الزائد أحدها .قد تعمل دهون الجسم كمخزن لتخزين فيتامين دال القابل للذوبان في الدهون، مما يقلل من توافره الحيوي.

الأهداف: تحديد نسبة انتشار نقص فيتامين دال وارتباطه بخصائص دورة الطمث وقياسات الجسم الأنثوي في مجموعة النساء في سن الإنجاب في بغداد / العراق.

المنهجية: تم إجراء دراسة مستعرضة، عن طريق اختيار 99 امرأة في سن الإنجاب، مقسمة إلى مجموعتين: 49 امرأة، مصنفة على أنها تعاني من قلة الحيض، وفترة طويلة من فترة انقطاع الطمث، حيث تم اختيارهن لدورة الحيض غير المنتظمة .أدرجت 50 امرأة مع دورة منتظمة كمجموعة للمقارنة. تم قياس الطول والوزن ومحيط الخصر، وتم حساب مؤشر كتلة الجسم .تم تحديد حالة فيتامين د من خلال قياس مصل 25 هيدروكسي فيتامين دال تم تعريف حالة فيتامين دال على أنها نقص إذا كان تركيز 25 هيدروكسي فيتامين دال اقل من 20 نانوغرام / مل، وعدم كفاية إذا كان بين 20–29 نانوغرام / مل، وكفاية إذا كان أكثر من 30 نانوغرام /مل.

النتائج: كان انتشار نقص فيتامين دال بين المشاركات عالياً بشكل كبير (93 ٪). لم تكن هناك علاقة ذات دلالة إحصائية بين حالة فيتامين د وعدم انتظام الدورة الشهرية، على الرغم من أن مجموعة النساء ذوات الدورات غير المنتظمة كانت أكثر عرضة للنقص من مجموعة الدورة الشهرية المنتظمة (93.9٪ إلى 92.0٪). لم تكن هناك علاقات ذات دلالة إحصائية بين حالة فيتامين دال والعمر، وعمر الحيض، والحالة الاجتماعية، وعسر الطمث، ومؤشر كتلة الجسم). لم تكن هناك علاقة ذات دلالة إحصائية بين حالة فيتامين دال ومحيط الخصر، ولكن كانت مجموعة النساء ذوات محيط خصر اعلى (متوسط = 83.8 سم) كان لديهم تركيز فيتامين د أقل مقارنة مع مجموعة النساء ذوات محيط خصر اقل (متوسط = 76.6 سم). كان تأثير مكملات فيتامين دال واضحا في تحسين حالة الفيتامين.

الاستنتاجات: كان انتشار نقص فيتامين دال عالية جدا بين المجموعة التي شملتها الدراسة .على الرغم من أن انتشار النقص كان أعلى بين مجموعة الدورات غير المنتظمة، إلا أن العلاقة لم تكن ذات دلالة احصائية. لم تكن علاقة حالة فيتامين دال مع المؤشرات الاخرى ذات دلالة احصائية.

الكلمات المفتاحية: نقص فيتامين (د)، معدل حدوث، دورة طمث غير منتظمة و مؤشر كتلة الجسم.

Introduction

Vitamin D is produced endogenously through exposure of skin to sunlight (Carl and others, 2012). Any reference interval for 25(OH) D should not be confused with the"optimal"or "healthy" range for 25(OH) D (Carl et al., 2012). Vitamin D nutritional status is best determined through the measurement of 25(OH) D, because 25(OH) D is the main circulating form of vitamin D, its longer day to-day variation, and because measurement of 25(OH) D is relatively (Lensmeyer et. al., 2006; Hollis, 2007; Hollis, 2008). Vitamin D deficiency is the most common medical condition worldwide (Holic et. al., 2007). The prevalence of vitamin D deficiency among adult population was reported to be 14-59% (Silva et. al, 2011).The prevalence varying widely by and within regions in developing countries between 30–90% (Asma et. al., 2010). The role of Vitamin D in reproduction is a new, active area of investigation (Lerchbaum and Obermayer, 2012; Luck et. al, 2012). Vitamin D receptor is expressed in the ovary, placenta, and the uterus (Baird et. al., 2013). The promoter region for the gene encoding anti-Müllerian hormone (AMH) contains a domain for the vitamin D response element (Malloy et. al., 2009), which provides a mechanism for vitamin D to influence ovarian function and menstrual cycle regularity (Lerchbaum and Obermayer, 2012). The normal menstrual cycle is mostly a reflection of ovarian events (Kieth, 2007). The mean age of menarche is 12.8 years (Asha and Stephen, 2011). Body fat may act as a reservoir for storage of the fat soluble vitamin D, reducing its bioavailability. Lower vitamin D status among obese people might be lower than average exposure of large body areas to the sun (Zoya, 2009).

Aim of the Study

To determine the proportion of vitamin D deficiency and its association with menstrual cycle characteristics and anthropometric measurements in reproductive age group women in Baghdad/Iraq.

Materials and Methods

Participants: In this cross-sectional study, 49 women were selected among the 15-44 years old women who referred to the gynecologic outpatient clinic at Al-Elwiya Maternity and Gynecology Teaching Hospital, Baghdad, Iraq from July 2017-March 2018 for having irregular menstrual cycle. Women were classified as having oligomenorrhea if the cycle lengths were greater than 35 days but less than 6 months, or having polymenorrhea if the frequency of menses was 21 days or less. Women having cramping pain during menses were referred as having dysmenorrhea. 50 women working at the Central Public Health Laboratory with regular menstrual cycle matched in age with irregular cycle group were included in the study as a control group. All the participants provided informed consent and completed a (self) administered questionnaire that includes reproductive, personal, and demographic history data.

Methods

Clinical and anthropometric characteristics: Height, weight, and waist circumference was measured at 08:00 a.m to 10:00 am after 12 hours of fasting. The BMI was calculated using the standard equation and it was classified into four categories; underweight <18.5, Normal 18.5 – 24.9, overweight 25 – 29.9, obesity 30 – 39.9 (Xavier et. al., 2000). Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest (WHO, 2008).

Laboratory measurements: Blood samples were collected in plastic tubes with separation gel and clot activator (WHO, 2010). The Serum was stored at -25˚C until analysis. 25(OH) Vitamin D was measured by VIDAS equipment using the (Enzyme Linked Fluorescent Immunoassay) technique. Calibration, using the calibrator provided in the kit, was performed every time a new lot of reagent was opened, and after 28 days. Quality control materials were tested with each run according to the laboratory local regulations (BioMérieux, 2015).

Statistical analysis: 25(OH) D was structured as a trichotomous variable, as a vitamin D deficiency if the 25(OH) D concentration was below 20 ng/mL, vitamin D insufficiency if the 25(OH) D concentration was between 21–29 ng/mL, and as vitamin D sufficient if the 25(OH) D concentration was between 30 – 100 ng/mL based on Endocrine Society Clinical Practice Guideline (Michael et. al., 2011). Data were reported as mean ± SD for continuous variables and as numbers (frequency) or percentage for categorical variables. Clinical and biochemical characteristics were compared using the Independent sample *t*-test or the chi-square test when the variables were continuous or categorical, respectively. The one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of two or more independent groups.
If $p<0.05$, the relation is considered significant. Statistical analyses were performed using SPSS software, version 24.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion:

The basic characteristics for the two studied groups are shown in (Table 1).

Table (1) Basic characteristics of the participants.

Variables	Regular cycle(n=50)		Irregular cycle (n=49)		P-value
	Mean	SD	Mean	SD	
Age (yr)	33.4	8.4	31.0	9.7	0.197*
Weight (kg)	71.6	13.4	68.6	13.7	0.285*
Height (cm)	159.8	5.8	158.9	7.2	0.536*
BMI (kg/m2)	28.06	5.1	27.08	4.7	0.325*
WC	85.4	10.9	82.6	10.6	0.205*
Marital Status	Single (13) 26% Married (37) 76%		Single(9) 18.3% Married(40) 81.7%		0.361**
Dysmenorrhea	(23) 46%		(40) 81.6%		0.000**
Vitamin D Supplementation	(3) 6.1 %		(23) 46%		0.000**
Age of Menarche	12.8	1.6	12.7	1.4	0.64*

*** Independent sample *t*-test was applied.**
**** Chi square test was applied**

The prevalence of vitamin D deficiency among participants was high (Figure 1) 93% of the participants were deficient with vitamin D (< 20 ng/mL).

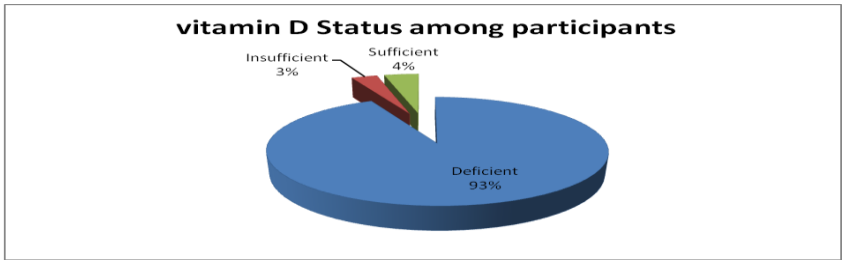

Figure (1) The prevalence of vitamin D deficiency among all participants.

The data collected by the National Health and Nutrition Examination Surveys within North America document a 4-fold increase in the prevalence of vitamin D deficiency over the past 10–15 years with as much as 36% of the USA population being affected (Nesby-O'Dell et. al, 2002; Looker et. al., 2008). The prevalence of vitamin D deficiency especially in developing

countries, varying widely by and within regions between 30 – 90% (Asma et. al., 2010). A study of Al-Hilali K. A. found that the prevalence of below optimal level for 25(OH) D in child-bearing women in Iraq was 90 % (Al-Hilali, 2016). Shiva Faghih and her colleagues concluded that 95.1 % of female university students in Shiraz, Iran were not sufficient with vitamin D (Shiva et. al., 2014). A very low serum level of Vitamin D which ranges between (4.0-15.8 ± 11.6 ng/ml) with a mean level of (<10 ng/ml) was observed in many countries of the Middle East and Africa (Mithal *et. al.*, 2009; Moussavi et. al., 2005; Siddiqui and Kamfar, 2007; Elsonbaty and Abdul-Ghaffar, 1996).

In our study, the prevalence of vitamin D deficiency in child bearing women was dramatically high (93.0%); a finding that is consistent with the above studies that were done in Iraq and in some of the neighboring countries suggesting that there are similar etiological factors for these findings.

Latitude, season, aging, sunscreen use, and skin pigmentation influence production of vitamin D3 by the skin. Only a few foods primarily fish liver oils, fatty fish, and egg yolks naturally contain significant amounts of vitamin D. Consequently most vitamin D in the body is that produced by synthesis in the skin. Circulating concentrations of 25(OH) vitamin D may be decreased by reduced availability of vitamin D, inadequate conversion of vitamin D to 25(OH) vitamin D, accelerated metabolism of 25(OH) vitamin D, and urinary loss of 25(OH) vitamin D. Reduced availability of vitamin D occurs with inadequate exposure to sunlight, dietary deficiency, malabsorption syndromes, and gastric or small bowel resection (Carl *et. al.*, 2012).

Therefore, we can consider women wearing style, those spending most of their time at indoor places, and those using sunscreen lotion to prevent the harmful effect of sunrays especially during summer, are vulnerable to vitamin D deficiency in our population in Iraq, as the main source of vitamin D is by its synthesis in the skin, putting in mind the dietary habits of Iraqi people that could be poor with vitamin D containing food. This may explain the high prevalence of vitamin D deficiency in our studied group.

There was no significant relationship between Vitamin D Status and menstrual cycle status (Table 2).

Table (2) The relation between Vitamin D Status and regularity of menstrual cycle.

Vitamin D status	Regular cycle(n=50)		Irregular cycle(n=49)		P-Value*
	Frequency	%	Frequency	%	
Deficient < 20 ng/mL	46	92	46	93.9	0.503
Insufficient 20-29 ng/ mL	1	2	2	4.1	
Sufficient 30-100 ng/ mL	3	6	1	2	

In our study, we found that irregular cycle group was more likely to be deficient with vitamin D than regular cycle group (93.9% to 92.0% respectively). However, there was no significant relationship between Vitamin D Status & Menstrual Cycle Status.

Anne Marie Z Jukic and her colleagues found that lower levels of 25(OH) D were not associated with short or long menstrual cycles (Anne et. al., 2015), a finding agrees with our study. Anne Marie Z Jukic and her colleagues did another study at 2018, a study showed different results. They concluded that lower levels of 25(OH) D are associated with longer follicular phase and an overall longer menstrual cycle, when they studied late reproductive-aged women in North Carolina (2010-2015) (Anne et. al., 2018). There is some, but limited, evidence for beneficial effects of Vitamin D supplementation on menstrual dysfunction in women with PCOS. While the results of human studies are contradictory the role of Vitamin D on human fertility and reproductive physiology merits further assessment by appropriate longitudinal studies. However, the effects of Vitamin D deficiency on human reproduction and fetal development are poorly studied (Dipanshu and Ratnabali, 2015). More than that,

among the total number of the participants (N=99) , only four(4) women have Sufficient level of 25(OH) D (30-100 ng/mL) which mean that Vitamin D. deficiency is a very common problem in our community , so studying the effect of vitamin D deficiency on other parameters could be difficult. The high prevalence of vitamin D deficiency in Iraq (Al-Hilali, 2016) and other neighboring countries in the Middle East (Shiva *et. al.*, 2014; Elrassi et. al, 2009; Moussabi *et. al.*, 2005; Siddiqui and Kamfar, 2007; Elsounbaty and Abdul-Ghaffar, 1996; Samira *et. al.*, 2016) makes it hard to find a significant relationship between 25-OH vitamin D deficiency and other factors such as irregular cycle, and this was the main limitation in our study. Another concept is that there is still no universal agreement about normal levels of vitamin D and its deficiency states (Samira *et. al.*, 2016).
Vitamin D Status did not differ significantly between 68.7% of the participants who had dysmenorrhea and 32.3% who did not suffer from dysmenorrheal (Table 3).

Table (3) The relation between Vitamin D Status and dysmenorrhea among participants.

Vitamin D status	Dysmenorrhea (n=68)		Dysmenorrhea (n=31)		P-Value*
	Frequency	%	Frequency	%	
Deficient < 20 ng/mL	62	91.2	30	96.8	0.301
Insufficient 20-29 ng/ mL	3	4.4	0	0	
Sufficient 30-100 ng/ mL	3	4.4	1	3.2	

Previous results showed that High dose vitamin D supplementation can reduce the prevalence of premenstrual syndrome and dysmenorrheal (Afsane *et. al.*, 2018).
There was a significant relationship between Vitamin D Status & Vitamin D Supplementation (Table 4).

Table (4) The relation between Vitamin D Status and Vitamin D supplementation.

Vitamin D status	Vitamin D Supplementation (n=8)		without Vitamin D supplementation (n=91)		P-Value *
	Frequency	%	Frequency	%	
Deficient < 20 ng/mL	3	37.5	89	97.8	0.000
Insufficient 20-29 ng/ mL	2	25	1	1.1	
Sufficient 30-100 ng/ mL	3	37.5	1	1.1	

This finding indicates that there is an important role for the vitamin D supplements on the vitamin D status, and further discussion about introducing these supplements to certain groups like pregnant, elderly, and children should be discussed.
Our study showed that there was no statistically significant effect for marital status of the participants on their vitamin D status (Table 5).

Table (5) The effect of marital status of the participants on their Vitamin D Status.

Vitamin D status	Single (n=22)		Married (n=77)		P-Value*
	Frequency	%	Frequency	%	
Deficient < 20 ng/mL	21	95.5	71	92.2	0.67
Insufficient 20-29 ng/ mL	0	0	3	3.9	
Sufficient 30-100 ng/ mL	1	4.5	3	3.9	

The prevalence of vitamin D deficiency was higher in single women (95.5%) compared the married women (92.2%). Previous studies showed controversial results regarding this finding. Saudi study showed that there was a statistically significant relationship between vitamin D level and marital status and between vitamin D level and educational level. Mean 25(OH) D level was low in single participants compared to married (P=0.014) (Ebtehal, 2012). Also a Turkish study found that vitamin D deficiency rate was almost double as severe in single participants (Hasan and Fevziye, 2017). In studies investigating marital status in the literature, similarly, it was shown that the vitamin D levels of married individuals are higher (Travas *et. al.,* 2009; Kilkkinen *et. al.,* 2008). Our study agree with a study done in Cameroon, a study showed that marital statuses did not have any significant effect on the median vitamin D levels (Delphine *et. al.,* 2018). Vitamin D level was not significantly correlated with marital status in another study in Pakistan (Mahmood *et. al.,* 2009). Also a study by Farid Ahmed Toor and his colleagues showed that when the Vitamin D deficiency was cross tabulated against marital status, statistically the difference was non-significant (Farid *et. al.,* 2016). A study showed that higher proportions of institutionalized subjects, in the lower educational level, single, divorced or widowed, reporting low levels of physical activity and presenting cognitive impairment were also found to be at risk of 25(OH) D deficiencies (Santos *et. al.,* 2017). Therefore, the effect of duration of indoor activities, educational level, physical activity, or even psychological status that may affect food habits can be considered as factors that may contribute to these controversial findings.

Our study showed that there was no statistically significant effect for the age of the participants on their vitamin D status (Table 6).

Table (6) The effect of the age participants on their Vitamin D Status.

Vitamin D status	Frequency	Age (Years)		P-Value*
		Mean	SD	
Deficient < 20 ng/mL	92	32.0	9.2	0.327
Insufficient 20-29 ng/ mL	4	38.7	9.2	
Sufficient 30-100 ng/ mL	3	30.0	4.0	

Baker MR and colleagues showed the decline in vitamin D status with age with the studied females to be between (20-96) years old (Baker et. al., 1980) . A significant inverse relationship between age and 25(OH) vitamin D levels was observed in other studies (Delphine et. al., 2018). Our finding may be explained that the upper limit for the studied group is 45 years. Another study showed that age groups from (51 – 70 years) and (> 70 years) had less 25 OH vitamin D concentrations than younger age groups (Bailey et. al., 2010).

Our study showed that there was no statistically significant effect for the age of menarche of the participants on their vitamin D status (Table 7).

Table (7) The effect of the age of menarche of participants on their Vitamin D Status.

Vitamin D status	Frequency	Age of Menarche (Years)		P-Value*
		Mean	SD	
Deficient < 20 ng/mL	92	12.7	1.5	0.341
Insufficient 20-29 ng/ mL	4	13.0	1.8	
Sufficient 30-100 ng/ mL	3	14.0	1.0	

Previous studies showed no relation between vitamin D status and age of menarche (Hua, 2016). Our study found that the mean age of menarche in the vitamin D deficient group is less than that of the vitamin D sufficient group. A previous study showed that girls with evidence of vitamin D deficiency; 25(OH) D <20 ng/mL) were twice as likely to reach menarche during the observation period of 30 months compared with girls who were vitamin D sufficient (Eduardo *et. al.*, 2011).

Our study showed that there was no statistically significant effect for the BMI of the participants on their vitamin D status (Table 8).

Table (8) The relation between Vitamin D Status and BMI of participants.

Vitamin D status	Frequency	BMI		P-Value*
		Mean	SD	
Deficient < 20 ng/mL	92	27.6	5.0	0.33
Insufficient 20-29 ng/ mL	4	29.4	2.2	
Sufficient 30-100 ng/ mL	3	23.8	4.0	

Vitamin D deficiency was more prevalent among obese participants (97%) than the normal weight participants (93.9%), but there was no significant statistical difference between the vitamin D status in the three weight categories (Table 9).

Table (9) Vitamin D status among the three weight categories of the participants.

Weight(n=99)	Vitamin D status(Frequency/Percent)			P-Value*
	Deficient < 20 ng/mL	Insufficient 20-29 ng/ mL	Sufficient 30-100 ng/ mL	
Normal(n=33)	31(93.9%)	0(0%)	2(6.1%)	0.125*
Overweight(n =33)	29(87.9%)	3(9.1%)	1(3%)	
Obese(n=33)	32(97%)	1(3%)	0(0%)	

The results of previous studies are controversial. Some studies showed that Vitamin D deficiency was significantly associated with a BMI of 85 kg/m2 or higher (Mohan *et. al.*, 2017; Zoya *et. al.*, 2009; Reinehr et. al., 2007; Bischof *et. al.*, 2006).

The association serum 25(OH) D is stronger with adiposity than that with body weight and BMI (Arunabh *et. al.*, 2003). The reason for that may be explained by the fact that BMI and body weight do not necessarily reflect the percentage of body fat. Athletes and well trained persons may have relatively high BMI and may be considered overweight or even obese, while they have quite low total fat mass (Ode *et. al.*, 2007). The above causes could contribute to the finding of our study.

The relationship between vitamin D status and waist circumference was not significant (p=0.075) (Table 10), although that the deficient group had higher waist circumference (Mean=83.8 cm) compared to the sufficient group (Mean=76.6 cm) (Figure 2).

Table (10) The relation between Vitamin D Status and Waist Circumference of Participants.

Vitamin D status	Frequency	WC (cm)		P-Value*
		Mean	SD	
Deficient < 20 ng/mL	92	83.8	10.8	0.075
Insufficient 20-29 ng/ mL	4	94.5	6.0	
Sufficient 30-100 ng/ mL	3	76.6	5.0	

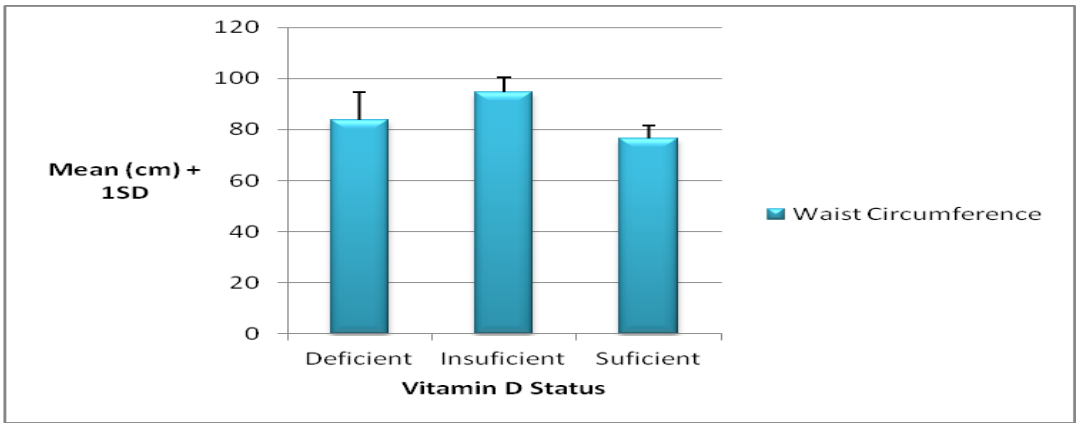

Figure (2) Waist Circumference in participants according to Vitamin D Status Category.

Previous studies done in eastern Africa showed that there was no significant correlation between central obesity and serum concentration of 25(OH) vitamin D, except in the males, which showed a significant negative correlation, despite the high prevalence of central obesity alongside a high prevalence of 25(OH) D insufficiency in the studied population (Gitahi and Festus, 2013). Many other cross-sectional studies did not report a correlation between obesity and vitamin D deficiency (Oosterwerff *et. al.*, 2011; Fariborz *et. al.*, 2013). The postulated decreased bioavailability of 25(OH) D from cutaneous stores due to deposition in body fat stores (Wortsman *et. al.*, 2000) was not evident in our population. More than that, females had a higher proportion of central obesity than males (Gitahi and Festus, 2013) so it is interesting that this correlation was not observed.

Our study showed that the relation between vitamin D concentration and waist circumference was more powerful that the relation between vitamin D concentration with BMI. The results of some studies can explain this finding. Dabhani and his colleagues showed that obesity, measured as BMI, and waist circumference were positively associated with vitamin D deficiency with stronger associations observed with waist circumference (Al-Dabhani *et. al.*, 2017). Similar results were found in both prospective and cross-sectional studies (Gagnon *et. al.*, 2012; Hypponen *et. al.*, 2008; Sadiya *et. al.*, 2014; Ardwai *et. al.*, 2011).

Conclusions

Conclusions: The proportion of vitamin D deficiency was very high among the studied group. Although that the proportion of deficiency was higher among irregular cycle group, the relation was not significant. Vitamin D deficient group had higher waist circumference compared to the vitamin D sufficient group. The relation of vitamin D status with other parameters was insignificant. The effect of vitamin D supplementation was obvious in improving the vitamin D status.

Recommendations

Adding more groups of population of different age, gender to study the prevalence of vitamin D deficiency and its related etiological factors in these groups.

1. Establishment of the reference ranges for vitamin D in the Iraqi population; take into consideration factors like age, gender.
2. The addition of fortified food containing vitamin D to the daily dietary regimes of people at risk of vitamin D deficiency.
3. Studying the response of vitamin D deficient women complaining from menstrual cycle irregularity to vitamin D supplementation within a specified time using prospective cohort study.
4. Measurement of other biochemical markers associated with the normal metabolic pathway of vitamin D such as serum calcium, and parathyroid hormone.
5. Finding the association between vitamin D status with other metabolic syndrome parameters which represent risk factors for cardio vascular diseases.

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C-reactive Protein as Both Specific and Non-Specific Marker for Toxic Thyroid Gland and Breast Cancer Diseases in Iraqi Women

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Abstract

This study indicate to investigate the possibility of using C-reactive protein (CRP) as a marker of toxic thyroid gland and breast cancer in Iraqi women by qualitative test of CRP and detected the level of CRP by using HPLC. The 60 blood samples were examined of Iraqi women patients , which divided in two groups about 30 patients with toxic thyroid gland and 30(100%) patients with breast cancer disease (aged between 14 and 60 years old). Qualitative test was done to detect the presence of CRP in the patient's serum. The qualitative test showed that 30 of patients with toxic thyroid gland disease given positive result to CRP. While 10(33.4%) of patients with breast cancer given positive result and 20(66.6%) given negative result. The HPLC analysis done to determine the concentration of CRP in patient's serum. The HPLC analysis showed that the level of concentration for CRP in serum of patients with toxic thyroid gland was between (7.8- 17.13) mg/l. When the level of concentration of CRP in serum of patients with breast cancer disease before therapy stage was between (27.9 – 45.85) mg/l, while in patients with breast cancer at therapy stage was under detection limit (UDL). According to the results of this study C-reactive protein consider a useful marker for patients with toxic thyroid gland while it consider as non-specific marker for breast cancer diseases.

KeyWords: Toxic thyroid gland, Breast cancer, C-reactive protein, Qualitative test and HPLC.

بروتين سي التفاعلي كمؤشر محدد وغير محدد لمرض الغدة الدرقية السامة وسرطان الثدي في النساء العراقيات.

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الخلاصة

تناولت هذه الدراسة إمكانية استخدام بروتين سي التفاعلي (CRP) كمؤشر لمرض الغدة الدرقية السامة وسرطان الثدي في النساء العراقيات عن طريق الفحص النوعي لـ بروتين سي التفاعلي (CRP) وتحديد مستوى تركيز CRP باستخدام HPLC.

تم فحص 60 عينة دم من النساء العراقيات اللواتي قسمن في مجموعتين 30 مريض بالغدة الدرقية السامة و 30 مريض بسرطان الثدي (تتراوح أعمارهم بين 14 و 60 سنة). تم إجراء الفحص النوعي للكشف عن وجود CRP في مصل المرضى. وأظهرت نتائج الفحص النوعي أن (100%) 30 مريض من المرضى المصابون بالغدة الدرقية السامة اعطى نتيجة إيجابية ل CRP في حين أن (33.4%) 10 مريض من الذين يعانون من سرطان الثدي قبل مرحلة العلاج اعطي نتيجة إيجابية و (66.6%) 20 مريض بمرحلة العلاج اعطى نتيجة سالبة. أظهرت نتائج تحليل HPLC لتحديد مستوى

تركيز CRP في مصل المرضى الذين يعانون من تسمم الغدة الدرقية كان ما بين (7.8 – 17.13 ملغم/لتر) عندما كان مستوى تركيز الـ CRP في مصل مرضى السرطان قبل مرحلة العلاج ما بين (27.9 – 45.85) mg\l، في حين كان مرضى سرطان الثدي في مرحلة العلاج هو تحت الحد الادنى للكشف UDL. وفقا لنتائج هذه الدراسة، يعتبر البروتين التفاعلي سي علامة مفيدة للمرضى الذين يعانون من الغدة الدرقية اي يمكن اعتباره مؤشر للإصابة بمرض الغده الدرقية السامة في حين يعتبر بروتين سي التفاعلي مؤشر غير محدد لمرض سرطان الثدي.

الكلمات المفتاحية: الغدة الدرقية السامة، سرطان الثدي، بروتين سي التفاعلي، الفحص النوعي و كروماتوغرافيا سائلة عالية الضغط.

Introduction

Toxic thyroid gland and breast cancer are most commonly forming of diseases in the Iraq country in this time, the rate of occurring in women are in high level. Toxic thyroid gland occur due to the inflammation caused by the autoimmunity and other thyroid diseases are considered to be unrelated with autoimmune processes. It was previously shown that some APRs (Acute-phase reactants) levels increase with several thyroid diseases (Duygu *et. al.*, 2013). (APRs) are known with their involvement as pro-inflammatory molecules in various inflammatory diseases. Most of the APRs generally elevate during inflammation C-reactive protein (CRP) is the well-known APRs . Most commonly used APR is C-reactive protein, which is a globulin type protein that use as marker in inflammation, produced by the liver (Maryam *et. al.*, 2018).

Chronic inflammation plays an important role in the initiation and progression of several cancers. Cancer considers one of the most important health problems of the current era and also a leading cause of death among population. Cancer can simply be defined as a malignant tumor or malignant neoplasm, it includes a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. It can also be defined as a group of disorders that are characterized by uncontrolled division of cells and the ability of these abnormal cells to spread (Blachford *et. al.*, 2002). Chronic inflammation is a key contributor in the development and progression of carcinogenesis. Inflammatory pathways play an important role in the causation of breast cancer (Sandahl *et. al.*, 2017).

Breast cancer is the second most common cancer worldwide and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Although early diagnosis has contributed to the success of therapy, breast cancer remains a major problem of women's health and its incidence is increasing in developing countries (Lanwei *et. al.*, 2015). C-reactive protein (CRP) is a sensitive and widely used systemic marker of inflammation (Lanwei *et. al.*, 2015).

For this reasons C-reactive protein consider one of the most important proteins in the medical field and in identifying disease states associated with inflammation. It belong to pentraxin family of proteins (Maryam *et. al.*, 2017).

The Cytogenetic location for CRP gene: 1q23.2 which is the long (q) arm of human chromosome1 at position 23.2, this position contains gene which encode to CRP (Maryam *et. al.*, 2017). It rapidly increases in cases of inflammation and tissue damage, and quickly returns back to normal levels as soon as the patient's recovery (Maryam *et. al.*, 2018). The normal level of this protein in serum for perfectly healthy individuals, was (1-5) mg/l and if it became more than this level this mean there is problem in the health of person (Maryam *et. al.*, 2018).

Materials and methods

Sample collection:

The 60 human patients with toxic thyroid gland and breast cancer disease were collected. Whole blood were obtained under aseptic conditions, three milliliter (3ml) from each sample by a vein puncture using a disposable syringe. The sera sample were obtained by putting the blood samples in a clean dry gel tubes separately. The tubes centrifuged at 3000 rpm for 10 min. for qualitative test and to determine the concentration of CRP where determine by HPLC. The sera samples were freezing at -20ºC (Qiling *et. al.*, 2013). All cases were diagnosed by the specialist doctor, table (1).

Table (1) The cases and the number of blood sample of each group.

No.	Cases	Number of patients
1	Toxic thyroid gland	30
2	Breast cancer	20
3	Breast cancer (at therapy stage)	10
	Total	60

Qualitative test:

The serum samples containing human CRP were obtained from patients with toxic thyroid gland and breast cancer, by using protocol in the Latex kit for CRP (Cat. No.: 850100A/ lorne laboratories). The process to determine qualitative CRP are as follows: Shake the CRP latex reagent gently and add 100µl to the circle on the glass slide and then add 100µl from the patient serum. Then Mix well by using disposable stirrer spreading the mixture over the whole test area and title the slide gently. Agitate for about 2 minutes by hand and observe for the presence or absence of agglutination. If an agglutination of the latex particles suspension will occur within 2 minutes this meaning positive result and if no agglutination will occur this meaning negative result**.** This kit indicating a CRP level of more than 6 mg/l.

Determine the concentration of the CRP in serum using high-performance liquid chromatography.

I. HPLC: The HPLC method is low cost and can be obtained at highest sensitivity using to measure very low concentrations in serum (Hall *et. al.*, 2013). Samples have been analyzed via the system of high performance liquid chromatography (HPLC), model Sigma LC-20AB equipped with binary delivery pump model LC-20AD. The eluted peaks have been observed with UV-Vis detector. The preparation conditions are depicted in table (2).

Table (2) Conditions of high performance liquid chromatography.

Parameter	Characteristic for CRP identification
Detector	UV spectrophotometer 280nm
Flow rate	1.1 ml/min
Volume injection sample	100µl
Type of column	C18-ODS (10µm,25cm*4.6mm)
Mobile phase	Methanol:deionized water:acetonitritle 80:19:1v/v
Temperature	24ºC

II. Preparation of sample: After 1min vortex, 100µl from sample (serum) was obtained and injected into HPLC.

III. Calculation: The region under a peak was utilized to calculate the concentration of a sample as the following formula (Maryam *et. al.*, 2018):-

$$CS = \frac{\mathbf{ASA}}{\mathbf{ASD}} \times SC$$

Where:-

CS is Concentration of sample (mg/l).
ASA is the area of the sample.
ASD is the area of the standard.
SC is standard concentration.

Results and discussion:

Qualitative test: Serum specimens were tested for presence of CRP using latex kit for CRP, Figure (1) shown the presence or absence of agglutination.

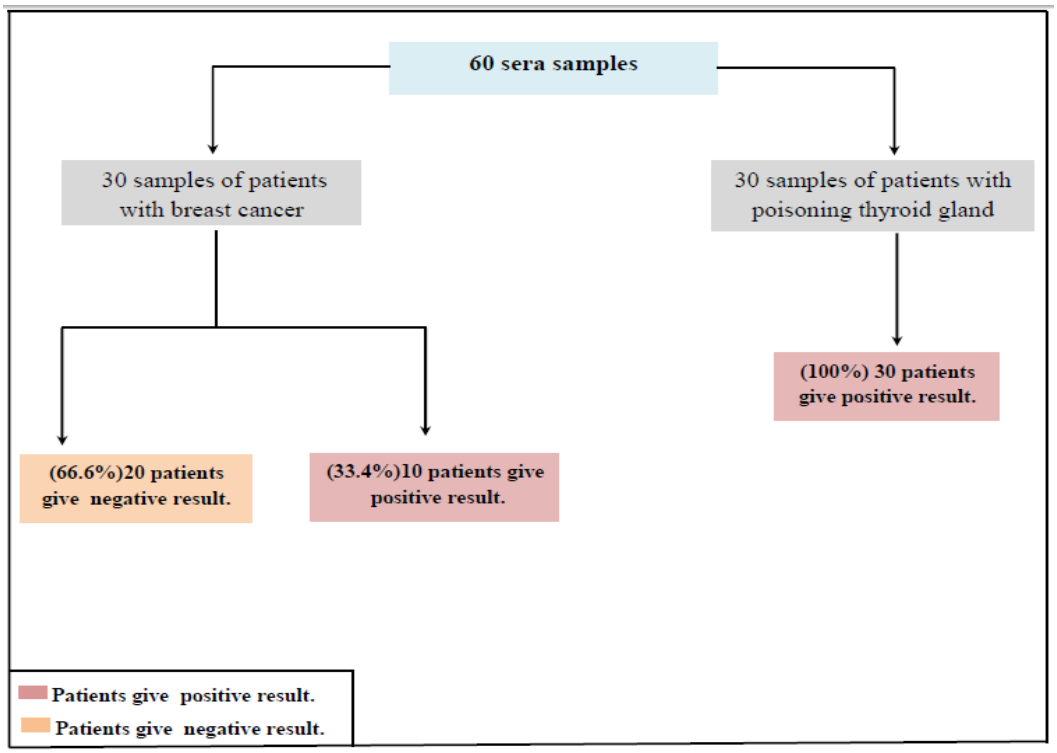


Figure (1) Shown the presence or absence of agglutination in sera samples by using latex kit of CRP.

The circles from 1 to 3 show the results of presence or absence of agglutination in serum samples of patients with breast cancer and only circle 2 gave positive result(presence of agglutination), while 1 and 3 circles gave negative results (absence of agglutination). The circles from 4 to 6 gave positive results or show the presence of agglutination in sera samples of patients with toxic thyroid gland. The results of qualitative test are shown in the, Figure (2). These included (60) sera sample of patients divided into two groups: first group consist of 30 patients with toxic thyroid gland. Second group consist of 30 patients with breast cancer some of them in therapy stage, recorded in table (3).

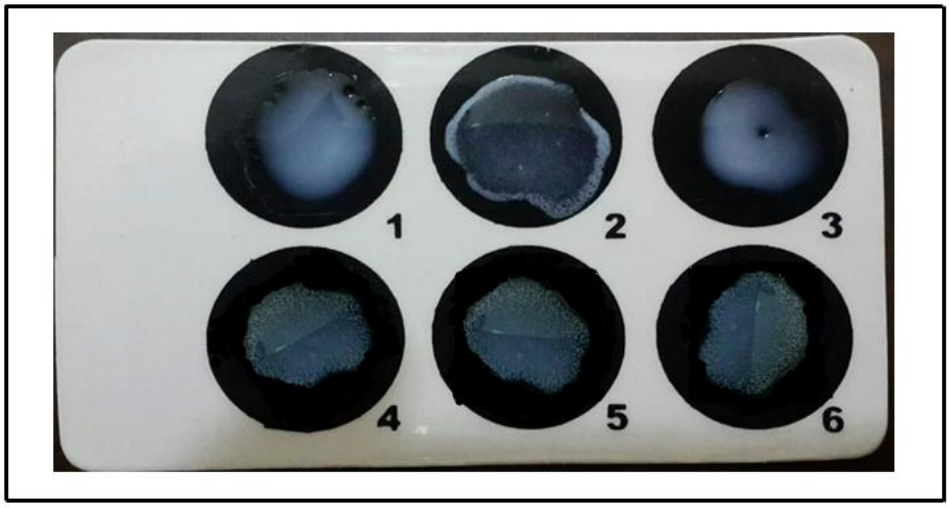
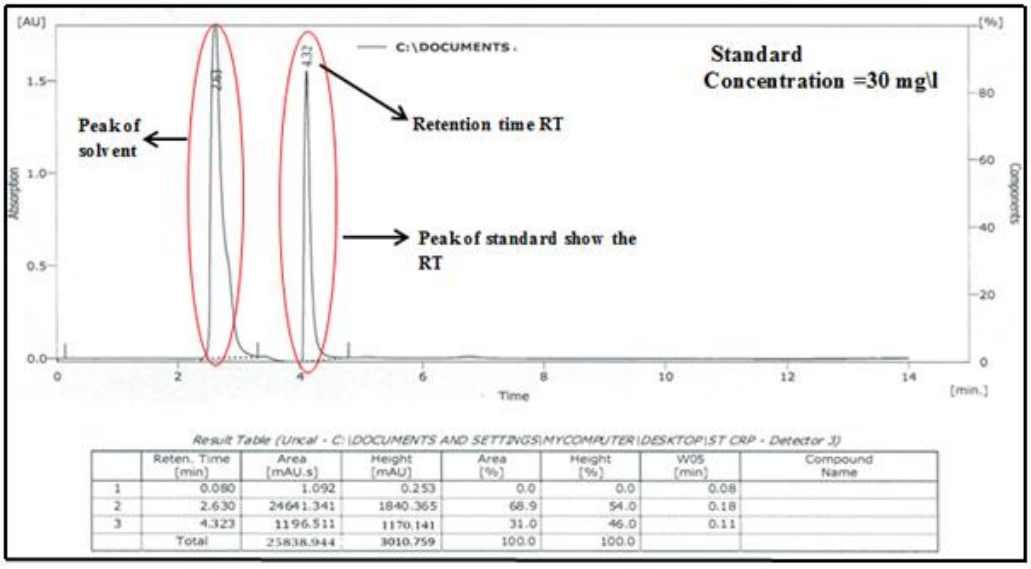


Figure (2) percentage of positive and negative samples to CRP in patient with Poisoning thyroid gland and cancer disease by qualitative test.

10 (33.4 %) patients give positive result and 20 (66.6%) patients with breast cancer in the therapy stage give negative result to CRP while the study of (Shilpa *et. al.,* 2015), Which proved that the CRP was act as marker for predictor and to identify the risk of breast cancer. 30 (100%) patients with toxic thyroid gland give positive result to CRP this result agree with study of (Manash *et. al.,* 2012), which significantly that higher rise in serum CRP level in patients with thyroid disease. The inflammation consider common causes of thyroid disease this make the CRP one of the most important marker of this disease (Manash *et. al.,* 2012), because in the case of response to inflammation the macrophage and neutrophil cells secretion the group of cytokines in the blood, the most notably interleukins IL-1, IL-6 and L-8. This make the liver responds and works to produce large amounts of CRP. While the level of CRP in the blood of the patients with cancer disease was decrease after the chemical and radiation therapy (Hall *et. al.,* 2013), this make the presence of CRP uncommon in all



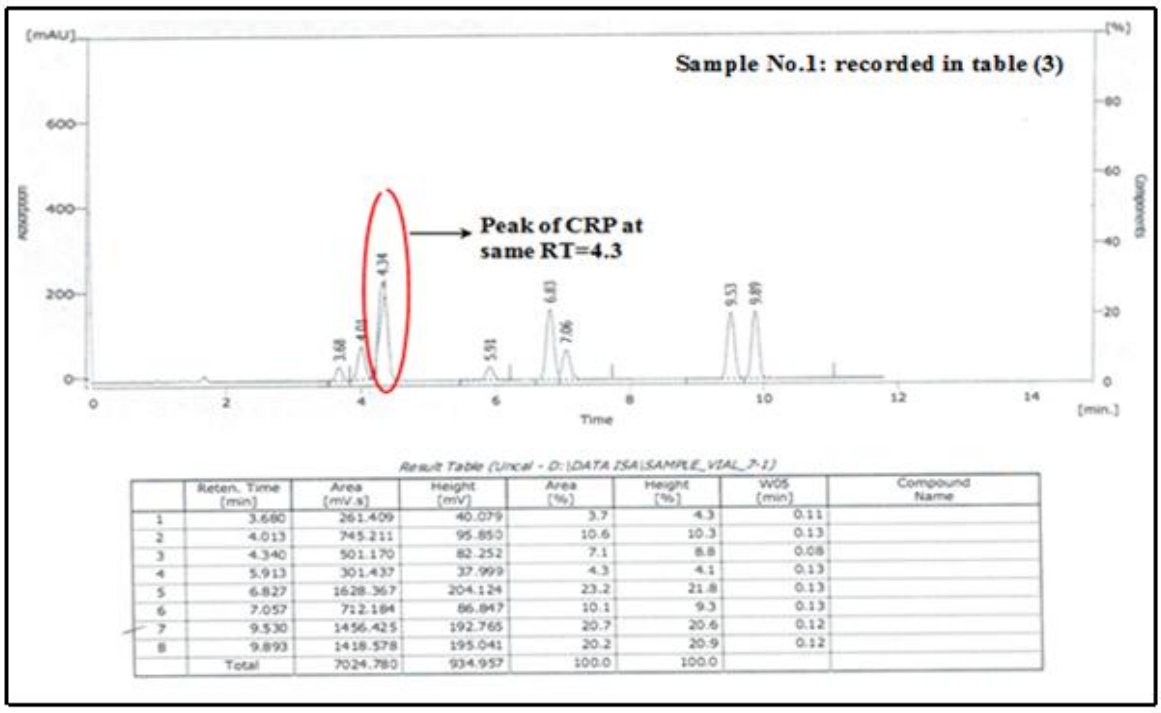
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.080	1.092	0.253	0.0	0.0	0.08	
2	2.630	24641.341	1840.365	68.9	54.0	0.18	
3	4.323	11196.511	1170.141	31.0	46.0	0.11	
	Total	25838.944	3010.759	100.0	100.0		

Figure (3) The HPLC Chromatogram signal of Standard CRP level, its retention time (RT) is 4.32 min

patients with cancer disease after therapy for this reason this protein doesn't act as good marker for cancer in therapy stage .

Serum CRP concentration: In the present study, it has been developed through examination to assess the CRP activity in serum of patients. Figure (3) shown that a complete baseline separation was obtained within CRP by HPLC.

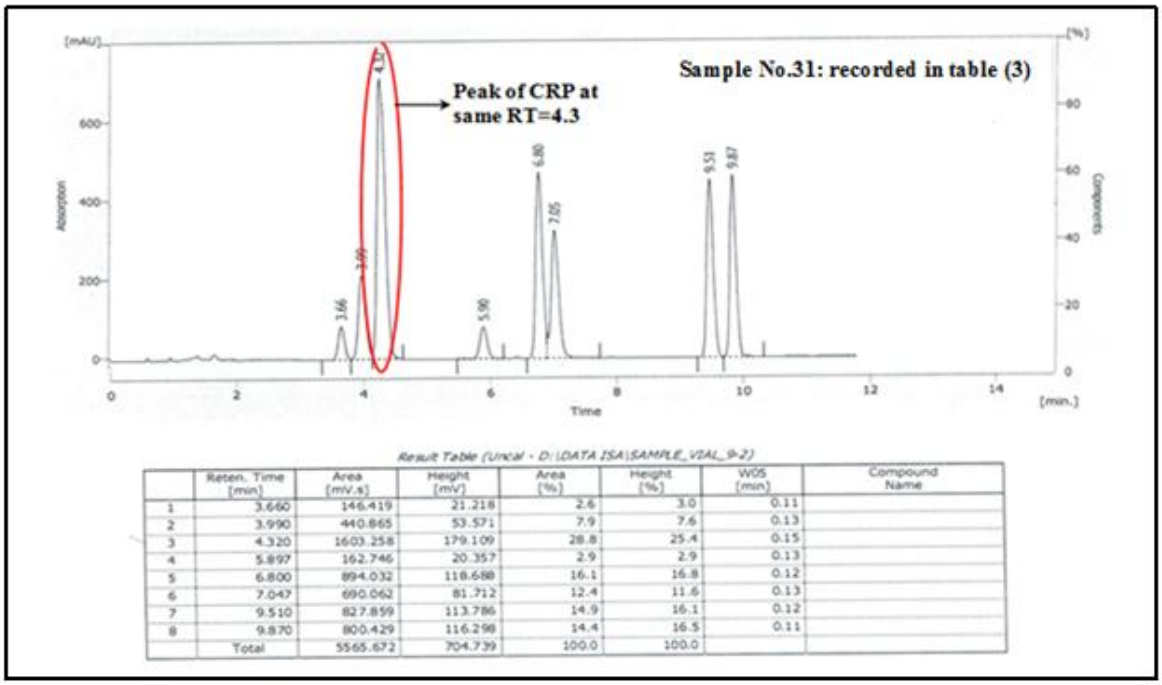
As it is shown in, Figures [(4), (5) and (6)] distinct difference exit obtained in the curves peaks between patients with poisoning thyroid gland and breast cancer, in comparison with the standard material CRP. Curved of peaks the standard material appeared when CRP analysis at minute (RT =4.32 min) which its retention time (RT) for the emergence of substance analysis using the above mentioned conditions. At the same time the concentration of CRP of patient with toxic thyroid gland less than samples with breast cancer.



Result Table (Uncal - D:\DATA ISA\SAMPLE_VIAL_7-1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.680	261.409	40.079	3.7	4.3	0.11	
2	4.013	745.211	95.850	10.6	10.3	0.13	
3	4.340	501.170	82.252	7.1	8.8	0.08	
4	5.913	301.437	37.999	4.3	4.1	0.13	
5	6.827	1628.367	204.124	23.2	21.8	0.13	
6	7.057	712.184	86.847	10.1	9.3	0.13	
7	9.530	1456.425	192.765	20.7	20.6	0.12	
8	9.893	1418.578	195.041	20.2	20.9	0.12	
	Total	7024.780	934.957	100.0	100.0		

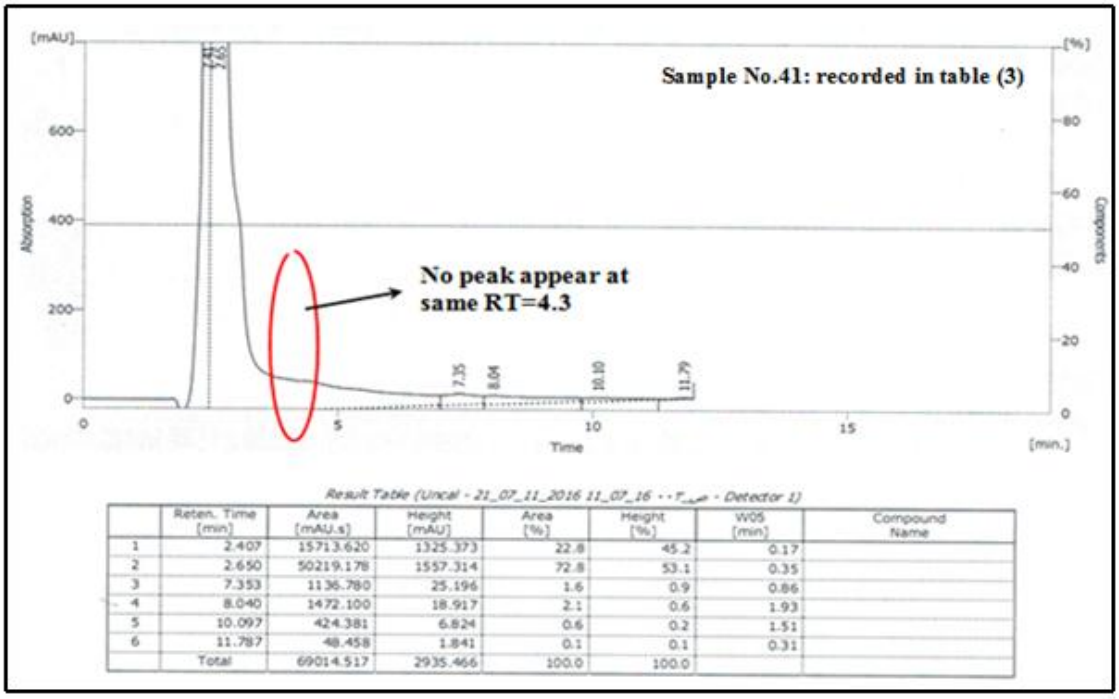
Figure (4) The HPLC Chromatogram signal of CRP Levels in Serum of patient with toxic thyroid gland and its Retention Time (RT= 4.34 min).



Result Table (Uncal - D:\DATA ISA\SAMPLE_VIAL_9-2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.660	146.419	21.218	2.6	3.0	0.11	
2	3.990	440.865	53.571	7.9	7.6	0.13	
3	4.320	1603.258	179.109	28.8	25.4	0.15	
4	5.897	162.746	20.357	2.9	2.9	0.13	
5	6.800	894.032	118.688	16.1	16.8	0.12	
6	7.047	690.062	81.712	12.4	11.6	0.13	
7	9.510	827.859	113.786	14.9	16.1	0.12	
8	9.870	800.429	116.298	14.4	16.5	0.11	
	Total	5565.672	704.739	100.0	100.0		

Figure (5) The HPLC Chromatogram signal of CRP levels in Serum of patient with breast cancer and its Retention Time (RT= 4.32 min).



Result Table (Uncal - 21_07_11_2016 11_07_16 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.407	15713.620	1325.373	22.8	45.2	0.17	
2	2.650	50219.178	1557.314	72.8	53.1	0.35	
3	7.353	1136.780	25.196	1.6	0.9	0.86	
4	8.040	1472.100	18.917	2.1	0.6	1.93	
5	10.097	424.381	6.824	0.6	0.2	1.51	
6	11.787	48.458	1.841	0.1	0.1	0.31	
	Total	69014.517	2935.466	100.0	100.0		

Figure (6) The HPLC Chromatogram signal of CRP levels in patient with breast cancer at therapy stage and no peak appeared at same standard RT.

The concentration of CRP in serum had shown increase in the patients with toxic thyroid gland and breast cancer more than normal range (1-5) mg/l, this agreement with study (Manash *et. al.*, 2012; Kao *et. al.*, 2006) which explain that the concentration of CRP increase during the inflammation and the main causes of cancer and thyroid disease was the inflammation typically the response to inflammatory was a cytokines. One of the cytokine binding with CRP synthesis was IL-6. Its appears to be the main regulator, by promoting synthesis of CRP via liver, during inflammation IL-6 produce this lead to CRP synthesis and

therefore concentration increase, as shown in table (3)**.** But the concentration of CRP in serum of patients with breast cancer increase more than level of CRP of patients with poising thyroid gland. And also the patient with breast cancer at therapy stage didn't give a peak at RT=4.32, as shown in table (3).

Table (3) The concentration of CRP in patients with toxic thyroid gland and breast cancer.

No.	Description	Concentration mg/l
1	Toxic thyroid gland	12.6
2	Toxic thyroid gland	11.27
3	Toxic thyroid gland	10.01
4	Toxic thyroid gland	8.62
5	Toxic thyroid gland	9.02
6	Toxic thyroid gland	10.57
7	Toxic thyroid gland	9.32
8	Toxic thyroid gland	11.58
9	Toxic thyroid gland	8.03
10	Toxic thyroid gland	12.83
11	Toxic thyroid gland	16.62
12	Toxic thyroid gland	13.74
13	Toxic thyroid gland	10.22
14	Toxic thyroid gland	12.99
15	Toxic thyroid gland	15.84
16	Toxic thyroid gland	7.87
17	Toxic thyroid gland	10.79
18	Toxic thyroid gland	11.59
19	Toxic thyroid gland	14.52
20	Toxic thyroid gland	16.67
21	Toxic thyroid gland	9.75
22	Toxic thyroid gland	15.12
23	Toxic thyroid gland	11.69
24	Toxic thyroid gland	8.57
25	Toxic thyroid gland	9.24
26	Toxic thyroid gland	9.3
27	Toxic thyroid gland	17.13
28	Toxic thyroid gland	9.47
29	Toxic thyroid gland	7.84
30	Toxic thyroid gland	10.79
31	Breast cancer	40.2
32	Breast cancer	33.8
33	Breast cancer	36.21
34	Breast cancer	34.4
35	Breast cancer	38.92
36	Breast cancer	31.76
37	Breast cancer	45.85
38	Breast cancer	41.6
39	Breast cancer	27.9
40	Breast cancer	29.5
41-60	Breast cancer at therapy stage	*U.D.L
***U.D.L: under detection limit**= less than 6mg/l		

In present study as shown in table (3) it's found that CRP has been significantly elevated in patients with toxic thyroid gland and breast cancer disease was more than normal level (1-5 mg/L). This was in agreement with other studies (Manash *et. al*., 2012; Kao *et. al.,* 2006) demonstrated that the normal level of CRP in serum sample was (1-5 mg/l) if concentration of CRP increase more than 5mg/l this meaning elevated in CRP level. The chronic inflammation may be a caused factor in a variety of cancers and thyroid disease. In general, the exposure to inflammation for long time, leads to the risk of cancer. The relationship between inflammation and cancer was that central role in the regulation of inflammatory and immune response was IL-6. Whereas Several studies have indicated that IL-6 plays important roles in cancer progression related to proliferation, migration, and angiogenesis. It was reported previously that, mainly CRP produced by liver in response to IL-6, this explained the relationships between CRP, inflammation and cancer disease.

And also this result in agreement with the study (Arkader *et. al.,* 2016) demonstrated that CRP was significantly elevated in patients with poisoning thyroid gland. The study of (Shilpa *et. al.,* 2015) Which proved that the CRP was act as marker for predictor and to identify the risk of breast cancer While the level of CRP in serum samples of patients with breast cancer at therapy stage was U.D.L (>6 mg/l) this was in agreement with study (Hall *et. al.,* 2013) Which proved that the level of CRP in the blood of the patients with cancer disease was decrease after the chemical and radiation therapy.

Conclusions

C-reactive protein consider useful marker for patients with poisoning thyroid gland and it can be act as marker for predictor and to identify the risk of this disease. While it is non-specific marker for breast cancer diseases depending on our samples. Significant changes in the levels of CRP for the patients with thyroid disorders observed in current study confirm that inflammation has an important role on pathogenesis of thyroid dysfunctions regardless of their thyroid dysfunction type. As well as the present study shown that the concentration of CRP in patients with cancer decrease in therapy stage this make the presence of CRP uncommon in all patients with cancer disease after therapy for this reason this protein doesn't act as good marker for cancer in therapy stage .

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Micro Spectrophotometric Determination Streptomycin Sulfate by Cloud Point Extraction in Pure form and Pharmaceutical Preparation

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Abstract

A new method for the determination of the drug Streptomycin Sulfate in some Pharmaceuticals using (UV Vis). Ag (I) should forms a chelating complex with Streptomycin Sulfate (STR- Ag I) at pH (1- 12) and the best pH for the formation of chelating complexes was pH 12. The method based on cloud point extraction (CPE) using Trtion X-114 as surfactant. The product was stabilized and measured at 404 nm. Beer's law is obeyed in the concentration range of 2.5-30 $\mu g \cdot ml^{-1.}$ Sandell's sensitivity was 0.284738041 $\mu g \cdot cm^{-1}$, the detection limit was 0.274576 $\mu g \cdot ml^{-1}$, and the limit of quantitation was 0.915254 $\mu g \cdot ml^{-1}$. All variables including the metal concentration, reaction time, color stability period, and mole ratio were studied in order to optimize the reaction conditions. The composition of the product was (1:1). The method was effectively useful to determine Streptomycin Sulfate in pharmaceutical dose form, and the attained results were in good agreement with the official and other method in literature .No interference was observed from the commonly encountered additives and excipients. The complex was extracted with ethanol. The mole-ratio method has been used to determine the structure of chelate (STR- Ag I) found to be 1:1 L:M (Ligand: Metal) .

Keywords: Antibiotic drug, Streptomycin, Cloud Point Extraction and Spectrophotometry chelating complex.

التقدير الطيفي المايكروي للستربتوا مايسين بواسطة الاستخلاص بنقطة الغيمة في المادة النقية والمستحضرات الصيدلانية

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الخلاصة

طريقة حديثة لتقدير، ستربتو مايسن في بعض العقارات الصيدلانية وذلك باستخدام تقنية الأشعة فوق البنفسجية المرئية. حيث تكون الفضة معقد كيليتي مع ستربتو مايسن في مدى من قيم pH تتراوح ما بين (1−12) و أن افضل قيمة لـ pH تعطي اعلى ممتصية كانت عند (pH =12) اعتمدت الطريقة على الاستخلاص بنقطة الغيمة (CPE) المادة السطحية المستخدمة هي ترايتون اكس 114 تم تثبيت المنتج وقياسه عند 404 نانومتر. اطاع قانون بير في مدى من التراكيز يتراوح بين 2.5−30 ميكروغرام / مل. كانت حساسية ساندل هي 0,284738041 مايكروغرام / مل، وكان حد الكشف 0.274576 مايكروغرام / مل، وكان حد التقدير الكمي 0,915254 مايكروغرام / مل. تمت دراسة جميع المتغيرات بما في ذلك تركيز الكاشف، زمن التفاعل، فترة استقرار اللون، ونسبة المول من أجل تحسين ظروف التفاعل. طبقت الطريقة بنجاح لتقدير عقار ستربتوا مايسين في المستحضرات الصيدلانية، من

خلال النتائج المستحصلة والخاصة بقيم الاسترجاعية المئوية اظهرت الطريقة ان عدم وجود تاثير للمتداخلات على عملية القياس. وان المعقد الناتج استخلص باستخدام الايثانول وكانت النسبة المولية للمعقد هي (1:1)

الكلمات الدالة: مضادات حيوية، ستربتو مايسن، استخلاص نقطة الغيمة، المطيافية و معقدات مخلبية.

Introduction

Antibiotics are the chemotherapeutic agents that kill or inhibit the growth of microorganisms. These chemical agent s are used to treat disease by destroying pathogenic microorganisms or inhibiting their growth at concentration low enough to avoid undesirable damage to the host. Antibiotics are drugs preparations which contain some chemical substances that are produced by microorganisms and by chemical synthesis. These substances at very low concentrations are known to totally destroy or partially inhibit microorganisms. Antibiotics have wide spread application in the treatment of bacterial disease (Nishant et. al., 2016) Streptomycin sulphate chemically is sulphate(STR),5-(2,4-diguanidino-3,5,6-trihydroxycyclohexoxy)-4-[4,5-dihydroxy-6-(hydroxymethyl)-3-methylamino tetra hydropyran -2-yl]oxy-3-hydroxy-2- methyl tetra hydro furan-3-carbaldehyde (Shafqat et. al., 2012) Molecular formula of Streptomycin sulphate is $C_{21}H_{39}N_7O_{12}$ Molecular Weight (1457.376 g/mol) Melting point $12^{O}C$ ($54^{O}F$), It is sparingly soluble in water , white to off-white powder, STR is one of the most widely used aminolglycoside antibiotics. It is used to treat infections in humans, veterinary medicine, as well as in plant agriculture. Which must be determined and all impurities must meet specified limits before a manufactured lot is used clinically. The current United States Pharmacopeia (USP 30, NF 25) compendial method for streptomycin sulfate measures streptomycin A as the primary antibiotic (2007-2003) The structures of drugs are shown in (Figure 1).

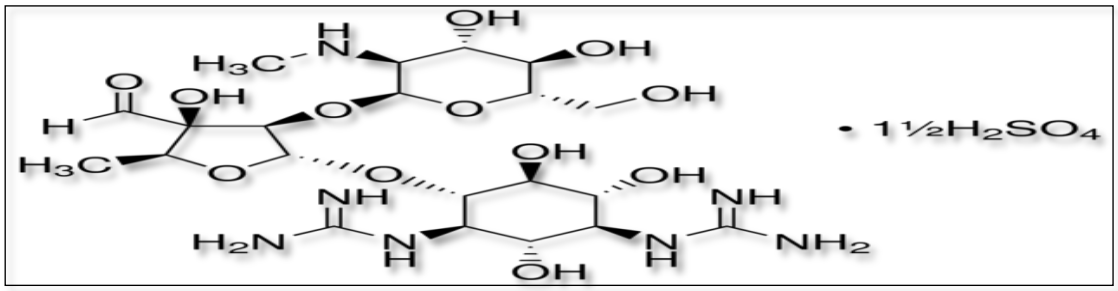

Figure (1) The structure of Streptomycin sulphate.

The cloud point procedure (CPE) is based on the following phenomenon: an aqueous solution of some surfactant be comesturbid and separates in to two isotropic phases if some condition such as temperature or pressure is changed or if an appropriate substance is added to the solution (Saadiyah and Sana, 2015) This salts can be extraction by used cloud point extraction method (Ibrahim et. al., 2018). Cloud point extraction (CPE) is based on the phase behavior of non- ionic surfactants in aqueous solution (Naeemullah et. al., 2013), which exhibit phase separation after an increase in temperature or the addition of a salting out agent (Jawad and Khaleel, 2015). Separation and pre concentration based on (CPE) are becoming an important and practical application of surfactant in analytical chemistry (Bakir and Dhahir, 2013).This method is easy, sensitive, experimental conditions are free as heating and environmental friendly because use a small particular for analysis (Dhahir, 2015).

Apparatus

- UV-Visible recording spectrophotometer (1986) Shimadzu Model (160A) (Japan) with a response time of 0.1s ,was used for spectrophotomatric determination A quartz cell of 5 ml internal volume and 1cm path length was used for absorbance measurements.
- *Hotplate Stirrer* (*Hotplate stirrer Model L-81 Labinco bv*).
- Electric Balance (Sartorius, 4digitals, made in Germany).
- Oven (Memmert, maximum temperature 250, made in western Germany).
- Water Bath **(**A thermostat water Bath, model Unitemp**)**
- Centrifuge (Triup International corp, TRIU 800 Centrifuge, made in Korea**).**
- PH-meter (model BP 3001).

Materials

- A pure grade of Streptomycin Sulfate was obtained from Drug Industries and Midical Appliance (SID) Samarra/ Iraq.
- All the chemical stock solution were prepared from analytical grade BDH.

Preparation of Standard Solutions:

All glassware used was cleaned with distilled water and dried at 50˚C for 30 minute prior to use. Batch experiments were carried out in to ensure the reproducibility of results and the average value. All metal used were of the highest purity and most solutions were prepared in distill water.

- 250μgml^{-1}Stock solution of Streptomycin sulphate was prepared by dissolving 0.025g from Streptomycin sulphate ($C_{21}H_{39}N_7O_{12}$) in distilled water and diluting to the mark in 100ml volumetric flask.
- A 10% (v/v) of Triton X-114 was prepared by diluting 10 ml with water in a 100 ml volumetric flask.
- A solution of 500 ppm of Ag^{+1} was prepared by dissolving 0.7874 gm of $AgNO_3$ in small amount of Water and complete the volume to 1000 ml by using volumetric flask.
- A standard stock solution of sodium hydroxide NaOH (1M) was prepared by dissolving (4g) of the solid product in 100 ml of distill water Then 10 ml of the stock solution was diluted to100 ml with distilled water to Prepare 0.1M solution.

Interference Solutions of 1000 ppm

An amount of 1000 μg ml^{-1} stock solution of interferences is prepared by dissolving 0.1g of the different organic compound such as [Lactose, Starch, Arabic Gum, Glucose and Talc] and inorganic compound such as of $Ca_3(PO_4)_2$ and $CaCO_3$ by 0.2579g and 0.2500g respectively in distilled water and diluting them to the mark in 100 ml volumetric flask

General procedure for CPE

A typical experiment of cloud point include the following steps: In 10ml volumetric flask and added the optimum condition of complex [0.5ml Streptomycin sulfate, 0.4 ml Ag 1.2 ml buffer pH 12 and 1.4ml of 10%(v/v)] Triton X114 then completed to the mark by distilled wate. The content of volumetric flask was transfer to centrifuge test tube then added the mixture in water bath 75^0C at 35 min and separated by centrifugation 3000 rpm at 10 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated. The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS. And the maximum wave length show in figure (1).

Results and discussion:

Absorption Spectra

The spectrum complex product of 0.5ml of (12.5 ppm) of Streptomycin sulphate with of 0.5ml of (25ppm) Silver metal ion which was adopted of CPE for the drug. The absorption spectrum of the complex product formed was also recorded against the corresponding metal blank between 220 to 1100 nm before obtaining optimum conditions according to the recommended CPE procedure show an absorbance at a wavelength at 404nm. The molar absorpetivity value is $3.4308\times10^{-4}Lmol^{-1}cm^{-1}$. The value of molar absorpitivity enables to carry out the quantitative analysis of Streptomycin sulphate in Pharmaceuticals directly as shown in the figure (2).

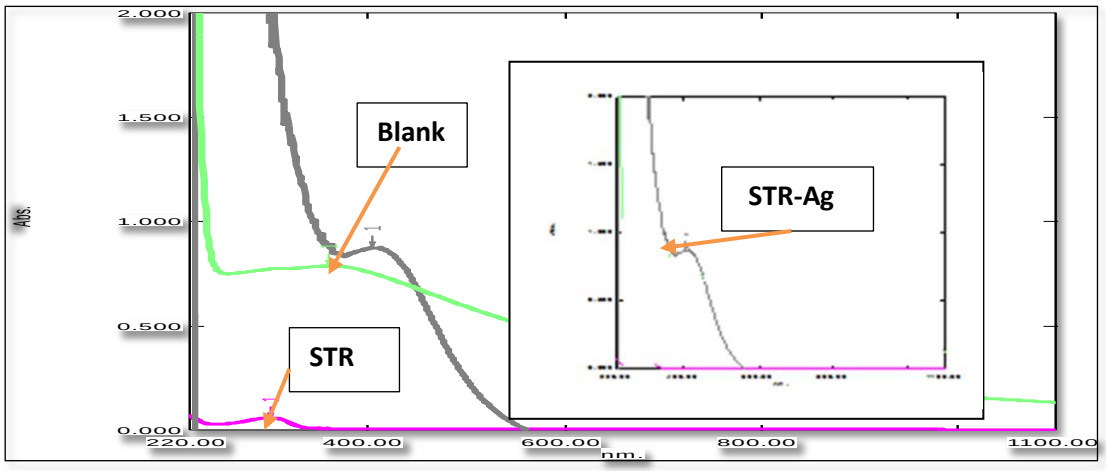

Figure (2) Absorbance spectra of the Resulting complex product.

Optimization of CPE Methodology

A series of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for drug under study using a classical optimization. The variables such as the concentration of metal ion, best of pH, best of buffer, best of volume buffer, Triton X-114 amount, equilibration temperature and incubation time.

Effect of metal ions concentration

The effect of Silver ion concentrations upon the absorbance values of the extracted complexes using (250µg/ml) of drug solution. The optimum concentration of the metal ions that gave maximum absorbance was 500µg/ml of the optimum concentration of Ag (I) ions were for complex The absorbance is measured and the absorbance results to Optimum Volume of (0.4 ml) metal ion.

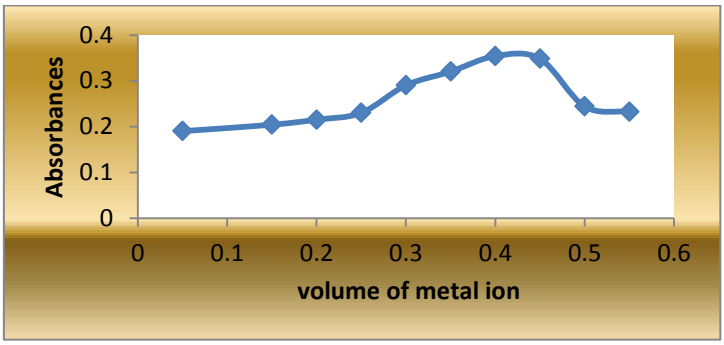

Figure (3) Effect of Optimum concentration on absorbance of (STR-Ag I) complex.

It is obvious that absorbance increase with increase the volume of metal, suddenly the absorbance decrease.

Effect of pH

Cloud point extraction yield plays a unique role on metal-ligand formation and subsequent extraction, and is proved to be a main parameter for CPE the described procedure The maximum sensitivity for CPE was obtained at pH12 to find the best acidic function of the ion extraction process different value of pH 1-14 The results are shown in Fig (4), the best separation was achieved at pH =12 for Ag(I) show the value of absorbance intensity for the complexes drug- Ag against the value of pH, the best values of pH recorded for the highest absorbance values were Plotting of the absorbance values versus the value of pH is shown in Figure (4).

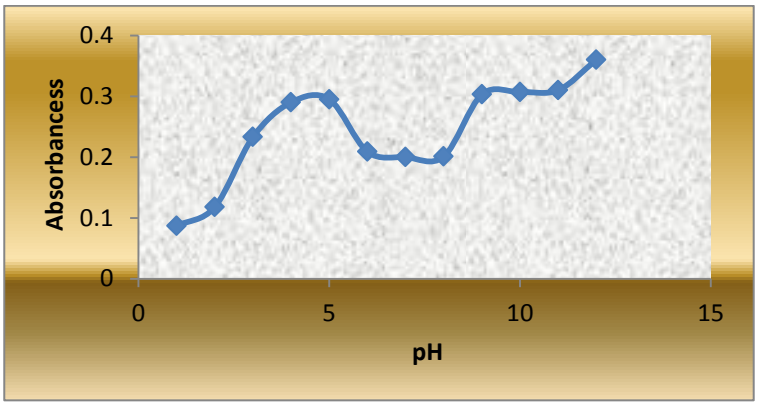

Figure (4) pH effect on absorbance of (STR-Ag I) complex.

Plotting of the absorbance values versus the value of pH is shown in figure (4). Cloud point extraction yield plays aunique role on metal a set of similar experiments in the pH range of 1.0 the described procedure. The maximum sensitivity for CPE was obtained at pH 12. In more basic solutions, deteriorate ion of the signal occurs due to the ligand protonation.

Effect of buffer solutions

The best values of buffer pH 12 recorded for the highest absorbance values were, the absorbance is measured the absorbance results are shown in table (1) for complexes (Ag+ STR)

Table (1) buffer pH 12.

Preparation buffer pH 12	Absorbance
Sodium hydrogen ortho phosphate	0.052
Potassium chloride buffer solutions	0.392

Effect of Volumes buffer solutions

Figure (5) show the value of absorbance intensity for the complexes drug- Ag against the value of buffer solutions, the best value of Potassium chloride buffer solutions recorded for the highest absorbance values.

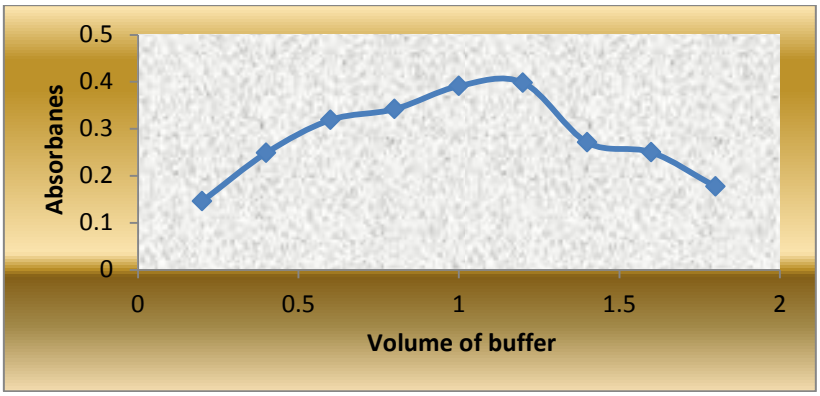

Figure (5) buffer of pH effect on absorbance of (STR-Ag I) complex.

It is evident that absorbance increase with increase the volume of buffer, but suddenly decrease the absorbance because the decomposition happen when increase basicity

Effect Type of Surfactant with metal

The type of surfactant plays very substantial role in cloud point extraction process where each surface owns spectral properties depend on practical basis of Micelles .Aliquots of 10ml of a solution contains [0.5ml Streptomycin sulphate, 0.4ml Ag, 1.2ml buffer pH 12] for Silver metal in 10ml volumetric flask and use different surfactant for each drug [Tween 20, Tween80, CTAP, SDS, Triton X-100, Triton X-114] at 50°C for 10 min for complex incubation time then it centrifugeted at 3000 rpm for 10min, separated the surfactant- rich phase and dissolved in 1ml ethanol then measured by UV-Vis at λ**max** = 404nm for Ag results shown in Table (2)**.**

It was observed that Triton X- 114 which have maximum absorbance at 404 nm. It is clear from the results that the nonionic surfactant Triton X-114 is of high absorbance and this surface increases the efficiency of the extraction process in cloud point extraction.

Table (2) Data of Absorbance to Type of Surfactant with Ag (I)
Plotting the absorbance values of the cloud point versus the type of surfactant is shown in Figure (6).

No	Surfactant	Absorbance at λmax =404 nm for Ag (I)
1	Tween 20	0.192
2	Tween80	0.132
3	SDS	0.225
4	CTAP	0.14
5	Triton X-100	0.031
6	Triton X-114	0.559

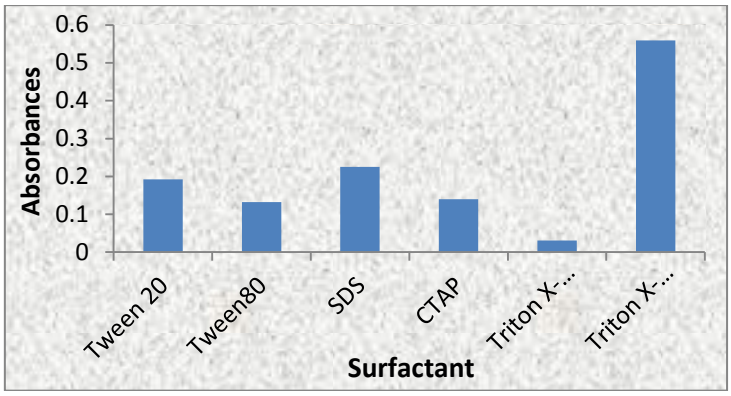

Figure (6) Effect of surfactant type on absorbance of (STR-Ag I) complex.

Effect of Triton X-114 Amount

Most studies confirm that the amount of an nonionic surfactant type TX-114 as an extracting medium plays an important role for maximizing the extraction efficiency by minimizing the phase volume ratio (Vs/Va) and therefore improving the pre-concentration factor of the CPE procedure Therefore, the amount of TX-114 was investigated by varying the volume of 10% TX-114 between (0.2-2.0 ml) for STR. The results are presented in Figure (7). It was noticed that the absorbance values of STR drug continued to increase dramatically and reached maximum at 1.4 ml of 10% TX-114 (i.e. 1. 4% TX-114 in 10 mL solution) for Ag metal. These values were selected as optimal amount and used in the proposed methods for the detection of STR Plotting the absorbance values of the cloud point versus the volume of Triton X-114 is shown in Figure (7).

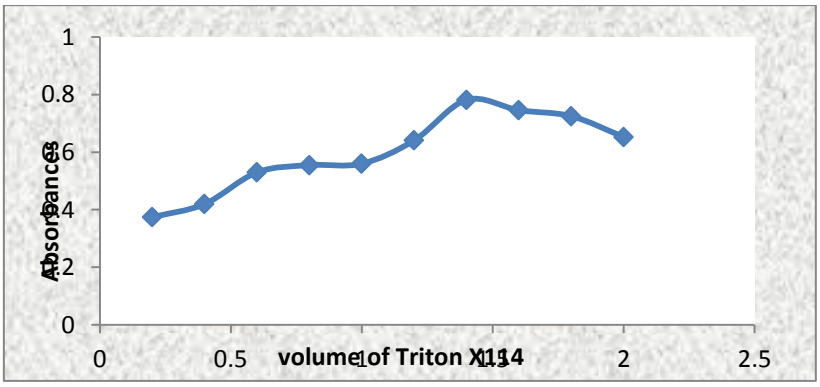

Figure (7) Effect of the TX-114 amount on absorbance of (STR-Ag I) complex.

Effect of the Equilibration Temperature and Time

In order to optimize the method, it was necessary to examine the effect of the temperature on cloud-point extraction. Temperature that enhances higher range of (35-90) °C and (5-50) min, respectively, while keeping all other parameters constant. Excellent absorbance was found at temperature 75°C as shown in figure (8); there fore choose75^0C higher than is probably due to the decomposition of the complex.

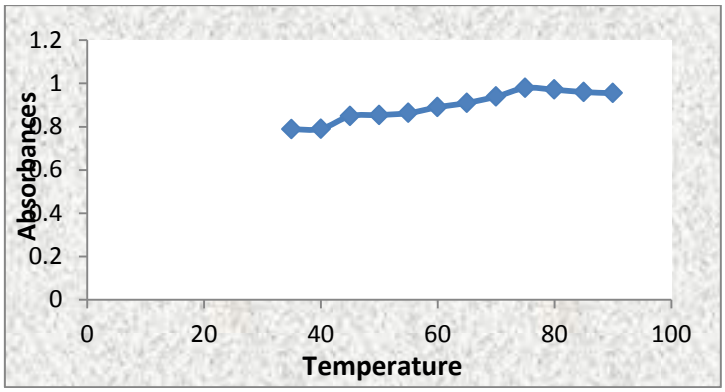

Figure (8) Effect of temperature on absorbance of (STR-Ag I) complex.

Incubation time was also investigated in the range of (5-50) min fig (9) Excellent absorbance found at 35min the time for 35 min was selected to fulfill efficient separation conditions.

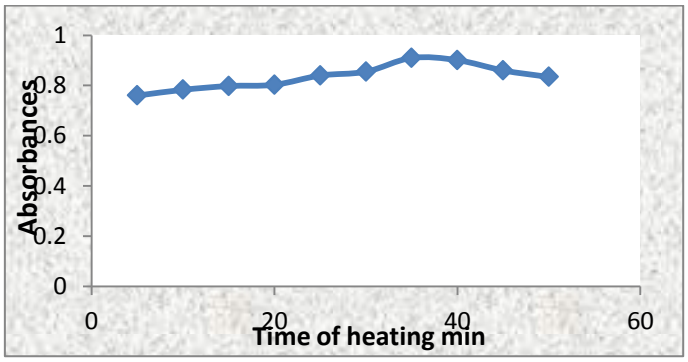

Figure (9) Effect of time on absorbance of (STR-Ag I) complex.

Order of Additions

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Fig (10) shows that the best order of addition is the number 1 for target analytes due to giving a highest absorption signal among the others. The absorbance is measured and the absorbance results are shown in table (3)

Plotting of the absorbance values versus the order additions is shown in Figure (10).

Table (3) Data of Absorbance to Order Additions.

No	Order Additions	Absorbance at λmax =404 for Ag(III)
1	*D+ M+B+T*	0.98
2	*M+D+B+T*	0.63
3	*D+B+M+T*	0.577
4	*M+B+D+T*	0.367

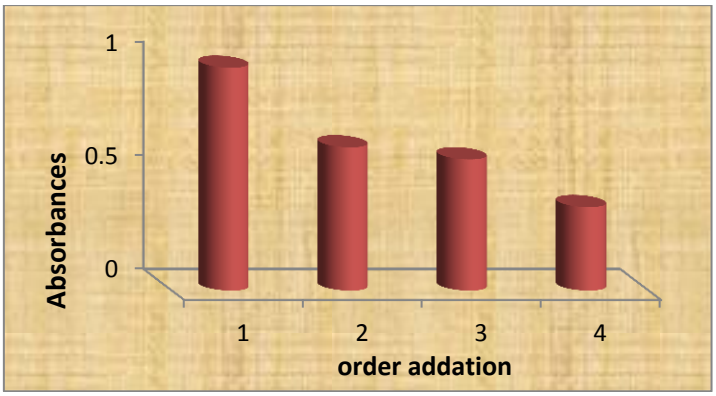

Figure (10) Effect of Order Additions on absorbance of (STR-Ag I) complex.

It is noted that the best addition is the first order of Ag (I) because if it's another order gets lost in the intensity of color and this order fixed in subsequent experiment

Effect of organic solvents

Different organic solvents (water, Ethanol, Methanol, Acetonitril, H_2O_2, chloroform, Acetyl aceton, Dimethy formamide, Dimethy phthalate, Dimethy malonate) are examined to evaluate their effects on the intensity of the resulting complex and Plotting of the absorbance values versus the solvent is shown in figure (11).

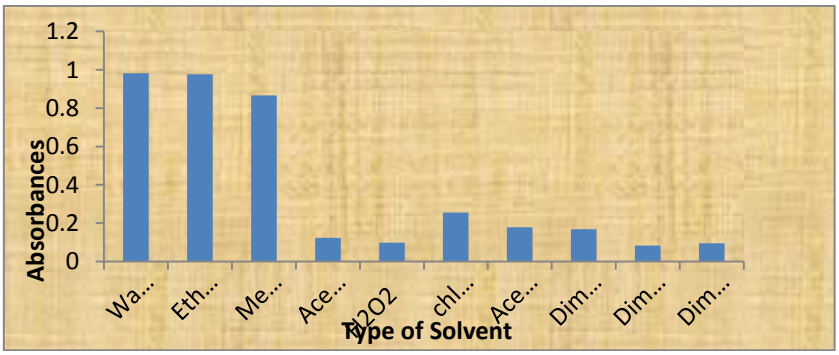

Figure (11) Effect of Solvents on absorbance of (STR-Ag I) complex.

It has been shown that water is the optimum solvent, economically, sensitivity method, cheap price, to provide and nontoxic. This solvent is fixed in subsequent experiment.

Effect of Interference

The effect of some foreign organic compounds and Inorganic compounds, which often found in environmental, were studied by adding 1ml of (100ppm) Equal amounts organic compounds, Inorganic compounds to 1ml of (100ppm) of complex. The color was developed following the recommended procedure described earlier.

Table (4) Effect of Interference.

100ppm interference	Absorbance at λmax =404 for Ag
With out	0.981
Lactose	0.055
Starch	0.231
Arabic Gum	0.093
Talc	0.045
Glucose	0.0001
$Ca_3(PO_4)_2$	0.145
$CaCO_3$	0.166

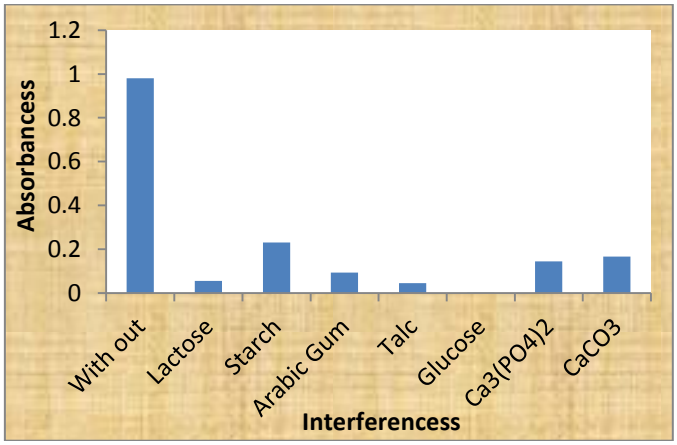

Figure (12) Effect of organic and inorganic Interferences on absorbance of (STR-Ag I) complex.

It was observed that the table (4) were not interfering with the determination at levels found in complex form.

Selected Optimum Conditions

After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product.

Table (5) The optimum conditions for the determination of STR.

Optimum	Concentrations	Range selected	Optimum quantities of complex (STR-Ag)
λ max(nm)	----	220-1100	404
Effect of volume of metal ion required	500 ppm	0.05-0.55ml	0.4
Effect of PH	0.1M(Na OH)	1-14	12
Buffer pH	----		
Effect of volume of Buffer	----	0.2-1.8ml	1.2
Effect of volume of triton x114 required	10%(v/v)	0.2-2 ml	1.4
Effect of time heating	----	5-50min	35min
STR solution required	250 ppm	0.1-1.2ml	0.5

The optimum conditions for the proposed procedure were summarized in (Table 5) and were used in all subsequent experiments.

Calibration Graph

Employing the optimum conditions described in the procedure of cloud point extraction,

liner calibration graph of STR with Silver was obtained Figure (13), which show Beer law obeyed over the concentration range of 2.5-30µg ml^{-1} with correlation coefficient equal to 0.9989.All other analytical characteristics data are summarized in table(6). Then it is completed to the mark by distilled water, are mixed, heated at optimum temperature in the thermostat water bath at optimum incubation time, to form cloud point then aqueous phase is separated by centrifugation at 3000 rpm for 10min, 1ml ethanol is added to the surfactant-rich phase to dissolve it then is measured by UV-Vis at λmax = 404 nm for Sliver, triplicate manner. The absorbance measurements are illustrated in table 6.

Table (6) The absorbance measurements of standard solutions of complex (STR-Ag).

Conc. ppm	Mean Absorbance	RSD%	Found	Recovery%
2.5	0.465	0.151903	2.2478	89
5	0.587	0.240513	4.8326	96
7.5	0.698	0.101087	7.1843	95
10	0.829	0.085245	9.9597	99
12.5	0.979	0.288615	13.1377	105
15	1.079	0.065503	15.2563	101
17.5	1.201	0.058852	17.8411	101
20	1.315	0.214926	20.2563	101
22.5	1.421	0.248719	22.5021	100
25	1.533	0.092191	24.875	99
27.5	1.645	0.042998	27.2478	99
30	1.767	0.199917	29.8326	99

The calibration curve was Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (STR-Ag) as shown in Figure (13).

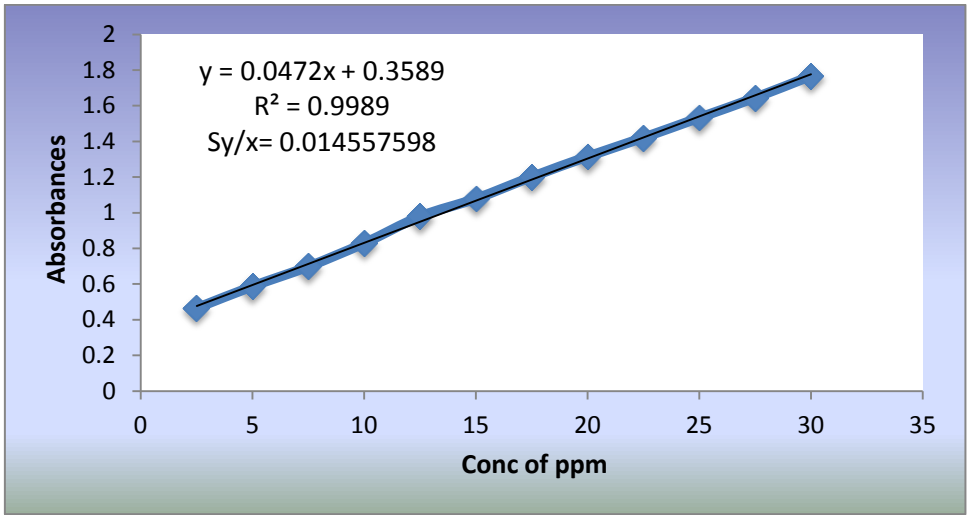

Figure (13) Calibration Curve of complex (STR-Ag).

Optical characteristics Features of the calibration curve

Table (7) shows the main features of the calibration curve and measuring the absorbance at 404 nm.

Table (7) Optical characteristic features of calibration curve.

Parameter	Complex (STR-Ag)
Wave length λ_{max} (nm)	404nm
Concentration rang (µg ml^{-1})	2.5-30 µg ml^{-1}
Regression equation	y =0.0472x +0.3589
Correlation coefficient(r)	0.9994
Correlation coefficient (r^2)	0.9989
Variation coefficient (%)	99.89
Limit of Detection (µg ml^{-1})	0.274576 µg ml^{-1}
Limit of Quantitation (µg ml^{-1})	0.915254 µg ml^{-1}
Sandell's sensitivity (µg cm^{-2})	0.284738041
Slope (m)	0.0472
Intercept (C)	0.3589
Molar absorptivity($L.mol^{-1}.cm^{-1}$)	2.5×10^{3}
Composition of product	**1:1**
C.L for slope (b±tSb) at 95 %	$0.0472\pm1.0858\times10^{-3}$
C.L for intercept (a±tSa) at 95 %	0.3589±1.93298
C.L for Conc. 5 µg ml^{-1}at 95%	$0.587\pm4.9653\times10^{-3}$
C.L for Conc. 15 µg ml^{-1}at 95%	$1.079\pm2.4826\times10^{-3}$
C.L for Conc. 25µg ml^{-1}at 95%	$1.533\pm4.9653\times10^{-3}$

Calculation of the stability constant (K) of complexes:

Stability constant of reaction product

The conditional or apparent stability constant of the 1:1 (Drug and metal) product was evaluated and described as shown A series of solution were prepared containing three different concentration of metal and Streptomycin Sulfate (1:1) the concentration (1×10^{-4}) $molL^{-1}$ for each (Sliver with Streptomycin Sulfate) are shown in Table (8).

Table (8) Stability Constant of the complex (Ag+ Streptomycin Sulfate) formed.

Vol of Streptomycin Sulfate	Absorbance at λ= 404nm				K (Average) ($l.mol^{-2}$)
	A_s	A_m	α	K ($l.mol^{-2}$.)	
0.3	0.590	0.597	0.01172	7×10^{7}	2.7×10^{9}
0.5	0.768	0.769	1.30039	5.9×10^{9}	
0.7	0.925	0.927	2.15749	2.1×10^{9}	

Stoichiometric Determination of Color complex:

Continuous Variation Method (Job`s method)

A series of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) ml of (1×10^{-4}) mol $L^{-1}$of the solution that contain Streptomycin Sulfate was pipette into each of 10ml volumetric flask then (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1) ml of (1×10^{-4}) mol $L^{-1}$of metal the absorbance of the solution was measured by UV-Vis Spectrophotometer at λ_{max} 404nm the stoichiometric ratio between Streptomycin Sulfate with metal 1:1 results are shown in the Table (9).

Table (9) The continuous variation method of Streptomycin Sulfate with metal (Silver) complex.

V D mL	V M mL	VD / VT	Absorbance at λ= 404 for Color compound
0.1	0.9	0.1	0.0245
0.2	0.8	0.2	0.078
0.3	0.7	0.3	0.14
0.4	0.6	0.4	0.187
0.5	0.5	0.5	0.262
0.6	0.4	0.6	0.209
0.7	0.3	0.7	0.175
0.8	0.2	0.8	0.111
0.9	0.1	0.9	0.056

Plotting the value of absorbance versus the VD / VT is shown in Figure (14)

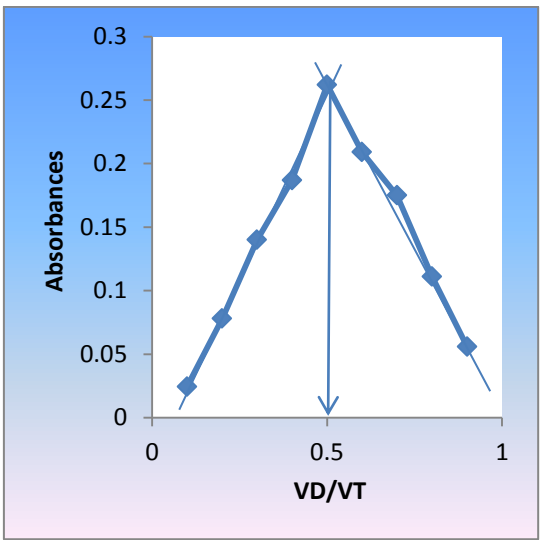

Figure (14) Continuous variation method plot.

VD: values of the compound (Streptomycin Sulfate)
V M: The values of the metal (silver).
VT: Total (V M+V D)

Mole – Ratio Method

Aliquots of 10 mL solution containing (1×10^{-4}) $molL^{-1}$ of (1mL) Streptomycin Sulfate and increasing concentrations (1×10^{-4}) mol L^{-1} of (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2) mL of (Ag) silver (2×10^{-6}--2×10^{-5}) mol L^{-1} metal. The absorbance of the solutions were measured by UV-Vis spectrophotometer versus blank at λ_{max}= 404 nm the stoichiometric ratio between 1:1 results are shown in the Table (10).

Table (10) The Mole - Ratio Method of the Streptomycin Sulfate with Silver.

CL	*CL / CM*	Absorbance at for λ=404 nm Color compound
2×10^{-6}	*0.2*	0.053
4×10^{-6}	*0.4*	0.098
6×10^{-6}	*0.6*	0.159
8×10^{-6}	*0.8*	0.208
1×10^{-5}	*1.0*	0.234
1.2×10^{-5}	*1.2*	0.231
1.4×10^{-5}	*1.4*	0.222
1.6×10^{-5}	*1.6*	0.214
1.8×10^{-5}	*1.8*	0.211
2×10^{-5}	*0.2*	0.201

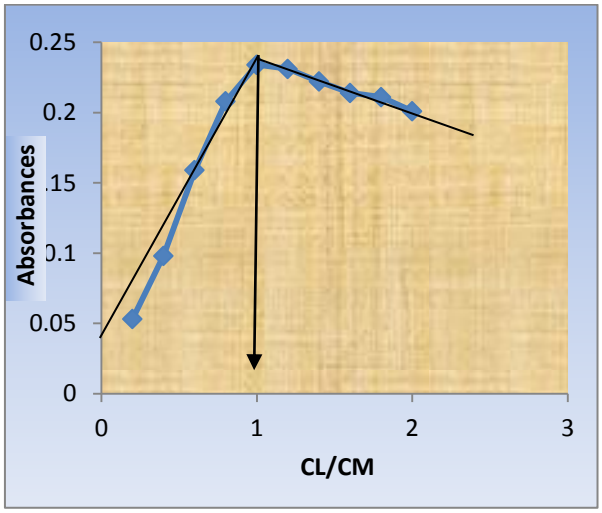

Figure (15) Mole - Ratio plot of Streptomycin Sulfate with Silver complex.

Analytical Application

The suggested methods was applied to the quantitative determination of Ag (I) in some Pharmaceuticals it was gave good accuracy and precision Table (11). Application of proposed method for determination of Ag (I).

Table (11) Data for Determination STR with Ag in the Pharmaceutical Preparation (STR) by CPE.

Amount of STR / µg ml^{-1}	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%
7.5	*0.680*	*0.328834*	*6.8029*	*90*	*97*	*-9.333*	*-7.64419*
12.5	*0.792*	*0.15464*	*11.2944*	*90*		*-9.68*	
17.5	*1.279*	*0.22795*	*19.4936*	*111*		*11.3714*	

[*]= Average of Five

Conclusion

CPE preconcentration is an easy, safe and inexpensive methodology for separation and Preconcentration of trace metals in aqueous solutions. The ligand was successfully to formed complex with the some metals ion by cloud point extraction. Is a stable, sensitive and selective complexion successfully to determination Ag (I) in some Pharmaceuticals, the method gives a very low limit of detection and good R.S.D. values and green chemistry.

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The Relationship of Estrogen, Progesterone and Mammaglobin-A Protein with Breast Cancer in a Sample of Iraqi Patient's Women

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Abstract

Breast cancer considered a serious health problem since it is the main cause of cancer death in women. This study was aimed to determine the association of estrogen, progesterone hormones, and mammaglobin-A protein serum levels with breast cancer in 60 Iraqi women with breast cancer compared to 28 apparently healthy volunteers. The results of hormonal study showed increase levels of estrogen hormone in 39/60 patient, representing 65% of patients group while a normal levels of estrogen were observed in 21 (35 %) patients ($p<0.01$). Weak association between progesterone elevation and breast cancer risk was appeared which is slightly elevated in ten patients only. The mammaglobin-A protien concentration was elevated in patients group (0.014- 10.28 ng/ml) when compared with control group (0.048-0.7 ng/ml), with mean equal (1.435 ng/ml) in patients group compared to (0.077 ng/ml) for control group ($p<0.01$). The current study found a positive relationship between mammaglobin-A levels and estrogen hormone since the mean of both elevated in patients group when compared with control group ($P<0.05$). No any correlation was observed between mammaglobin A and progesterone hormone.

Key Words: Breast cancer, Estrogen, Progesterone and Mammaglobin-A

علاقة هرموني الاستروجين والبروجستيرون وبروتين الماماكلوبين بسرطان الثدي في عينة من المريضات العراقيات

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*البحث مستل من رسالة ماجستير للباحث

الخلاصة

يعد سرطان الثدي مشكلة صحية خطيرة كونه المسبب الرئيسي لوفيات السرطان في النساء. هدفت هذه الدراسة لتحديد العلاقة بين مستويات هرموني الاستروجين والبروجستيرون و بروتين الماماكلوبين بسرطان الثدي عند 60 امرأة عراقية مصابة بسرطان الثدي مقارنة مع 28 امرأة متبرعة اصحاء ظاهريا . أظهرت نتائج الدراسة الهرمونية ان هرمون الاستروجين مرتفع عند 60/39 مريضة ويمثلن 65% من مجموعة المرضى ,بينما وجدت معدلات طبيعية لهرمون الاستروجين عند 21 (35%) مريضة مع وجود فروق عالية المعنوية، وقد اظهر هرمون البروجسترون علاقة ضعيفة مع سرطان الثدي بأرتفاع طفيف في 10 مريضات فقط. ان تركيز بروتين الماماكلوبين مرتفع في مجموعة المرضى (0,014 −10,2 نانوغرام/مل) بالمقارنة مع (0,048− 0,7 نانوغرام/مل) لمجموعة السيطرة بمعدل مساوي ل 1.435 نانوغرام /مل في مجموعة المرضى بالمقارنة مع 0.077 نانوغرام /مل في

مجموعة السيطرة ($p<0.01$). وجدت الدراسة الحالية علاقة ايجابية بين مستويات بروتين الماماكلوبين وهرمون الاستروجين كون معدلاتهما مرتفعة في مجموعة المرضى بالمقارنه مع مجموعة السيطرة ($P<0.05$). ولم تلاحظ اي علاقة بين بروتين الماماكلوبين وهرمون البروجسترون.

Introduction

Breast cancer is the most common cancer in women and second most common cancer overall, both in the developed and developing countries (Kan'an, 2018). Breast cancer risk is affected by several reproductive and hormonal factors. One of these factors is blood level of certain sex hormones, particularly estrogen (AL-Thwani and Rashid, 2015). Estrogen is frequently a key initiator of the process that transforms normal breast cells into cancerous cells. It can also be an important promoter of cancer. The mechanisms of carcinogenesis in the breast caused by estrogen include the metabolism of estrogen to genotoxic, mutagenic metabolites and stimulate tissue growth. Together, these processes cause initiation, promotion, and progression of carcinogenesis. Also blood levels of progesterone appear to be inversely associated with the risk of breast cancer (Yager and Davidson, 2006; Colditz, 2015).

Mammaglobin-A protein is a member of the uteroglobin proteins family found in mammary tissue and can be detected in serum. This protein has been proposed as a biomarker for breast cancer diagnosis, patients exhibit an increased amount of the protein in serum and tumor tissue, while a low levels may be seen in normal breast tissue, expression is increased dramatically in breast cancer and is correlated with higher grade. Number of subsequent studies described the detection of mammaglobin at high levels in primary breast cancers, while it was either undetectable in non-breast tumors or present at low levels in healthy breast tissue, but not in other tissues, making it a suitable candidate for diagnosis of breast cancer (Galvis *et. al.*, 2013; AlJoudi, 2014).

Materials and method:

Subjects and Samples collection

Sixty Iraqi patient with breast cancer who attended Baghdad hospital and oncology teaching hospital during the period extended from the first of October /2015 to the end of February/2016 with age ranged from 25-86 years, and 28 apparently healthy volunteers, at age matched the patients group were enrolled in this study.

Amount of 3 ml venous blood was placed into clot activator and gel serum separation tubes and left to stand at room temperature (18-22°C). Then, the serum separated by centrifugation at 3000 rpm for 15 minutes. Later, it was divided into three aliquots and kept at -80 Cº until used.

Detection of Serum Estrogen and progesterone levels

Enzyme-linked immunosorbent assay (ELISA) is a biochemical assay that uses antibodies and an enzyme-mediated color change to detect the presence of either antigen (proteins, peptides, hormones, etc.) or antibody in a given sample (Gan and Pate, 2013). The quantitative determination of serum estrogen and progesterone concentration applied by Monobind Inc. ELISA kit by a microplate enzyme immunoassay. The principles of this assay were delayed competitive enzyme immunoassay and competitive enzyme immunoassay respectively.

Detection of serum Mammaglobin A levels

According to instruction of Cusabio Company, ELISA assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for *SCGB2A2* has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any *SCGB2A2* present is bound by the immobilized antibody. After washing, avidin

conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SCGB2A2 bound in the initial step. Then the intensity of the color was measured.

Results and Discussion:

Serum Estrogen and Progesterone Levels

Levels of some female steroid hormones have been associated with the risk of breast cancer such as estrogen, which has a critical role in the pathogenesis and progression of breast cancer (Surekha *et. al.*, 2007).

The present results showed that elevated levels of estrogen were observed in 39/60 (65%) of patients group while the remaining 21 patients have normal estrogen levels ($p<0.01$) as shown in Table (1). On the other hand only 4/22 cases representing18% of the control group have elevated estrogen levels.

Table (1) Estrogen Levels of the Studied Groups.

Groups	Estrogen level (pg/ml)	No.	(%)
Patients group	Elevated	39	65.00
	Normal	21	35.00
	Total	60	100%
	Chi-square	---	9.613 **
	P-value	---	0.001
Control group	Elevated	4	18.18 %
	Normal	18	81.82 %
	Total	22	100%
	Chi-square	---	13.275 **
	P-value	---	0.0001
** (P<0.01).			

The results found a percentage of 89.74 % of the patients who have elevated levels of estrogen were postmenopausal, while only 4/39 cases representing (10.25) % of the cases were premenopausal.

The hypothesis that breast cancer risk is positively associated with circulating concentrations of estrogen in postmenopausal women supported the outcome of this work. In a collaborative reanalysis of individual participant data from seven prospective studies included data for up to 767 women with breast cancer and 1699 controls in the risk analyses conducted by Collaborative Group of Hormones, Endogenous, and Breast Cancer in 2013, concluded that Circulating estrogens and androgens are positively associated with the risk for breast cancer in premenopausal women (CGHFBC, 2013).

The results of serum progesterone concentrations showed that progesterone levels were slightly elevated in only 10/60 patients, while it's decreased in only one patient, this revealed a weak association between progesterone elevation and breast cancer risk. None of the control group showed abnormal progesterone level but when the mean of the patients and control groups were compared, the patients group showed lower mean than control group ($p<0.05$) (Table 2).

The low mean of progesterone concentration in patients group may be due to estrogen elevation since estrogen and progesterone work to balance each other in the body, when estrogen levels increase for any reason, progesterone levels may drop because high estrogen levels suppress progesterone production (Camacho, 2012).

Table (2) The Mean of Progesterone Hormone in the Studied Groups

Group	No.	Mean ± SD of progesterone (ng/ml)
Patients	60	1.665 ± 0.23
Control	22	2.958 ± 1.03
LSD value	---	1.251 *
P-value	---	0.050
* (P<0.05):significant differences		

The preceding findings come in agreement with many Iraqi studies, one of them is a recent study conducted by Al-Thwani and Rashid (2015), who has found that estrogen levels were elevated in 62% of the studied patients which is nearly similar to the present results, but they reported that progesterone levels was decreased in 59% of the studied cases. Also this results supported by Ghanim (2009), who found that elevated levels of estrogen were observed in 63% of the studied cases of breast cancer patients. Another study conducted by sadkan (2009), showed that elevated levels of estrogen were observed in 70% of breast cancer patients.

Many studies found strong associations between breast cancer and elevated estrogen levels in postmenopausal women (Kaaks *et. al.,* 2005; Zhang *et. al.,* 2013).

This is likely to be due to non-ovarian estrogen synthesis being prominent in subcutaneous fat after menopause, the relationships have been reported to potentially explain the association of breast cancer with body mass index in postmenopausal women (Folkerd and Dowsett, 2013). Also a recent study concluded that estrogen and progesterone promoted breast cancer cell proliferation by inducing the expression of cyclin G1 (Tian *et. al.,* 2018).

The response of breast tissue to estrogen may related to the nature of breast tissue, hormone-sensitive breast cancer cells contain proteins known as hormone receptors that become activated when hormones bind to them. The activated receptors cause changes in the expression of specific genes, which can lead to the stimulation of cell growth and lead to breast cancer (Dunnwald *et al.,* 2007). The previous accumulative data give a strong evidence that female steroid hormone especially estrogen involve in breast cancer risk especially after the age of menopause.

Mammaglobin-A portion levels

The results revealed that mammaglobin-A portion concentration was elevated in patients group with mean equal to (1.435 ng/ml) in comparison to (0.077 ng/ml) for control group ($p<0.01$) as displayed in Table (3). Whereas the Concentration of Mammaglobin-A protein in patients group ranged from (0.014- 10.28) ng/ml while it ranged (0.048-0.7) ng/ml in control group.

Table (3) The Mean of Mammaglobin-A Protein concentrations in Patients and Control Groups

Group	No.	Mean ± SD of Mammaglobin
Patients	60	1.435 ± 0.26 A
Control	28	0.077 ± 0.02 B
LSD value	---	0.769 **
P-value	---	0.0007
** (P<0.01).		

Current results agreed with Fanger *et. al.* (2002), who detected mammaglobin in sera of breast cancer patients, they found elevated levels, ranged from 0.07 to 9.6 ng/ml, compared to 0- 0.07 ng/ml in healthy individuals.

Zehentner *et. al.*, (2004) used ELISA assay to analyze blood samples of 79 breast cancer patients for mammaglobin-A protein, reported that circulating mammaglobin protein was found in 68 % of the breast cancer sera, but 38 % showed significantly elevated protein levels in comparison to control group. Also Galvis *et. al.*, (2013) concluded that mammaglobin-A may be a stable biomarker for breast cancer in a study that used ELISA to detect serum concentrations of mammaglobin-A in 51 patients suffering from breast cancer and 51 control individuals.

It was found that the expression of mammaglobin is significantly increased during the proliferation of the cells in the breast, with production ceasing after the differentiation of epithelial cells in the breast, which is also observed during lactation. This suggests that the synthesis of mammaglobin is involved in the proliferation of epithelial breast cells, which could explain the overexpression of mammaglobin observed in breast cancer (Goedegebuure *et. al.*, 2004).

It was expected that mammaglobin secreted from breast tumors and to elicit production of autoantibodies detectable in the serum of breast cancer patients and women at high risk of breast cancer. The detection of mammaglobin-A in peripheral blood of breast cancer patients may depend on the number of cancer cells present in the blood and also on the mammaglobin expression level by individual cells (Aristizábal-Pachón *et. al.*, 2015).

The Relationship between Mammaglobin-A Protein and Estrogen Hormone

A positive association were observed between mammaglobin-A protien levels and estrogen hormone since the mean of both where elevated in patients group when compared with control group ($p<0.05$) (Table 4). Also when the individual data of each case notified, the results revealed that patients with highest mammaglobin−A concentrations have an elevation of estrogen hormone levels especially in postmenopausal women.

The elevated levels of estrogen may be a risk factor to develop breast cancer, and that may lead to increase the mammaglobin-A levels.

Table (4) Association between Mammaglobin-A Protein and Estrogen Hormone

Group	No.	Mean ± SD of Estrogen	T-Test (P value)
Patients	60	295.15 ± 29.31	39.483 * (0.0416)
Control	22	232.06 ± 24.86	
Group	**No.**	**Mean ± SD of Mammaglobin**	**T-Test (P value)**
Patients	60	1.435 ± 0.26	0.279 * (0.0227)
Control	28	0.077 ± 0.02	
* (P<0.05).			

Classen *et al*., (2012) suggested that expression of mammaglobin is controlled by steroid hormones in a study designed to prove hormonal regulation of mammaglobin. When Primary endometrial epithelial cells were cultured with 17β-oestradiol and promegestone, mammaglobin was detected in human endometrial tissue, with peak expression observed during the luteal phase in 64% of breast carcinomas.

In this study no correlation were observed between mammaglobin-A protien and progesterone hormone.

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The Effects of Human Cytomegalovirus on Placenta of Aborted Women

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Abstract

Abortion is the most common complication of human gestation. This study was designed to search for some causative and risk factors in 70 aborted women who had history of abortion as case group compared with 70 healthy pregnant women as control group in two hospitals: Al-Hilla Teaching Hospital and Babylon Hospital for Childbirth and Children in Hilla, between December 2017 and April 2018. These women were subjected to investigate the presence of anti-Cytomegalovirus IgM and IgG antibodies by using the ELISA technique. The results display that out of 70 samples, (18.5%) for recurrent spontaneous abortion (RSA) and (30%) for control were positive for anti-CMV IgM antibodies, and (94.2%) for RSA and (93.3%) for control were positive for anti-CMV IgG antibodies. Out of 70 samples, (81.4 %%) for RSA, and (70 %) for control were negative for anti-CMV IgM antibodies, and (5.7 %) for RSA and (6.6%) for control were negative for anti-CMV IgG antibodies. The study has been found that the highest prevalence in the first trimester for CMV infection. The results showed that anti-HCMV IgM positivity was 13 (18.5%) among aborted women and 66 (92 %) were anti-HCMV, IgG positive antibodies by ELISA. The rate anti-HCMV, IgM antibodies seropositivity among primary abortion was 6 (8.5%) out of 36 with the highest rate 4 (5.7 %) out of 15 was recovered among with the age group (26-36) and the recurrent abortion was 3 (4.2%) out of 14 among the age group (26-36).Recurrent abortion rank 7 (9.8 %) of the total 34 abortions in this study. The morphological of placenta obtained from aborted women that placental enlargement, thickened and weight increased at abortion of gestational stage could result from fibrinoid deposition that form to compensate for hypoxia and leukocytic infiltration, calcification and necrosis (histological features).

Key words: abortion, HCMV and placenta.

تاثير فايروس المضخم للخلايا البشري على المشيمه لدى النساء المجهضات

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بابل_العراق

الخلاصة

صُممت هذه الدراسة للبحث عن بعض العوامل المسببة والمخاطر في 70 امرأة لديهن تاريخ الإجهاض متكرر تلقائي عينه دراسه مقارنة مع 30 امراة سليمة حامل كعينه مقارنه في كل من مستشفى الحلة التعليمي ومستشفى بابل للولادة والأطفال في الحلة وللفتره من ايلول عام 2017 الى نيسان عام 2018 تم إخضاع هؤلاء النساء للتحقق من وجود بعض العوامل المناعية مثل مضاد (Cytomegalovirus IgM) و IgG الأجسام المضادة باستخدام تقنيةELISA.تم جمع عينات من الأمصال والأنسجة (المشيمة) من كلا النساء المجهضات والسيطرة. تم استخدام فحص الاليزا (ELISA) لتقييم وجود أجسام مضادة محددة ضد CMV. في حين تمت معالجة خزعات من

المشيمة لفحص النسيجي مع hematoxylin –صبغة ايوسين. من 70 عينة كانت (18.5 ٪) من عينه الاجهاض المتكرر و (30 ٪) عينه سيطرة كانت إيجابية للأجسام المضادة IgM المضادة ل CMV، و (94.2 ٪) ل RSA و (93.3 ٪) للسيطرة كانت إيجابية للأجسام المضادة IgG المضاد لل CMV. من 70 عينة، (81.4 ٪) لـ RSA، و (70 ٪) للسيطرة كانت سلبية للأجسام المضادة لـ CMV IgM، و (5.7 ٪) ل RSA و (6.6 ٪) للسيطرة كانت سلبية لـ Anti-CMV IgG الأجسام المضادة. وجدت الدراسة أن أعلى معدل انتشار في الأشهر الثلاثة الأولى من الإصابة CMV. وقد خضعت عينات المصل المتسلسلة التي تم جمعها من النساء المجهضات في فترات الحمل المختلفة في مستشفى الحلة التعليمي ومستشفى بابل للولادة والأطفال في محافظة الحلة. تم اختبار مجموعه 70 عينة. تم تطبيق تقنية الاليزا لتقييم الحالة المنطقية المناعية بين جميع الظروف من خلال قياس مضادات الفيروس المضخم للخلايا، IgM، IgG antibodies، أظهرت النتائج أن إيجابية IGM المضادة لـ HCMV كانت 13 (18.5٪) بين النساء المجهضات وكان 66 (92 ٪) مضاد ل HCMV، الأجسام المضادة إيجابية IgG بواسطة ELISA. نسبة مضادات HCMV، الأجسام المضادة IgM إيجابية للإجهاض بين الإجهاض الأولي كانت (8.5٪) 6 من 36 مع أعلى معدل (5.7٪) تم اختيار 15 بين الفئة العمرية (26–36) والإجهاض المتكرر كان (4.2 ٪) 3 من أصل 14 بين الفئة العمرية (26–36). معدل الإجهاض المتكرر (9.8 ٪) 7 من مجموع 34 عملية إجهاض في هذه الدراسة. الصفات الشكلية للمشيمة التي تم الحصول عليها من النساء المجهضات تضخم المشيمة، وزيادة الوزن عند الإجهاض وذلك ممكن أن ينتج عن ترسب fibroid الذي يتشكل للتعويض عن نقص الأوكسجين وانتشار الكريات البيض والتكلس والتنخر.

الكلمات المفتاحيه: الاجهاض، المشيمة و فايروس المضخم للخلايا البشري.

Introduction

Abortion is defined as termination of pregnancy resulting in expulsion of an immature fetus. A fetus of less than twenty week's gestation or a fetus weighing less than 500 gm is considered an abortus (Chan and Johnson, 2006).

There are two types of abortion, the induced abortion and spontaneous abortion (miscarriage); (1) Induced Abortion is the intentional termination of a pregnancy before the fetus can live independently, it may be elective or therapeutic (Finer and Henshaw, 2003). (2) Spontaneous Abortion (Miscarriage) is that type of abortion which is not induced (Hughes *et. al*., 2007).

Abortion has been established that Cytomegalovirus (CMV) have direct effect on the fetus leading to spontaneous abortion, stillbirth or congenital anomalies (Jones *et. al*., 2001 and Stagno, 2001). The risk and severity of the fetus infection depend partly on the timing of the mother's infection. Studies suggest that when mothers are infected with CMV, especially in the first trimester of pregnancy, frequently results in severe damage to the nervous system of the fetus or abortion (Britt, 1996 and Jones *et. al.*, 2003).

The human cytomegalovirus (CMV) extremely common human pathogens. Depending on hygiene standards and habits, socioeconomic parameters and population demographic structure, the prevalence rates of CMV in different countries vary between 40–100 % and 20–70 % respectively. CMV is transmitted by close contact between infected subjects, via blood or blood products, sexual intercourse, or congenital (Novotná *et. al.*, 2005).

Materials and Methods

Whole blood and tissue sample (placenta) were collected from 100 person 70 patients suffering from abortion pregnancy losses and 70 pregnant women with two or more successful and uncomplicated pregnancies as control at Al-Hilla teaching hospitals and

Babylon Hospital for Childbirth and Children in Hilla , between December 2017 and April 2018.

The pathological cases diagnosed by a specialized physician. Study group: A 70 patients with abortion .The patient with (RM) had at least three spontaneous miscarriage and no history of successful pregnancy. All selected patient were with primary abortion having no live child and the age range from 16-40. Control group: A 70 women (with at least two live birth and no history of miscarriage). Exclusion Criteria uterine abnormalities, diabetes mellitus, essential hypertension and patients with thyroid dysfunction.

Blood sample from aborted mother (5 ml) were collected, 2 ml of the 5 ml were collected by tubes made of plastic or (gel tube), and centrifugation of blood samples for 5 minutes at 4,000 rpm for IgG and IgM ELISA tests. Tissue from 50 placenta were taken for morphological and histological study. Ethical Approval**:** A verbal consent have been taken from patients

Results and Discussion

The ELISA test was done for all samples under present study. The ELISA test was done to detect Anti-HCMV IgM and IgG antibodies, as show in the table (1) 66 out of the 70 samples (94.2 %) were positive for anti-HCMV IgG antibodies. Where as in the table (2) 13 samples out of 70 samples (18.5%) were positive for anti-HCMV IgM antibodies by ELISA test**.**

Table (1) Seropositivity of anti-HCMV IgG among aborted women.

Cytomegaloviruses	Abortion group n=70	Control group n=30	P value
CMV +	66 (94.2 %)	28 (93.3%)	0.85370682
CMV -	4 (5.7 %)	2 (6.6%)	

Table (2) Seropositivity of anti-HCMV IgM among aborted women.

Cytomegaloviruses	Abortion group n=70	Control group n=30	P value
CMV +	13 (18.5 %)	9 (30%)	0.20618687
CMV -	57 (81.4 %)	21 (70 %)	

These results are in agreement with Sotoodeh *et. al.*, 2009 who found that the number and percentage for anti-HCMV-IgG was 235 (94%) and the average age was 25.6 ± 7.6 years. The majority of women were anti-HCMV IgG positive, a result similar to what was stated by Munro *et. al*., 2005 and Ross *et. al*., 2006 who found that IgG increases within aborted women. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection; reactivation or new infection lead to high percentage of seropositivity.

For anti-HCMV IgM antibodies seropositivity, Sotoodeh *et. al.*, 2009 found that the percentage was 5.2 % in pregnant women underwent abortion while it was 5% in their counter part control group.

Bodeus *et. al.*, 2001 and Bodeus *et. al.*, 2002, concluded that the women with positive serology for IgG and negative for IgM antibodies were considered immune and their

primary infection with CMV was assumed to have taken place before the current pregnancy.

Enders *et. al*., 2001 showed that women with negative serology for IgG and IgM anti-HCMV antibodies were not infected with CMV and were susceptible to infection (primary infection). In these situation there is a risk for transmission of the virus to the fetus during the pregnancy.

Wreghitt *et. al*., 2003 stated that women with positive serology for IgG and IgM antibodies, at the same time, were considered to be possibly infected with CMV during the current pregnancy or an earlier infection which can be confirmed by IgG avidity test because antibody binds to the antigen with less avidity during acute infection than chronic infection. Goodrich *et. al.,* 2007 showed a high IgM anti-HCMV antibodies seroprevalence in young female population with abortion in Santo State in Brazil, and the IgM was considered an indicator for recent HCMV infection. The distribution of HCMV infection in aborted women is high, which refers to the wide spreadness of this virus among women. Increase in HCMV-IgM antibodies in aborted women confirms the presence of a new cases of viral infection or reactivation of previous infection (Gaytant *et. al*., 2002). Occurrence of CMV viral infection during pregnancy put a risk on the life of the embryo and may cause abortion (Adler and Marshall, 2007).

Table: (3) show the Distribution of anti-HCMV IgM antibodies among primary and recurrent abortion. The rate of anti-HCMV IgM antibodies seropositivity among primary abortion was (8.5%) 6 out of 36 with the highest rate (5.7 %) 4 out of 15 was recovered among with the age group (26-36) and the recurrent abortion was (4.2%) 3 out of 14 among the age group (26-36).Recurrent abortion rank (9.8 %) 7 of the total 34 abortions in this study (Razieh *et. al*., 2006). Mentioned that according to numerous studies approximately 50% to 80% of all pregnancy loses depended on the maternal and gestational age at the time of loss. Distribution of anti-HCMV IgM antibodies among primary and recurrent abortions were studied in table (3).

Table (3) Distribution of anti-HCMV IgM antibodies among primary and recurrent abortion.

Age groups	Primary Abortion		Total	Recurrent Abortion		Total
	IgM +	IgM - *		IgM +	IgM - *	
15-25	1 (1.4%)	18 (25.7%)	19	3 (4.2%)	10 (14.2%)	13
26-36	4 (5.7%)	11 (15.7%)	15	3 (4.2%)	11 (15.7%)	14
37-47	1 (1.4%)	1 (1.4%)	2	1 (1.4%)	6 (8.5%)	7
Total	6 (8.5%)	30 (42.8%)	36	7 (9.8%)	27 (38.6%)	34

Recurrent abortion revealed a high IgM seropositivity (9.8 %) 7 than that with primary abortion (8.5%) 6. This difference was not significant $p > 0.05$.

For primary abortion, the age group of highest IgM seropositivity was (26-36) year.This was due to new infection of this young group. The high percentage of IgM seropositivity among those with recurrent abortion when compered with primary abortion probably related to accumulation of new or reactivation of old infection (Stango and Britt, 2006).

In figure (1) the anti-HCMV, IgM antibodies seropositivity among aborted women in the first trimester was (14.2 %) 10 out of (total samples examined) regarding IgM seropositivity among aborted women having abortion in second trimester was (4.2 %) 3 out of total sample size.

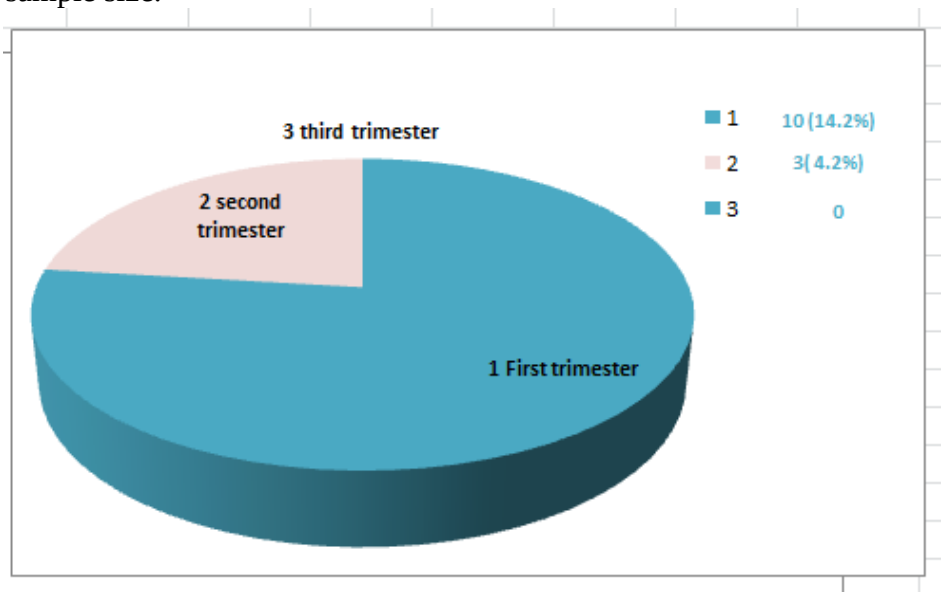

Figure (1) Distribution of positive cases according to the stages of pregnancy.

Chandler *et. al.,* (2000) studied a group of women suffering from different degree of CMV infection and they had differences in the values of IgM between the 1st and the 2nd trimesters.

Another study conducted by Massimo *et. al*., (2009) stated that the rate of anti-HCMV IgM antibodies was high in those underwent abortion at the 1st trimester of gestation.

These controversies in the time of occurrence of abortion and its relationship to anti-HCMV IgM antibodies seropositivity could give impression those anti-HCMV IgM antibodies seropositivity is of a significant role in causation of abortion. It had no definite relationship to the time of pregnancy when abortion occurred or reactivation of a latent infection happen.

The diagnosis of a recent CMV infection is possible with a blood sample that can be accurately tested for IgM antibodies to CMV, the avidity and quantity of IgG antibodies to CMV, and CMV DNA. If a maternal infection is confirmed or if there are ultrasound findings of fetal involvement or growth restriction, amniocentesis may be appropriate. (Nigro *et. al*., 2005; Nigro *et. al*., 2003) women who had anti-cmv IgM and anti-CMV IgG positivity or who sero converted to CMV IgG positivity were classified as having primary infection (Lazzarotto *et. al*., 2011).

On gross examination, the placenta with HCMV infection were found to be increased in weight and in gross measurement in comparison to the control group and type coiling of umbilical cord lean in positive group while control group was hyper coiling as figures (2,3,4 and 5)

Initial observations suggest that placental enlargement could result from fibrinoid deposition and small vascularized villi that form to compensate for hypoxia in utero, primary maternal CMV infection should be associated with increased placental weight at birth. A previous study of 27 placentas of newborns or fetuses with congenital infection whose mothers had CMV infection of unknown status observed a tendency towards increased placental weight (Garcia *et. al.,* 1989). Increase weight, thickened and number of cotyledons of placenta have been previously associated with increased

fetal and perinatal mortality, maternal diabetes chromosomal abnormalities, maternal and fetal anemia fetal heart failure, and congenital nephrotic syndrome and fetal hydrops. (Benirschke and Kaufmann, 2000).

Thus, a thickened placenta is none a specific parker of fetal disease (Elchalal *et. al*., 2000). My work agree with this study. In our study placental measurement and comparing it to reference values should be helpful in the prenatal diagnosis of maternal CMV infection.

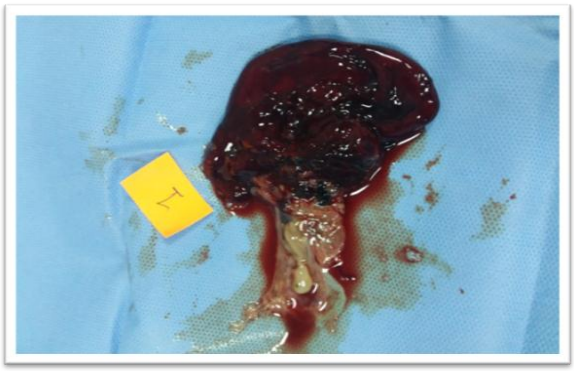

Figure (2) Placenta of aborted women with positive HCMV at first trimester.

Figure (3) placenta of aborted women with negative HCMV at first trimester.

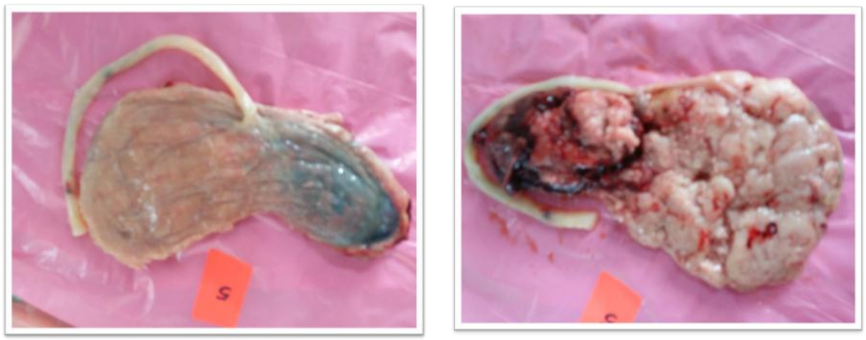

Figure (4) Maternal surface with infracted area of a placenta with umbilical cord from aborted women with negative HCMV at second trimester. (Left). Fetal surface of a placenta from aborted women with negative HCMV revealed a marginal insertion and non-coiled umbilical cord. (Right).

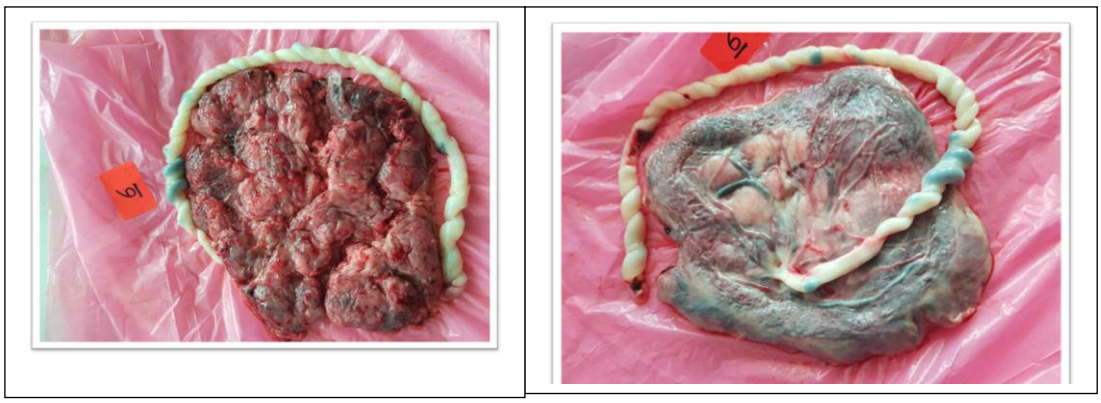

Figure (5) Maternal surface of a placenta from aborted women with positive HCMV revealed increase number of cotyledons and fibrnoid. (Left), Gray color of fetal surface of a placenta revealed Eccentric insertion and hyper-coiling umbilical cord from aborted women with positive HCMV. (Right).

The present study investigated placentas from aborted women primarily and recurrent infected by CMV with a positive diagnoses. Histological analysis of biopsy specimens and characterization was performed on the placenta. The predominant target cells of CMV infection were epithelial and endothelial cells, and this probably explains the more frequent CMV infection in placenta. Figure (6, 7and 8). Showed numerous syncytial knot were found, fibrin deposits within the villi (intra villous fibrin deposition) and between the villi (perivillous, intervillous fibrin deposition) were noted to be increased in the placentas compared to the none infected HCMV. Leukocytic infiltration and necrosis hall marks of CMV infection – were evident. The parenchyma showed diffuse chronic villitis with necrosis and hydrops. We considered not only non-functional villi showing necrosis with inflammatory infiltrate, but also these with hydrops , because stromal oedma reduced local vascular flow. Study by (Joshi *et. al.*, 1996) Stromal oedema reduces local vesicular flow perivillous fibrin deposition were marked in the subchorionic areas and near the basal plates. The entrapped villi were sclerosed, lacked syncytial lining and many were a vascular. som fibrin masses contained grooves or sheets of chiant (x-cells) these perivillus fibrin deposition might be acting as a barrier between fetal and maternal circulation , thereby reducing the transfer of the essential nutreints to the fetus. Stromal edema reduces local vascular flow. These features suggest hypoxia and reduced perfusion. Increase the lymphocytes associated with the severity of infection and histological lesion. Recurrent study is in agreement with literature reports that high viral loads may be associated with symptomatic or asymptomatic HCMV infection (Lazzarotto *et. al*., 2011).

Histological analysis of biopsy specimens from placentas showed numerous syncytial knots were found these resembled defects observed in placentas with hypoxia and intrauterine growth restriction. Fibrin deposits with in the villi (intra villous fibrin deposition) and between the villi (perivillous, intervillous fibrin deposition) were noted to be increased in these placentas compared to the none infected HCMV. Leukocytic infiltration, calcification, and necrosis—hallmarks of CMV infection—were evident. Many very large fibrinoids encased floating villi, replacing trophoblasts and fetal blood vessels. Their study also observed placental inflammation consisting of diffuse vascular inflammation, villitis, necrosis, and calcifications (Garcia *et. al.*, 1989). Previous study said the parenchyma showed diffuse chronic villitis with necrosis and hydrops. (Roberts, 2008). Another study that severe placental infection may cause severe tissue damage leading to impaired placental function with reduced oxygenation of the developing fetus (Baze and Schlauch, 2010). Another study was observed correlations

between inflammatory infiltrate HCMV and tissue damage of placenta (Gabrielli *et. al.*, 2009).

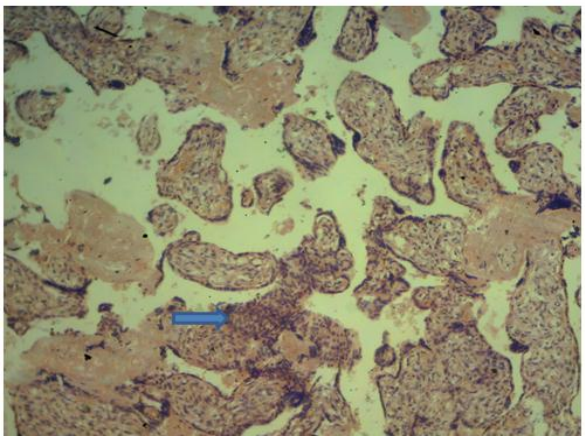

Figure (6) Microscopical appearance shows diffuse villitis and necrosis recognizable as a severe infiltrate of lymphocytes (arrow) in the chorionic villi. (HE stain 10x).

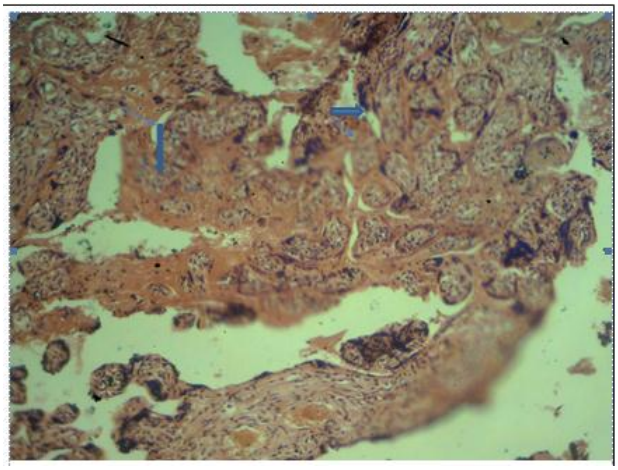

Fig (7) Placental with cytomegalovirus (CMV) infection showing intervillous fibrin deposition, absence and degeneration of terminal villi with syncytiovascular membranes with a decreased surface area diffusion that can lead to hypoxia then abortion. (HE stain 10x).

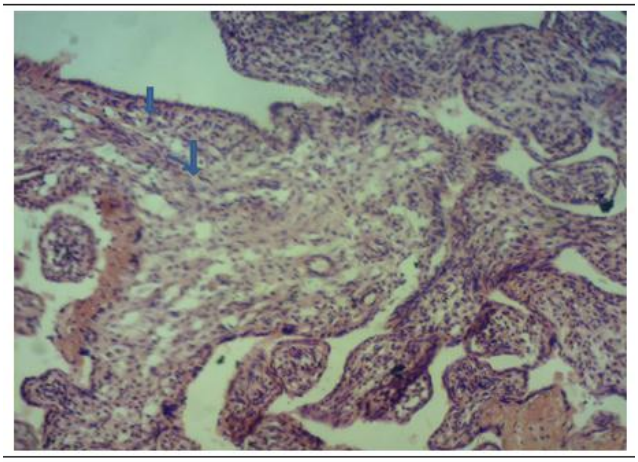

Figure (8) Placental tissue with cytomegalovirus (CMV) infection shows moderate villous edema and hydropic changes of the chorionic villi. (HE stain 40x).

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Estimation of Serum Testosterone Hormone According to Anthropometric Class in Adult Men

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Abstract

Evaluate the serum concentration of testosterone hormone in some healthy adult's men according to their characteristics of anthropometric measurements. This study included ninety-eight volunteer's adult's men, their ages (20-50) years, the blood samples were taken from subjects at the morning during 8:30-10:30 o'clock. ELISA kit was used to assay the serum level of testosterone hormone and the anthropometric measurements have been done such as; body mass index (BMI), waist circumference (WC) and percentage of body fat (BF%) by a special formula and the subjects were assorted depending on classes of anthropometric measurements. The results showed a significant decrease in serum level of testosterone hormone ($p<0.05$) in obese groups compared with the leaner groups. The class of the anthropometric measurements predicts the state of testosterone hormone level.

KeyWords: Testosterone Hormone, Anthropometric class and Adult men.

تقدير هرمون التستوستيرون في الدم وفق لفئة الجسم البشري في الرجال البالغين

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الحلة – العراق

*البحث مستل من رسالة ماجستير للباحث

الخلاصة

اجريت هذه الدراسة لتقييم تركيز مصل هرمون التستوستيرون في بعض الرجال البالغين الأصحاء وفقاً لخصائص قياسات الجسم البشري. وشملت هذه الدراسة ثمانية وتسعون شخصًا من المتطوعين البالغين، أعمارهم (20–60) عامًا، تم أخذ عينات الدم من الأشخاص في الصباح خلال الساعة 8:30 إلى 10:30، وتم قياس هرمون التيستوستيرون باستخدام جهاز Elisa وتم قياس معايير الجسم مثل: وزن الجسم، وطول الجسم وتم حساب كتلة مؤشر الجسم، وتم قياس محيط الخصر (WC) والنسبة المئوية من الدهون في الجسم (BF) باستخدام معادلة خاصة وأظهرت النتائج انخفاض معنوي في مستوى مصل هرمون التستوستيرون $p <0.05$ في الاشخاص البدناء وفقا لتصنيف المعايير الجسمانية. نستنتج بان تركيز هرمون التيستوستيرون تأثر بقياس نسبة الدهون ومحيط الخصر ووزن الجسم لأشخاص مجتمع الدراسة.

الكلمات المفتاحية : هرمون التيستوستيرون، الرجال البالغين و القياسات الجسمانية.

Introduction

The stable body weight depends on an equal balance calories intake from food and expenditure of calories, due to a lot of intake calories without accompaniment expenditure energy, the extra calories will store in the fat cells present in adipose tissue and cause overweight and leads to obesity (Hall *et. al*., 2012), as previous literature has suggested that endocrine and genetic, physiological, behavioral factors play a significant role in the etiology of obesity in turn obesity is associated with multiple alterations in an endocrine system including the abnormal level of circulating blood hormone. (Cheung and Mao, 2012).

Testosterone is a steroid hormone from the androgen group which has a significant influence on important aspects of life such as physical appearance, behavior, mentality, abilities, sexuality and social status and plays a significant role in obesity, glucose homeostasis, and lipid metabolism (Saad and Gooren, 2011; Van Anders *et. al*., 2015) As it's known for some time that testosterone has a major influence on body fat composition and muscle mass in the male (Kelly and Jones, 2013) then the component of body fat is an essential in the diagnosis of obesity and used as an indicator of health risk and which type of body fat is closely connected with the metabolic disorder, in particular, the visceral adipose tissue, likely the bioavailability of testosterone molecule correlated independently with visceral adipose tissue (Nielsen *et. al*., 2007; Przybylska *et. al*., 2012) this reasonable to estimate body fatness by measures the body fat percentage depending on the classification in which listed in the table 1.

Table (1) General body- fat percentage categories in adult men (Muth, 2009).

Description	Fat%
Essential fat	**2-5 %**
Athletes	**6-13 %**
Fitness	**14-17 %**
Acceptable	**18-24 %**
Obese	**25 % +**

Accordingly, the current study was conducted to figure out the grade of testosterone hormone that influence by the body fatness.

Materials and Methods:

Subjects and blood collection

The present study was conducted in the college of science for women, university of Babylon, subjects enrolled in this study included (98) volunteer apparently healthy non-smoking with ages between (20-50) years. Those selected adult men were assorted into subgroups according to their body mass index and body fat percentage and waist circumference classes.

Anthropometric measurements

Body Mass Index (BMI) was calculated by the following equation.

BMI= weight (kg)/square height (m^2), and the ranking of body mass index named according to WHO (2004) criteria. While the Body fat percentage was calculated by the following equation (Chumlea *et. al*., 2002):

Lean body weight =94.42+1.082(weight in pound)-4.15(waist in inches)

Body fat %= (body weight –lean body weight *100)/body weight.

Waist in inches = waist in cm /2.54

Weight in pound = weight in kg *2.2

Protocol to measure waist circumference were based on the procedure by Ma *et. al*., (2013). However, the cut- off point values of WC for men 90 cm associated with BMI of 25kg/m^2 which was applied in our study by reason that action level of WC is more opportune with demographic factors with our study population and provided more significant data. Accordingly, our population were assorted into two groups, one of them included individual with a large (WC)≥ 90 cm, the other group included the subjects with a small (WC) < 90 cm.

Determination of serum testosterone concentration

Human testosterone concentration was measured by Enzyme Linked Immune Sorbent Assay as mentioned in procedure of Elabscience Biotechnology company kit.

The standard curve of testosterone determination was plotted in figure1 as below:

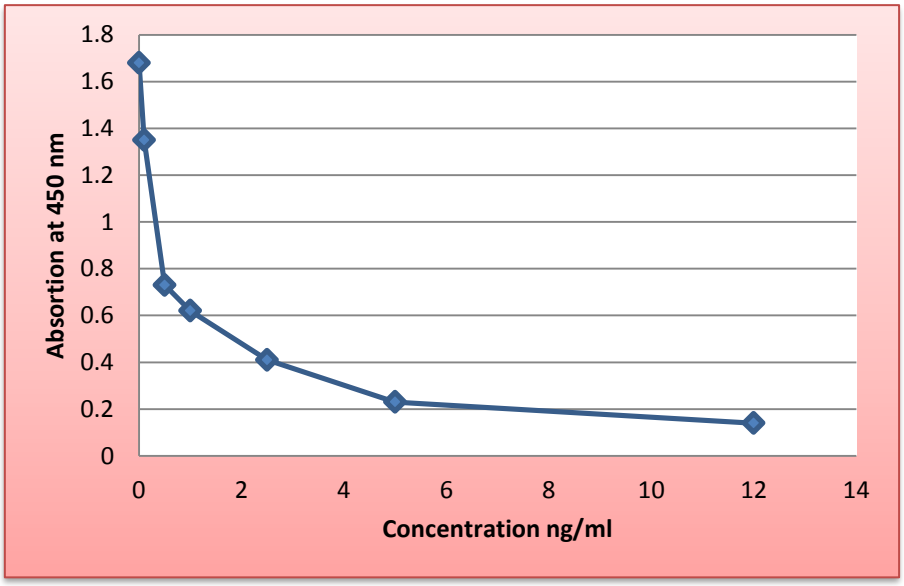

Figure (1) The standard curve of Testosterone concentration.

Statistical Analysis

Data analysis were performed on SPSS (version 18.0) software, data are being expressed as mean ± SD, a nova and independent -sample T test were used to determine any statistical difference for investigated parameters among subjects, post hoc test applied to multiple comparison among investigated characteristics, the ($p < 0.05$) were considered statistically significant.

Results

Our results revealed that testosterone level was significantly lower ($p<0.05$) in obese subjects (5.2±1.8ng/ml) than in subjects with normal weight (7±2.2ng/ml) as showed in figure 2.

According to body fat percentage the results were showed that serum testosterone level has significantly lower ($p<0.05$) in obese group compared with those subjects in athletes' group

In addition, subjects with large waist circumference recorded lower significant of testosterone level($p<0.05$) than subjects who have wider waist circumference as exhibited in figure 4.

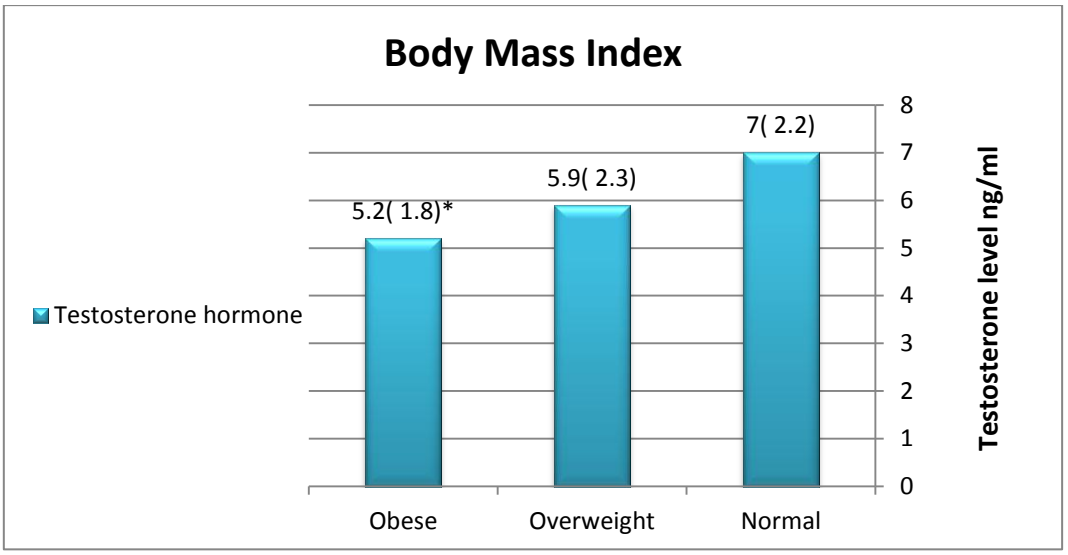

Figure (2) values of testosterone hormone according to Body Mass Index (BMI) categories.
*p< 0.05 vs. normal weight group.

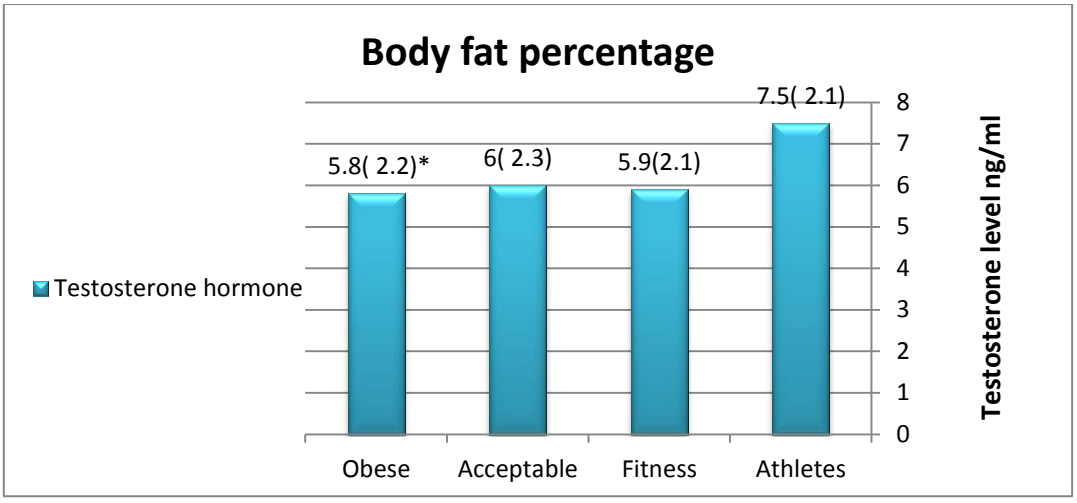

Figure (3) Values of testosterone hormone according to Body fat percentage categories.
***p< 0.05 vs athletes group**

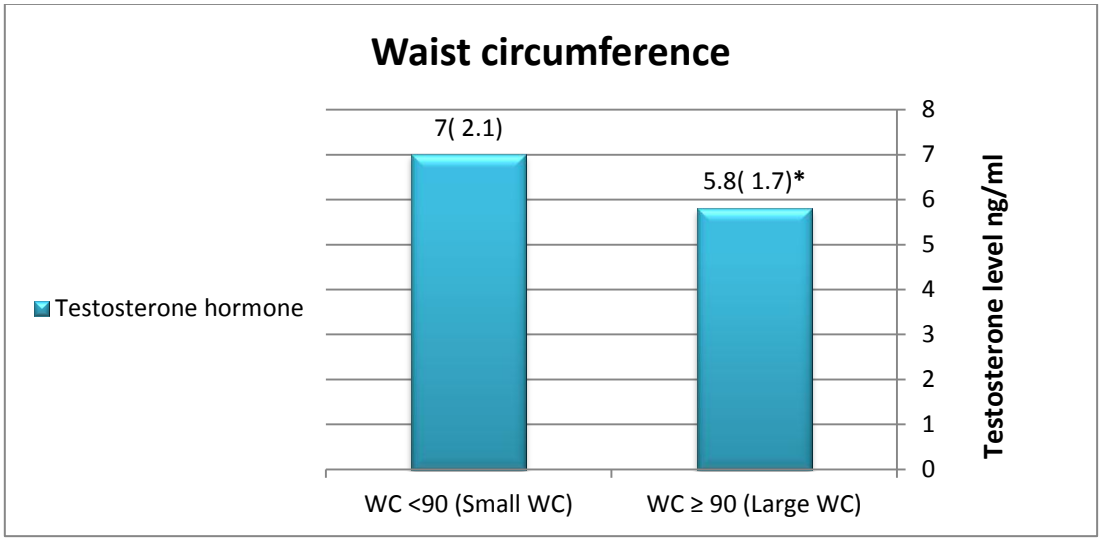

Figure (4) Values of testosterone hormone according to Waist circumference categories.
***p< 0.05 vs small WC group.**

Discussion

We assumed that low circulating testosterone hormone level contribute to the development of obesity.

Inconsistent with this hypothesis our data shows that low testosterone level associated with greater body fat percentage, body mass index and wider waist circumference, this obtain agrees with a hypothesis by Kelly and Jones (2013) who suggested that testosterone has a major influence on body fat composition and muscle mass in the male and the insufficiency of this hormone is related with an increased fat mass particularly central obesity moreover, other literature work confirms that obesity impairs testosterone levels then the low testosterone promote increased fat deposition was primarily proposed as the hypogonadal obesity cycle (Fui *et. al*., 2014) while other explain in another research about the role of sex hormone concluded that testosterone hormone represent a potentiate leptin signaling as a central mechanism to suppress lipid synthesis and promote lipolysis as a peripheral mechanism (Yanase *et. al*., 2015).

Testosterone is converted to 17β estradiol (E2) by the enzymatic activity of aromatase in adipose tissue (Stocco, 2012), hence higher adipocyte expression of aromatase makes a subsequent reduction of circulating testosterone, in turn, this falling in testosterone level is induced increasing adipocytes number and fat deposition, which gradually leads to a further lowering that one hormone (Kelly and Jones, 2013), therefore the insufficiency of the amount or action of steroid hormone causes the obesity (Yanase *et. al*., 2015)

Conclusion

Reduction in testosterone concentration contributed to obesity occurrence, in other words, obesity has an enhanced role to reduce the secretion of testosterone in the body.

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A Case Study of Wound Myaiss in an Adult Women's Foot in Baghdad

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Abstract

Wounds Myiasis is an accidental infection that occurs when fly eggs or larvae are deposited in opened wounds due to injuries. The present case study report a case of wound myiasis in a foot of an adult women.

KeyWords: Wounds Myiasis and Cutaneous myiasis.

دراسة حالة نغف الجرح في قدم امراة بالغة من بغداد

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بغداد العراق

الخلاصة:

نغف الجروح من الاصابات العرضية التي تحدث في حالة وضع البيوض او اليرقات من قبل الذباب في الجروح المفتوحة بسبب الاصابات. الحالة المرضية الحالية هي تقرير عن مثل هذه الاصابات المرضية من النغف في قدم امراة بالغة العمر في بغداد.

Introduction

Myiasis is a term derived from a Latin word Muia which means fly, iasis which means disease and the term was christened by Hope in 1849 and explained by Zupmt (Sharma et. al., 2008; Felices and Ogbureke, 1996; Zupmt, 1956). It is a pathological condition in which there is growth of dipterous larvae in a living individual which at least for a certain period of time feed on the host's dead or living tissues and develop as parasites (Sharma *et. al.*, 2008; Hope, 1840).

myiasis can be classified depending on the condition of the tissues that are involved as: obligatory, where the parasite cannot complete its life cycle without its parasitic phase, which may be specific, semi specific or opportunistic and facultative (accidental) when it is not necessary to complete the life cycle of the parasite in the host and perhaps a normally free-living larva accidentally gains entrance to the host or it may be classified depending on the tissues that are involved as cutaneous (the most common), ocular: nasopharyngeal; urogenital and intestinal. Cutaneous myiasis can be further differentiated based on its clinical manifestations: furuncular myiasis; wound myiasis and migratory myiasis (Gomez et. al., 2003; Aguilera et. al., 1999; Robbins and Khachemoune, 2010; Solomon et. al., 2016). Wound myiasis describes infection of an open wound or mucous membrane (PHaro et. al., 2017). In the human beings the most common site of myiasis is nose, ear, vagina, and skin (Hall and Wall, 1995).

Case presentation

An adult person**'s** foot infected with wound myaisis (figures 1 and 2) which show very clearly the larvae (maggots) present in the necrotic tissue of the foot before this time the patient was injured and due to using profuse water to perform some duties and

because of not paying any attention to the treat the wound, this played as a predisposing factors leading to wound inflammation, tissue necrosis and maceration and make a good environment for attracting dipterous fly to deposit her eggs into the wound hatched later into larvae.

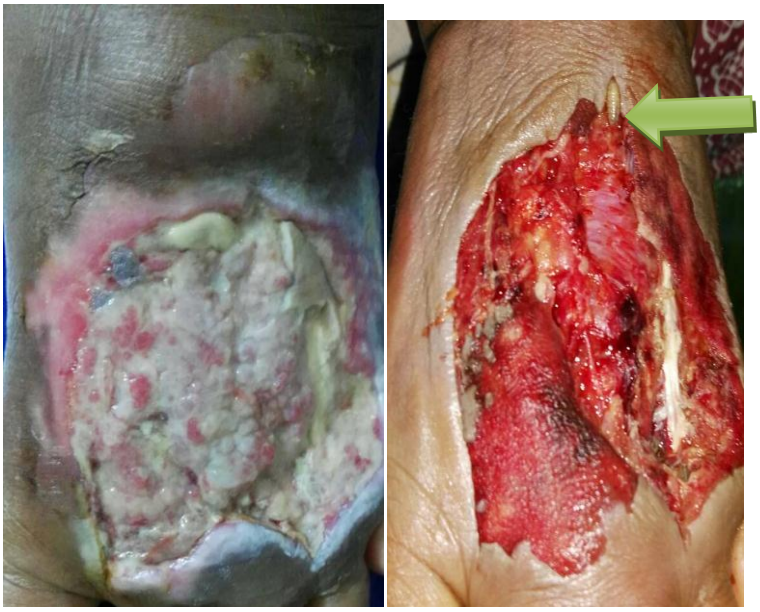

Figure (2) to the right shows the larva at the top of the wound (green arrow).

Other factors lead to make the situation more worse is the using of a bad choice of treatment (hydrogen peroxide) in order to get rid of the necrotic tissue and to treat the myiasis infection by a medical staff in one of the capital hospitals and this did not help to treat the case but make it worse and the larvae still alive in the wound And makes a tunnels, patient felt the movement of the larvae in her subcutaneous tissue of the foot.

The ex- treatment substituted with another choice depending on the principals used by Francesconi and Lupi (2006). So by using Povidine (10% PVP iodine) injected into the tunnels in order to make the larvae suffocated, keeping taking antibiotic (cefotaxine/1gm) to prevent secondary bacterial infection, scraping the necrotic and macerated tissue with keeping covering the foot with gauze to prevent attracting more flies, after month of this treatment the patient get well and a scar tissue developed at the site of the wound (figure 3).

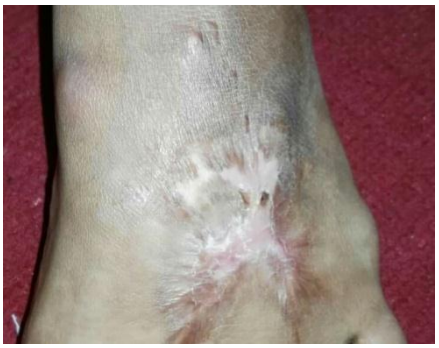

Figure (3) shows the improvement of wound healing after treatment changing.

Conclusion

Cutaneous myiasis is the infestation of the skin or mucous membranes by larvae of the order Diptera. Wound myiasis affect the skin with a previous lesion and it may consume both dead and living tissue.

It is a parasitic infestation of vital tissue of humans or other mammals by dipterous larvae. Human myiasis is a rare clinic condition but more frequently seen in tropical and subtropical areas (Bayndr *et. al.*, 2012).

Another critical factor is an abundance of exposed preexisting suppurative lesions that attract and stimulate the deposit of eggs by the female insect. For specific species, habits of the population, such as sitting or lying on the ground and some religious rites, and climatic conditions influence the occurrence of myiasis (Francesconi and Lupi 2006, 2012).

Myiasis can be prohibited by avoiding contact with infected hosts such as livestock, taking precautions while outdoors in rural areas (wearing clothing that covers arms and legs), keeping to a routine of adequate personal hygiene, and screening doors and windows to avoid contact with flies, these precautions should be especially extended to young children, who tend to exert less caution when playing outdoors and thus may be more likely to contact hosts or contaminated foliage (PHaro *et. al.*, 2017). Field control of flies is extremely important. All available methods should be used, including aerial sprays, destruction of animal carcasses, elementary sanitary and hygiene practices, and clearing of debris near houses. Individual actions should also be taken seriously and include emptying and steam cleaning dumpsters on a regular schedule, washing food and making a visual inspection of the food before consumption, storing food in adequate receptacles, making sure wounds are cleaned and suitable dressing ;sleeping nude, outdoors, and on the floor should be avoided. Appropriate precautions will help avoid infestations; the use of screens and mosquito nets is essential to prevent flies from reaching the skin. Adequate sanitation can be done by combination of official and civil efforts (Caissie *et. al.*, 2008).

Any kind of myiasis should be consider of a big importance and should be treated like other medical cases so more orientation and knowlegment about this issue in medical colleges and other public health educational programs should be implemented to prevent such cases in future.

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