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Original Article

Role of cytokine in Pathogenesis of *Streptococcus agalactiae* in placentitis of aborted women

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ABSTRACT

S. agalactiae has been appearing as a vital human pathogen and a gradually important cause of aggressive infections in immunocompromised adults and older, the aim of the study was to find out if placental infection with *S. agalactiae influenced* the levels of interleukin 2 (IL-2) in aborted women. A total of 100 aborted women aged between (16 - 42) years, were involved in this study. Placentas specimens were cultured to isolate the *Streptococcus agalactiae*, the level of cytokine in the serum was measured by commercial ELISA tests. Our results showed that there are 21 streptococcal isolates from Placentas specimens, Specific isolation and identification were done for *S. agalactiae*. Out of the 7 S. agalactiae isolates, significant difference could be found in serum levels of IL-2 ($P \leq 0.05$) between these two investigated groups (infected and uninfected with S. agalactiae).

Keywords: *Streptococcus agalactiae,* Abortion, IL-2, pregnancy, ELISA, cytokine

INTRODUCTION

Group B Streptococcus (GBS) is the most common cause of serious infections in newborns and young infants, with a case fatality ratio of 5-8%. It is also a prominent cause of peripartum maternal infections, causing an estimated 50,000 cases annually. [1-2]. In addition, recent reports have documented the importance of GBS as a cause of infection in adults with certain underlying medical conditions. The attack rate for GBS disease among neonates has been estimated at 1.8 cases per 1,000 live births , and the adult rate of infection at four cases per 100,000 population [3-4].Importantly, It has been observed that GBS reported as pathogens in pregnant woman causing reproductive tract infections, including: postpartum endometritis (10 to 20%), puerperal sepsis , chorioamnionitis and postpartum complication including :urinary tract infection, bacteremia, wound infections associated with caesarean delivery, and meningitis [5].GBS genital colonization lead to a possible cause of premature deliveries and premature rupture of membranes , Several studies have also reported that GBS colonization may play important role in the occurrence of intrauterine deaths, late abortions and low birth weight infants [6]. GBS can pass through placental membranes and weaken its strength. As a result of these routes, GBS may entrance the fetus within the amniotic cavity, cause placental membrane break or activate premature delivery. After aspiration of infected amniotic or vaginal fluid, the newborn lung is the key effort of GBS infection. From lung, the organism can enter into the bloodstream and then spreads to various organs and tissues [7]. Peptidoglycan and other GBS components related to the cell wall without the surface PSC, appear to be the most inflammatory agents in eliciting host cytokine cascades, in actual the proximal mediators TNF-α and IL-1. [7]. Normal pregnancy was accompanied by a decrease in Th-1 productive capacity, together with an increase in Th-2 production, most notably in the third trimester, The cause of recurrent pregnancy loss (Three or more consecutive spontaneous miscarriages) are unexplained in the majority of women and it is thought that abnormalities in the immune system may have a role in idiopathic recurrent abortion [8]. The aim of the study was to investigate if placental infection with *Streptococcus agalactiae* could influence the serum levels of IL-2 in pregnant women with the clinical symptoms of the abortion.

MATERIALS AND METHODS

Patients: The study enrolled 100 aborted women, admitted at the Department of Gynecology and

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Obstetrics at the Clinical Center in Samawah city, Clinical signs of aborted women were recorded by physician.

***Bacterial isolation*:** For specific isolation of *Streptococcus agalactiae* from Placentas specimens, pieces of placenta were cultured on blood agar then incubated at 37°C for 24 hr , after slicing with sterile scissors dipped in ethanol and flamed, then purified by sub cultured on blood and macconkey agar . The identification tests for the isolate, including cultural, morphological and biochemical characteristics was done for each isolate.

Serum cytokine: Serum samples preparation and immunoassay for IL-2: Venous blood was collected from all patients during the first 24 hours after the first symptoms of abortion. Serum was obtained by centrifugation at 4000g for 15 minutes and stored at -20 C before use. Measurements of cytokines in the serum were performed by ELISA test (R&D Systems). Absorbance was measured in duplicates with a micro plate reader (Beckman Coulter). The final concentration was expressed in pg/ml.

Statistical analysis: Statistical analysis was conducted by using Chi-square (χ^2) test to determine the statistical differences among different groups by using a design statistical package for social science (SPSS 19). The probability of ($P \leq 0.05$) was considered to be statistically significant. The investigated parameters were presented in terms of means ± standard errors (S.E.), and differences between means of patients and controls were assessed by ANOVA test and the Least Significant Difference (LSD). The difference was considered significant when the probability (P) value were (≤ 0.05, ≤ 0.01).

RESULTS AND DISCUSSION

***Bacterial isolation*:** our result showed that different type of bacteria were isolate from study samples.out of 21 streptococcal isolates, 7 samples were positive for *S. agalactiae*. The current results same that obtained by (Chaudhry et al., 2001) whom mentioned that Out of a total of 200 vaginal samples of pregnant women, 17 (8.5%) specimens were positive for GBS [9, 10]. (McDonald & Chambers, 2000) said that GBS was a key pathogen in unsuspected intrauterine infections, underlying spontaneous mid gestation abortions [11, 12]. But (El Kersh *et al.*, 2002) found that no correlation between the presence of group B streptococci and a history of repeated spontaneous miscarriages [13, 14].

Identification of St .agalactiae *:Streptococcus agalactiae* cultured recognized on the basis of colonial morphology, which appeared smooth, 3-4mm in diameter, convex, rounded, β-hemolytic colonies and grayish – white colour on blood agar as illustrated in Figure (1 and 2) and don't grow on macconkey agar. The present results like that mentioned by [15].

Biochemical tests: *S. agalactiae* was negative for catalase, oxidase, Gelatin liquefaction & resistance for bacitracin as shown in table (2) and Figure (3). The current results of this study were in agreement with [16, 17].

Serotyping test: The present study shows all isolate agglutination with B group of a plastic card of Masta group kit. The appearance of agglutination denotes that specific interaction of carbohydrate antigen extracted from the cell wall of bacteria with monoclonal antibodies as shown in Figure (4). (Niazy, 2011) reported that (49%) of the specimens were positive with group D antisera, and (23%) gave positive results with group B antisera [18]. In 1933, Lancefield noted that many *Streptococci* could be serologically identified and grouped according to their cellular carbohydrates or so-called *C* substances [19, 20].

Serum cytokine IL-2): The results of cytokine production were calculated by using the equation from the standard curve in the same assay. Serum IL-2 concentration revealed a significant increase ($P< 0.05$) in serum of aborted women infected with *S .agalactiae* (1605.81 ± 25.68) pg/ ml compared with aborted woman that non infected with *S .agalactiae* (824.63± 153.77) pg / ml as shown in table (4). Our result agreement with Ahmed who reported that highly significant increase in the serum level of IL-2 in aborted women as compared with control groups [21].Th-1 cytokines are considered to be detrimental to pregnancy, via direct embryo toxic activity, or via damage to the placental trophoblast, or possibly by activating cells that are deleterious to the conceptus, whereas Th-2 cytokines may directly or indirectly contribute to the success of pregnancy by down regulating potential Th-1 reactivity [22- 23].

CONCLUSION

The data of this study strengthen the possibility that high level of IL-2 in aborted women due to placental infection with *S . agalactiae* may explain the role of type-1 cytokines in the pathogenicity of *S . agalactiae* and progress of placental changes then abortion.

Table (1):Type of kite that used

No.	Type of kit	Manufacturing company	Origin
1.	Blood agar base	Oxoid	UK
2.	MacConkey agar	Oxoid	UK
3.	Brain heart infusion broth	Oxoid	UK
4.	Gelatin agar	Himedia	India
5.	API 20E kit	Biomerieux	France
6.	Mastastrep kit	Merseyside	UK
7.	Interleukin(2)kit	Elabscience	China

Table: (2) Biochemical tests of *S. agalactiae* isolates.

No.	Type of test	Result
1.	Gram stain	+
2.	Catalase test	-
3.	Oxidase test	-
4.	Growth on macconkey	-
5.	CAMP test	+
6.	Lancefield group	B
7.	Gelatin liquefaction	-

Table:(3) Api20 Strep for *S. agalactiae* isolate.

No	**Test**	**Reactions/Enzymes**	**Result**	**Color**
1	VP	Acetoin production (Voges Proskauer)	+	Pink-Red
2	HIP	Hydrolysis (HIPpuric acid)	+	Dark blue/Violet
3	ESC	ß-glucosidase hydrolysis (ESCulin)	-	Colorless Pale yellow
4	PYRA	PYRrolidonyl Arylamidase	-	Colorless or very pale orange
5	Agal	Alpha galactosidase	-	Colorless
6	BGUR	Beta glucuronidase	+	Blue
7	BGAL	Beta galactosidase	-	Colorless or Very pale violet
8	PAL	Alkaline Phosphatase	+	Violet
9	LAP	Leucine AminoPeptidase	-	Colorless
10	ADH	Arginine Dihydrolase	+	Red
11	RIB	Acidification (RIBose)	+	Orange/ Yellow
12	ARA	Acidification (ARAbinose)	-	Red
13	MAN	Acidification (MANnitol)	-	Red
14	SOR	Acidification (SORbitol)	-	Red
15	LAC	Acidification (LACtose)	+	Orange/ Yellow
16	TRE	Acidification (TREhalose)	+	Orange/Yellow
17	INU	Acidification (INUlin)	-	Red
18	RAF	Acidification (RAFfinose)	-	Red
19	AMD	Acidification (AmiDon)	-	Red
20	GLYG	Acidification (GLYcoGen)	-	Red or Orange

Table (4): The Concentration of IL-2 in patients and controls.

Group	number	Serum level of IL-2		
		Mean±SD	Minimum	maximum
Patient	7	1605.81 ± 25.68	1542.22	1733.33
Control	81	824.63± 153.77	79.26	12170.04

Figure 1: colonial Morphology of St. *agalactiae*. On blood agar showing round, translucent, smooth, small, with β- hemolytic colonies.

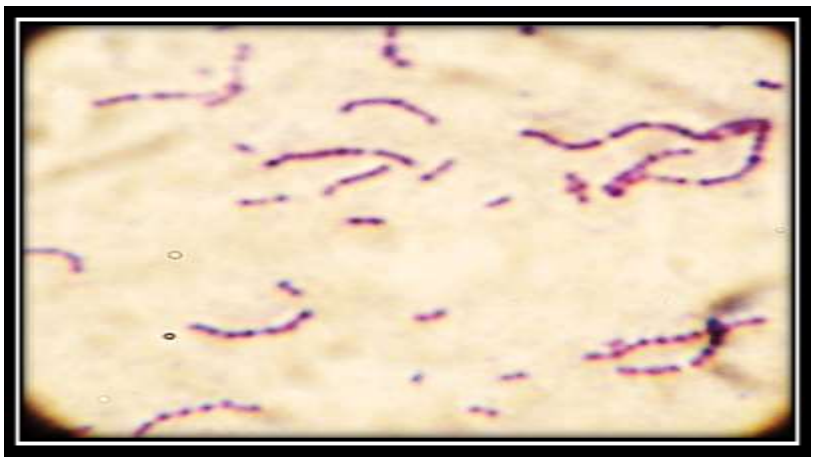

Figure 2: Microscopical morphology of S. *agalactiae* Showing gram positive cocci, arranged as chin or pairs.

Figure 3: API 20Strep System for *S .agalactiae* isolates**.**

Figure 4: Masta group kit for serological diagnosis of *S. agalactiae.* Showing agglutination of our isolates with B group of aplastic card.

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