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Association of Interleukin-6, Interleukin-17 and Angiopoietin in Different Infections, Thyroid Stimulating Hormone, Demographic and Socioeconomic Characteristics in Women with Bad Obstetric History, Kirkuk, Iraq.

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Abstract
Background: Bad obstetric history [BOH] is associated with microbiological infections and abnormal immunological responses.
Study design: Descriptive Case Control Study**.**
Aim: to determine the association of interleukin-6 (IL-6), interleukin-17 (IL-17) and angiopoietin with infectivity in female with Bad obstetric history, in Kirkuk city, Iraq.
Materials and method: The study included 547 Bad obstetric histories with Bad obstetric history and 291 female with normal pregnancy outcome. The test performed to detect IL-6, IL-17 and angiopoietin in 60 women with BOH, of them 20 were pregnant and 20 were non-pregnant at time of enrolment in the study. In addition, 20 female with inevitable abortion be included in the study. Also the study included 28 women with normal pregnancy as control and 14 of them were pregnant and 14 were non-pregnant. Serological study carried out to determine IL-6, IL-17 and Angiopoietin using ELISA kits.
Results: IL-17 significantly lower in parvovirus 19 acute infection. Angiopoietin was significantly lower in Toxoplasma gondii infected women, while IL-6 was significantly higher in acute rubella infection as compared to non-infected. Angiopoietin was significantly higher in women with positive TPO autoantibodies. Smoking, animal exposure and low haemoglobin (<11) were associated with decrease in IL-6, while IL-17 was significantly higher in women with history of animal exposure.
Conclusion: IL-6, IL-17 and angiopoietin may be influenced by infections in women with BOH, however, this need to be evaluated in a large scale study.
Key Words: BOH, IL-6, IL-17, Angiopoietin, women.

Introduction

Pregnancy outcomes are influenced by multiple factors that include immune responses effects, inflammatory and infections [1, 2]. Interleukin-6 is an interleukin

which act as anti-inflammatory and proinflammatory cytokine [3]. Previous studies suggest that IL-6 dysregulation may disturb the implantation of pregnancy and associated with recurrent abortion in animal model and human [4,5]. Interleukin-17 is a pro inflammatory cytokines, with a numerous immunoregulatory functions that including immune/autoimmune related diseases [6]. Angiopoietin-1 plays a vital role in the development of vasculogenesis and angiogenesis in human placenta [7]. Previous studies performed in Iraq indicated a significant association between infections, autoantibodies induction and hormones and bad obstetric outcomes [8-18]. Thus this study was conducted to determine the association between IL-6, IL-17 and angiopoietin in women with BOH in relation to some infections, TPO, TGA, TSH, SES and demographic characteristics.

Materials and methods

Study design and settings

The study plan be a Descriptive Case Control Study and was perform in Kirkuk General Hospital. Study proposal was approve by Tikrit University College of Science ethical committed and Kirkuk Health Authority Research Committee. Informed consent taken from each female included in the study.

Aim: to determine the association of IL-6, IL-17 and angiopoietin with infectivity in female with Bad obstetric history, in Kirkuk city, Iraq.

Study population:

Study population is female with childbearing age. Study population wis recruited from Kirkuk General Hospital. 838 female with age vary from 14 to 48 ware included in thi study. Of the sum, 547 females were with bad obstetric history and 291 female witc normal previous pregnancies as control group. The demographic information of these groups is showed im Table 1. For serological analysis, 5-10 mL of venous blood was collected in a sterile container with strict aseptic precautions from each studi subject. The serum way separated and store in numbere aliquots at -20 ^{o}C till assayed. All the serum simples collected from the study And control groups were tested for IL-6, IL-17 and angiopoietin by commercially- available (ELISA) kits. The results read by a Microwell reader and compared in a parallel manner with controls; optical density read at 450 nm on an ELISA reader.

The test performed on samples from 60 women with BOH, of them 20 was pregnant and 20 were non-pregnant at time of enrolment in the study. In addition, 20 women with inevitable abortion were included in the study. Also the study included 28 women with normal pregnancy as control and 14 of them were pregnant and 14 were non-pregnant, Table 2.

Determination of IL-6, IL-17 and angiopoietin:

ELISA was used for determination of IL-6, IL-17 and angiopoietin in serum and the test was performed according to manufacturer instructions. The kit purchased from Biotech, Inc, 323 Vintage Park Dr, Foster City, CA 94404 .

Statistical analysis:

The proportion and the mean value were computed im appropriate situations. To find out Any association between categorical data, Chi square test was employed using the Spas (Version 16). The studi finding data were presented as frequency and percentage.

Table 1. Study population

Group		Number	Mean age ± SD in years
Female with bad obstetric history	Pregnant	292	28.35 ± 7.25
	Non pregnant	255	28.24 ± 6.81
	Sum	547	
Female with normal pregnancy	Pregnant	140	27.40 ± 6.24
	Non pregnant	151	28.06 ± 10.51
	sum	291	
Grand total		838	28.42 ± 7.72
P value	ANOVA		>0.05

Results

IL-6, IL-17, and angiopoietin in different Infections.

Angiopoietin was significantly (P=0.027) lower in women with remote infection with T. gondii (73.5 pg/ml) as compared non infected (92.42 pg/ml) women. However, there was no significant difference in IL-6 and IL-17 between T. gondii infected and non infected women, Table (2).

Table 2. Mean values of angiopoietin1, IL-6, and IL-17 in women with *T. gondii* infection.

Variable	Mean ± SD					
	IgM			IgG		
	Negative No=88	Positive No=0	P	Negative No=77	Positive No=11	P
Angiopoietin	90.06±26.61	0 [0]	-	92.42±26.13	73.50± 25.04	0.027
IL- 6	23.15±13.38	0 [0]	-	23.15± 3.59	23.11 ± 12.37	NS
IL-17	117.4±86.06	0 [0]	-	117.95±88.8	113.53± 72.77	NS

In women with rubella acute infection, angiopoietin mean values was lower (83.77 pg/ml) than in women with negative (90.2 pg/ml) rubella infection, but the difference was not significant. In addition, IL-6 mean value was significantly (P=0.044) higher in women with acute rubella (27.77 pg/ml) as compared to women with rubella negative infection (23.04 pg/ml). Furthermore, IL-17 mean value was higher in women with negative rubella (117.46 pg/ml) acute infection compared to rubella positive (114.93 pg/ml) acute infection, but the difference not reach a significant level, Table(3). The same pattern was demonstrated in women with remote infection for angiopoietin, IL-6 and IL-17 mean values and without significant difference.

Angiopoietin, IL-6 and IL-17 mean values were higher in women with remote CMV infection (93.43, 23.53, 121.28 pg/ml, respectively) than in women with negative CMV infections (88.13, 22.93, 115.19 pg/ml, respectively), but the difference was not significant, Table (4).

Table 3. Mean values of Angiopoietin1, IL-6, and IL-17 in women with Rubella infection.

Variable	Mean ±SD					
	IgM			IgG		
	Negative No=86	Positive No=2	P	Negative No=40	Positive No=48	P
Angiopoietin	90.20±26.74	83.77±27.02	NS	91.47±28.59	88.88± 25.09	NS
IL- 6	23.04± 13.52	27.77 ±1.67	0.044	22.66±12.09	23.55± 14.48	NS
IL-17	117.46±87.6	114.93 ± 0.89	NS	133.83±1.13	103.72±70.54	NS

Table 4. Mean values of Angiopoietin1, IL-6, and IL-17 in women with CMV infection.

Variable	Mean ±SD					
	IgM			IgG		
	Negative No=88	Positive No=0	P	Negative No=32	Positive No=56	P
Angiopoietin	90.06± 26.61	0 [0]	-	93.43±31.33	88.13± 23.59	NS
IL- 6	23.15 ±13.38	0 [0]	-	23.53± 12.53	22.93± 13.95	NS
IL-17	117.4 ±86.6	0 [0]	-	121.28± 74.95	115.19 ±93.18	NS

Women infected with HPV-16 demonstrated a lower mean value of angiopoietin (69.33 pg/ml) and IL-6 (18.5 pg/ml) than in HPV-16 non infected women (angiopoietin=90.79 pg/ml; IL-6=23.31 pg/ml), but the difference was not significant. In contrast, IL-17 mean value was higher in HPV 16 infected women (146.4 pg/ml) as compared to HPV-16 negative (116.38 pg/ml) women, but not reaches a significant level. The same pattern for IL-6 and IL-17 mean values were demonstrated in women infected with HPV-18, however, angiopoietin mean value was about the same in infected (117.36 pg/ml) and non infected (118.54 pg/ml) women, Table (5).

Table 5. Mean values of Angiopoietin1, IL-6, and IL-17 in women with HPV-16 and HPV-18 infection

Variable	Mean ± SD					
	HPV 16			HPV 18		
	Negative No=85	Positive No=03	P	Negative No=85	Positive No=3	P
Angiopoietin	90.79±26.73	69.33±11.47	NS	98.66±26.77	101.28±22.58	NS
IL- 6	23.31±13.58	18.5± 3.72	NS	23.22±13.58	21.02± 6.43	NS
IL-17	116.38±87.18	146.4±75.74	NS	117.36±87.13	118.54±85.84	NS

Women with acute parvovirus infection show a significant (P=0.034) lower IL-17 mean value (98.3 pg/ml) than those without infection (137.39 pg/ml). In addition, IL-6 mean value was higher (P>0.05) in acute parvovirus infected women

(24.48 pg/ml) as compared to non infected (21.75 pg/ml) women. While angiopoietin mean value was lower in acute parvovirus infected women (78.95 pg/ml) than those non infected (92.26 pg/ml) women, but the difference was not significant, Table (6). Women with remote parvovirus infections demonstrated a higher angiopoietin (91.27 pg/ml), IL-6 (23.25 pg/ml) and IL-17 (118.06 pg/ml) mean levels than in women without parvovirus infection (angiopoietin =79.37 pg/ml; IL-6 = 22.22 pg/ml; IL-17=111.6 pg/ml), but the difference were not significant, Table 6.

Table 6. Mean values of Angiopoietin1, IL-6, and IL-17 in women with parvovirus 19 infections.

Variable	Mean ±SD					
	IgM			IgG		
	Negative No=43	Positive No=45	P	Negative No=9	Positive No=79	P
ANG	92.26±30.19	78.95±22.82	NS	79.37±26.93	91.27± 26.47	NS
IL- 6	21.75± 1.06	24.48±15.28	NS	22.22± 7.22	23.25± 13.94	NS
IL-17	137.39±100.47	98.30±66.56	0.034	111.6±51.24	118.06 ±82.95	NS

ANG= Angiopoietin 1

IL-6, IL-17, and angiopoietin in relation to anti thyroid peroxidase (TPO), anti thyoglobulin (TGA) and thyroid stimulating hormone (TSH) positivity.

Angiopoietin mean value was significantly (P=0.0016) higher in women positive for TPO (87.98 pg/ml) than in women who were negative for TPO (28.01 pg/ml). However, TPO positive women was with lower mean value of IL-6 (22.74 pg/ml) and IL-17 (109.24 pg/ml) as compared to TPO negative women (IL-6=30.46 pg/ml; IL-17=123.85 pg/ml), Table (7). Women positive for TGA was with lower mean values for angiopoietin (85.21 pg/ml), IL-6 (21.88 pg/ml) and IL-17 (102.96 pg/ml) as compared to TGA negative women as mean value was 97.11 pg/ml for angiopoietin, 23.12 pg/ml for IL-6 and 129.74 pg/ml for IL-17, Table (7). Women with abnormal TSH demonstrated a higher mean value for angiopoietin (93.78 pg/ml) and IL-17 (125.81 pg/ml) than women with normal TSH as the mean value was 89.23 pg/ml for angiopoietin and 115.53 pg/ml for IL-17. However, the mean value of IL-6 was about the same for women with normal (23.14 pg/ml) and abnormal (23.16 pg/ml) TSH, Table (8).

Table 7. Mean values of Angiopoietin1, IL-6, and IL-17 in women with TPO and TGA

Variable	Mean [SD]			
	TPO		TGA	
	Negative No=2	Positive No=67	Negative No=40	Positive No=31
Angiopoietin	28.01± 16.14	‡87.98±25.76	97.11± 27.34	85.21±27.46
IL- 6	30.46± 4.29	22.74± 3.55	23.12 ±12.32	21.88 ± 10.61
IL-17	123.85 ±51.43	09.24±82.85	129.47 101.7	102.96 ±54.11

‡P = 0.0016

P value for others was NS

Table 8. Mean values of Angiopoietin1, IL-6, and IL-17 in women with TSH.

Variable	TSH Mean [SD]	
	Normal ; No=72	Abnormal ; No=16
Angiopoietin	89.23± 26.38	93.78± 28.22
IL- 6	23.14± 13.53	23.16 ±13.12
IL-17	115.53± 78.08	125.81±120.61

IL-6, IL-17, and angiopoietin in relation to demographic, and socioeconomic characteristics.

Angiopoietin mean value was higher in small size family (93.2 pg/ml) as compared to large size (>3 crowding index) family (86.29 pg/ml). In addition, IL-17 mean value was higher in small size family (119.67 pg/ml) than those with large size family (114.68 pg/ml). However, IL-6 mean value was higher in large size family (24.9 pg/ml) as compared to small size family (21.68 pg/ml), although, the above difference were not significant, Table (9). Angiopoietin mean value was significantly (P= 0.028) higher in women with abortion of <3 (98.88 pg/ml) as compared to women with abortion of more than 3 times (85.72 pg/ml). In addition, IL-6 mean value was higher in women with <3 abortion (25.16 pg/ml, P>0.05) as compared to women with ≥3 abortion (22.16 pg/ml). Furthermore, IL-17 mean level was higher in women with <3 abortion (122.8 pg/ml, P>0.05) than in women with ≥3 abortion (114.75 pg/ml), as shown in Table (9).

Smoking women were with lower mean value of angiopoietin (85.74 pg/ml) than none smoking women (91.43 pg/ml), but, the difference was not significant. In addition, IL-6 mean value was significantly (P=0.029) lower in smoking women (17.77 pg/ml) compared to none smoking women (24.94 pg/ml). However, IL-17 mean value was not significantly higher in smoking women (137.3 pg/ml) than in those not smoker (110.77 pg/ml), Table (9). Animal exposure seems have not an effect on angiopoietin mean level as it was about the same in exposed (89.91 pg/ml) and non exposed (90.12 pg/ml) women. However, IL-6 mean value was significantly (P=0.025) lower in exposed women (18.49 pg/ml) as compared to non exposed (25.32 pg/ml) women. In contrast, IL-17 mean level was significantly (0.013) higher in exposed (150.87 pg/ml) women than in non exposed (101.83 pg/ml) women, Table (9). In women with haemoglobin of ≥11 g/dl, angiopoietin mean value was lower (87.72 pg/ml, P>0.05) than in women with haemoglobin of <11(95.95 pg/ml). However, IL-6 mean value was significantly (P=0.05) lower in women with haemoglobin of <11 (18.82 pg/ml) as compared to those with haemoglobin of ≥11 (24.86 pg/ml). In addition, IL-17 mean value was lower in women with haemoglobin

of <11 (117.69 pg/ml, P>0.05) than in women with haemoglobin of ≥11 (121.26 pg/ml), Table (9).

Angiopoietin mean value was higher in women with age of < 30 years (91.65 pg/ml, P> 0.05) as compared with women with age of ≥30 years (88.15 pg/ml). In addition, IL-17 mean value was higher in women with age of < 30 years (119.06 pg/ml, P>0.05) as compared to women with age of ≥30 years (115.41 pg/ml). However, IL-6 mean value was about the same in women with age of <30 (23.28 pg/ml, P>0.05) and those with age of ≥30 years (23.04 pg/ml), Table (8). Angiopoietin mean value was higher in rural women (91.72 pg/ml, P>0.05) as compared to urban women (88.9 pg/ml). In addition, IL-6 mean value was higher in rural women (23.73 pg/ml, P>0.05) as compared to urban women (22.74 pg/ml). In contrast, IL-17 mean level was not significantly higher in urban (120.1 pg/mL>0.05) women than in rural (113.03 pg/ml) women, Table (9). Angiopoietin mean value was higher in housewife women (90.83 pg/ml, P>0.05) as compared to working women (81.1 pg/ml). In addition, IL-6 mean value was higher in housewife women (23.18 pg/ml, P>0.05) as compared to working women (22.72 pg/ml). In contrast, IL-17 mean level was not significantly higher in working (130.69 pg/mL>0.05) women than in housewife (116.25 pg/ml) women, Table (9). Angiopoietin mean value was with no significant difference between educated and none educated women. IL-17 was higher in uneducated women compared to educated (115.59 pg/ml) women. In addition, IL-6 mean value was higher in uneducated (24.16 pg/ml, P>0.05) than in educated (22.23 pg/ml) women. However, Angiopoietin mean value was higher in educated (91.5 pg/mL>0.05) as compared to uneducated (88.47 pg/ml) women, Table (8). Women with history of congenital anomalies demonstrated a high angiopoietin mean value (110 pg/ml, P>0.05) than those without (89.59 pg/ml). In addition, IL-6 mean value was higher in women with history of congenital anomalies (30.57 pg/mL>0.05) as compared to women without history of congenital anomalies (22.97 pg/ml). In contrast, IL-17 mean value was lower in women with history of congenital anomalies (92.59 pg/mL>0.05) than those without (117.98 pg/ml), Table (9).

Table 9. Mean values of Angiopoietin1, IL-6, and IL-17 in relation to demographic and socio-economic variables.

Variable [Number]		Mean ±SD		
		Angiopoietin	IL-6	IL-17
Crowding Index	< 3 [48]	93.2 ± 27.17	21.68 ± 11.29	119.67 ± 95.93
	≥ 3 [40]	86.29 ± 25.75	24.90 ± 15.49	114.68 ± 75.01
Smoking	Absent [66]	91.43 ± 28.69	24.94 ± 14.31	110.77 ± 67.04
	Present [22]	85.74 ± 19.50	17.77 ± 8.24‡	137.30 ± 128.87
Animal exposure	No exposure [60]	90.12 ± 28.67	25.32 ± 14.3‡	101.83 ± 53.30‡
	Exposed [28]	89.91 ± 22.03	18.49 ±9.86	150.78 ± 127.52
Haemoglobin	≥ 11 [63]	87.72 ± 27.48	24.86 ± 14.89	121.26 ± 90.03
	< 11 [25]	95.95 ± 23.78	18.82 ± 7.01	117.69 ±78.18
Age	≥ 30 [40]	88.15 ± 26.96	23.28 ±12.78	115.41 ± 89.66
	< 30 [48]	91.65 ± 26.50	23.04 ± 13.99	119.06 ±84.88
Residence	Rural [36]	91.72 ±24.05]	23.73 ±14.05	113.03 ± 88.86
	Urban [52]	88.90± [28.42]	22.74 ±13.02	120.10 ± 85.78
Occupation	Housewife [81]	90.83± 27.05	23.18 ± 13.66	116.25 ± 87.03
	Working [7]	81.16± 2.29	22.72 ± 10.41	130.69 ± 86.85
Education	Educated [46]	91.50± 27.12	22.23 ±13.44	115.59 ± 61.26

	Uneducated [42]	**88.47 ±26.28**	**24.16 ±13.41**	**119.39 ± 108.57**
Abortion	**≥ 3 [59]**	**85.72 ± 24.07‡**	**22.16 ± 13.60**	**114.75 ± 90.96**
	< 3 [29]	**98.88 ±29.66**	**25.16 ± 12.92**	**122.80 ±78.23**
Congenital anomalies	**Absent [86]**	**89.59 ± 6.56**	**22.97 ±13.16**	**117.98 ±87.53**
	Present [2]	**110.00 ± 21.21**	**30.57 ±27.35**	**92.59 ±5.41**

‡ P < 0.05

Discussion

IL-6, IL-17, and Angiopoietin in Different Infections.

IL-6 play a critical role in resistance to *T. gondi* [19,20], and although the mechanism by which IL-6 promotes resistance to this pathogen remain unclear, these findings are consistent with its role as a proinflammatory factor [21]. Recent studies have highlighted the importance of IL-6 signaling in promoting the generation of IL-10-producing $CD4^+$ T cells that are essential for limiting infection induced pathology [22]. The present study has not reveal a significant difference between women infected with *T. gondi* and those who are not infected. However, Joanna et al [23] reported that IL-6 was two-fold higher in women infected with *T. gondi* as compared to healthy subjects. The difference between the findings of the two studies may be attributed to the differences in sample size and study design, as our study population included women with BOH. In the present study women with normal pregnancy outcomes are with significant higher mean serum IL-6 as compared to those with BOH, which are consistent with reported studies [24-26]. These findings indicate that IL-6 is with anti inflammatory effects that are exaggerated in the absence of appropriate regulation by suppressor of cytokine signaling family [27], this inhibitory effect may represent a physiological function of IL-6. In mice (gb130 Y757F) challenged with *T. gondii*, IL-6 that is produced become an efficient antagonist of IL-12 production that is essential to control this parasite. It appears that *trans* signaling provides a significant contribution to the IL-6-mediated susceptibility to *T. gondi* in these mutant mice [21]. The present study confirmed such hypothesis as it indicated that mean serum level of IL-6 was lower in women with BOH, which may lead to increase susceptibility to infection in such population. There is an evidence that IL-6 is part of regulatory loop that is important to initiate inflammation but can also act to limit this response in a chronic setting [21].

Previous studies have implicated T cell production of IL-17 in resistance to *T. gondi* as well as the development of immune mediated pathology during this infection [28]. Our study did not indicate a significant difference between women infected with *T. gondi* and control. However, other studies reported that serum IL-17 was higher in women with *T. gondi* infection than in control [29], while other group reported that it was higher in non infected women [30].

This study indicated that Angioipoietin-1 was significantly lower in women with remote *T. gondi* infection as compared to those who were not infected. This finding was consistent with that reported in other type infection [31]. In addition, Angiopoietin-1 mean serum levels were not significantly different in women infected with rubella, HPV-16, HPV-18, CMV and parvovirus 19. IL-6 and IL-17 mean serum

levels were not significantly different in women infected with rubella, CMV, HPV-16, HPV-18 and remote parvovirus 19 from those in control. However, mean serum IL-17 level was significantly higher in control as compared to women with acute parvovirus 19 infection.

IL-6, IL-17, and Angiopoietin in Relation to Demographic, Socioeconomic Characteristics

The present study demonstrated that serum mean level of IL-6 was significantly lower in smoking women than in non smoker women. This finding did not agree to that reported by Mosson et al [32] in cases of pancreatitis. However, studies of cigarette smoke related increases in IL-6 yielded inconsistent results [33]. Smoking produces an IL-6 exposure-response in patients with chronic obstructive airway disease , both from cumulative smoking and current smoking intensity. Persons who ceased smoking may have continuing inflammation related to their prior cumulative exposure, while those continuing to smoke manifest a pro-inflammatory gradient from active smoking, but did not exhibit effect moderation from the prior smoking burden [33]. Thus the variation in the serum/ plasma levels of IL-6 in different studies may be due to such cumulative exposure difference in studied population. Smoking is a major risk factor for many human diseases [34]. Toshihiro and Chiki suggest that a crucial role was played by interactions between inflammatory cytokines and tobacco smoke in the induction of endothelial dysfunction [35].

IL-6 mean serum level was significantly lower in women with haemoglobin of < 11 g/dl than those with ≥11, and animal exposed women than none exposed. Studies in human and mice suggest that the iron-regulatory hormone hepcidin is the principal mediator of anaemia of chronic disease and/or inflammation [36].

Hepcidin is a liver-produced acute-phase peptide who's over production leads to iron-limited erythropoiesis. Hepcidin binds to the cell membrane iron exporter ferroportin and induces its internalization and degradation, thus decreasing iron release from macrophages and enterocytes. Inflammatory cytokines increase the expression of hepcidin, [37] leading to decreased absorption of iron from the intestine, and block the release of iron from the reticuloendothelial system and the liver [38]. Infusion of IL-6 in human volunteers resulted in increased excretion of urinary hepcidin and the development of hypoferremia [37]. IL-6 is a potent inducer of hepcidin expression through a signal transducer and activator of transcription 3–dependent transcriptional mechanism [39]. In addition, macrophages express hepcidin in response to microbial stimulation [40]. A pathogenic cascade for the development of anemia of inflammation has been proposed that leads from IL-6 to hepcidin to hypoferremia and, as a consequence, to anemia of inflammation [41].

The inflammatory response surrounding the abortus material and endothelial cell is part of the abortion process. In the interactions between the fetus (placenta) and uterine tissue cells and the reactive cells of the microenvironment, there are high levels of cytokines, such as IL-6, IL-10, and the chemokine thymus and activation-regulated cytokine among others. Local production of these cytokines results in elevated systemic levels in the peripheral blood, and these cytokines are responsible for the development of systemic symptoms and laboratory abnormalities that are correlated with disease prognosis. IL-6 is among the cytokines most strongly associated with anemia [42].

This study indicated that mean serum IL-6 concentration was higher in non educated and rural living women, a finding that was consistent with that reported by others [43] who suggested that serum IL-6 was higher in individuals with low socioeconomic standard.

Ang-1 mean serum level was significantly lower in women with ≥ 3 repeated abortions. Immunohistological studies demonstrated an increased Ang-1 mRNA expression in the decidua basalis in healthy, late compared to early first trimester pregnancies [44]. Decidual tissues of healthy pregnancies and miscarriages show an altered Ang-2/Ang-1 ratio based on different oxygen levels in both groups, since hypoxia enhanced Ang-2 transcription and destabilizes Ang-1 in healthy pregnancies [45, 46]. As hypoxia was a key regulating mechanism for Ang expression, it is reseaonable to hypothesize that any change in the hypoxic conditions of early pregnancy (such as pregnancy failure), could alter Ang expression and ratio in the placenta or uterus [47].¨ These alterations might be reflected in maternal serum levels [48]. The present study finding was consistent with such hypothesis, as Ang-1 serum mean values were lower in women with BOH and inevitable abortion than women with normal pregnancy outcomes.

Ang-1 and 2 bind to the same endothelial cell-specific tyrosine kinase receptor, which was activated by Ang-1 but blocked by Ang-2, Placental expression of Ang-1 increases, whereas that of Ang-2 and Tie-2 decreases in the course of pregnancy and subsequently was reflected in maternal serum. However, once local inflammation occurred in pregnancy failure cases placental expression of Ang-1 reduced, followed by reduction of Ang-1 level in maternal blood [46].

IL-17 mean serum level was significantly higher in women exposed to animal. This could be due to induction of zoonotic infection in those set following animal exposure. Th17 cells were directly involved in chronic inflammatory processes, by secreting IL-17, which recruited neutrophil to tissue through induction of granulocyte colony stimulating factor and IL-8 [49]. An interesting finding of our study is that mean serum IL-17 level was lower in women with age of ≥ 30 as compared to those <30 years. This finding was consistent with that reported that serum IL-17 mean value was lower in elderly [50].¨ However Tobias et al [51] reported that IL-17 measured in amniotic fluid was not influenced by maternal age and some cytokines in amniotic fluid including IL-17 were varying in concentration by gestational week.

Interleukin -6, Interleukin -17, and Angiopoietin in Relation TPO, TGA and TSH positivity.

In the present study Ang-1 serum level was higher in women who were positive for TPO antibodies and those with abnormal TSH. However, women with TG positive antibodies were with lower mean serum level of Ang-1 as compared to those who were negative. Angiopoietines dysregulation has been associated with a variety of autoimmune disorders [52]. The data suggesting that the angiopoietin system might contribute to the pathogenesis of autoimmune diseases was derived from patients with autoimmune thyroid disease, in whom monocyte over expression of the Tie-2 receptors appeared to enhance the chemotactic response to Ang-1 and 2 overproduction by thyroid follicular cells, leading to an inflammatory cell infiltrate in the thyroid parenchyma [53]. Our study indicated that Ang-1 significantly correlated with TG antibodies ($r=0.36, P<0.01$) and negatively correlated to TPO antibodies ($r= -0.4, P<0.01$).

Reported studies suggest that IL-6 was increased in autoimmune diseases [54] when compared with a control group, suggesting that IL-6 was involved in the development of the autoimmune diseases. However, the present study contrasted that finding; IL-6 mean serum value was lower in women who were sero-positive for TPO and TG. It might be that the variation in serum IL-6 concentration is a systematic manifestation of immune activation, which might affected by other factors; however, the clinical significance of our observation was uncertain.

Although TSH levels in our studied population with BOH were within the normal range in 42.5% of cases, the studied group was characterized by significantly higher TSH concentrations than the controls.IL-6 serum levels were significantly [$P<0.05$] inversely correlated with TSH serum levels in women with BOH [$r= - 0.32$]. However, there were no correlations between IL-6 and anti thyroid antibodies.

In our study, higher levels of TSH were detected in the studied group and they correlated positively with TPO antibodies. In addition, there was no significant correlation between concentrations of IL-6 and TPO antibodies in the peripheral circulation of women with BOH. This suggested that the two phenomena might not be directly related to each other. However, Maha et al [55]) reported a significant correlation between IL-6 serum levels and Anti TPO in patients with thyroid nodular goiter, but not in Hashimoto thyroiditis.

The newly discovered Th17 cells, a new class of CD4+ cell subsets, also have important regulatory role in autoimmune diseases and infectious diseases [56]. Although Th17 cells secrete IL-17, IL-17F, IL-22 and other cytokines, its main function is the secretion of IL-17 [46]. ·Several cytokines are involved in the differentiation of human Th17 cells [57-60]. Th17 cells and IL-17 play an important role in various autoimmune diseases [61-63].

The present study showed that IL-17 was lower in women with positive TPO and TG antibodies, a finding that was not consistent with that reported in patients with autoimmune thyroiditis [64]. ·However, this study findings demonstrated that mean value of both serum IL-17 and TG antibodies were significantly higher in women with BOH as compared to those with normal pregnancy outcome. In addition, TPO antibodies were higher in women with BOH than in control, but did not reach a significant level.

In women with BOH, TSH serum levels did not demonstrate a significant correlation with Ang-1 and IL-17 serum levels, however, IL-6 showed a significant correlation with serum TSH levels. Furthermore, the mean serum values for Ang-1 and for IL-17 were higher in women with abnormal TSH, but did not reach a significant level. The exact role of antibodies against thyroid peroxidase was unclear but it was likely that they promoted the release of a variety cytokines including IL-6[65].¨

In conclusion: IL-6, IL-17 and angiopoietin may be influenced by infections in women with BOH, however, this need to be evaluated in a large scale study.

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