Njum and Hamed, J Clin Trials 2018, 8:4
DOI: 10.4172/2167-0870.1000353

Journal of Clinical Trials
ISSN: 2167-0870

Research Article Open Access

Role of Inflammatory Cytokines and Immune Reactive Molecules in Pathogenesis of Streptococcus Agalactiae in Aborted Women

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Abstract

Streptococcus. 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 the effect of inflammatory cytokine (interleukin 8) and immune reactive molecules (CD79 and CD54 molecules) on pathogenesis of S. agalactiae that isolated from aborted women. A total of 100 aborted women aged between (18 - 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, while CD molecules was estimated by immunohistochemistery assay. Our results showed that there was streptococcal isolates from Placentas specimens, Specific isolation and identification were done for S. agalactiae. Significant difference could be found in serum levels of inflammatory cytokine (P≤ 0.05) between these two investigated groups (infected and uninfected with S. agalactiae) in addition to high expression for CD79 and CD54 in infected women as compare with non S. agalactiae infected women .

Keywords: CD Molecules; Inflammatory cytokines; Placentitis; S. agalactiae

Introduction

Streptococcus agalactiae is Gram-positive, oxidase- and catalase-negative. Streptococcus agalactiae has been divided serologically into 9 serotypes (Ia, Ib, and II-VIII) due to various in capsular polysaccharide Streptococcus agalactiae or group B streptococcus (GBS) is a commensal organism in humans, but can cause life threatening infection in susceptible hosts such as neonates, pregnant women and non-pregnant adults with chronic illnesses [1]. The structure of the human vaginal flora is precious by some host factors, plus, phase, high parity, health care workers, high Body Mass Index, chronic diseases as diabetes, sensual action, gestation and the custom of contraceptives, antibiotics, as well as separate ways such as antiseptic-douching sterility [2].

Group B Streptococcus (GBS) has become the major cause of bacterial infections in the perinatal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as focal and systemic infections in newborns. It is a relatively rare cause of infection in older children and non-pregnant adults [3].

Macrophage and monocytes in neonatal blood and in the urinary tract respond to GBS with a pro-inflammatory cytokine release, involving Interleukin (IL) IL-1α, tumor necrosis factor (TNF) and IL-6. Interleukin-8 secretion is elevated by oxidative stress, which in that way causes the enrolment of inflammatory cells, induces a higher in oxidative stress mediators, producing important parameter in localized inflammation [4]. If a pregnant woman has high levels of interleukin-8, there is an important risk of schizophrenia in her progeny. The preterm labor is related with the raised uterine construction of pro-inflammatory cytokines, IL-1β IL-6, IL-8 and TNF, where believed to excite uterine action, either straight or through a rise in prostaglandin construction, the pull of leukocytes, and tissue transformation [5]. Decreasing the inflammatory penetrate or preventing cytokines discharge in these cells might be energetic in the dealing of preterm labor. In the matching way, these mediators can cause abortion.

The CD79 particle forms part of the membrane immunoglobulin complex on B lymphocytes. CD79 itself is required for signal transduction [5]. CD79 is found on all mature B lymphocytes in the blood stream and on B lymphocytes at all phases of development in the bone marrow. CD79 appearance is absent after distinction of B lymphocytes into plasma cells [6].

Materials and Methods Materials

Mastastrep kit was provided by Merseyside/UK. API strep kit and Vitek 2 system kit were supplied by BioMerieux/France Company.

Methods

Samples collection: Clinical signs of aborted women were recorded by physician to show pregnancy period, and clinical sings.

Aborted Placentas: Hundred placenta samples from aborted women have been obtained, at maternity and children hospital of Al- Samawa, after curtag operation (by gynecologists) placenta samples were cultured as described by Saini et al. [7], isolation and identification of S. agalactiae was performed by API strep kit and Vitek 2 system kit then determent groups of isolates by Mastastrep kit, where S. agalactiae positive for group B.

Cytokine assessments (IL-8): Cytokine assessments (IL-8) in serum were performed by ELISA kit: Provided by Elabscience\ China.

Immunohistochemistery for placenta specimens: It was performed as described by (Abcam, UK), The expression of CD79 and CD54 were measured as the same scoring system used by Mao et al. [8)]. The positivity of cells for expression of CD79 and CD54 were seen as brown staining. It was graded as four grad of the cells staining positive for CD79 and CD54.

Score	0	1+	2+	3+	4+
Positive Cells	<10%	10-25%	25-50%	50-75%	>75%

Table 1: 4-Statistical Analysis: All data were analyzed using the statistical package for social science (SPSS) for Windows program on the computer. Chi-square was used to compare between the frequencies. Student t test was used to compare between means of groups. The significance was accepted as P value < 0.05.

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Received date: October 01, 2018; **Accepted date:** October 15, 2018; **Published date:** October 22, 2018

Citation: Njum AA and Hamed AK (2018) Role of Inflammatory Cytokines and Immune Reactive Molecules in Pathogenesis of Streptococcus Agalactiae in Aborted Women J Clin Trials 8: 353.

Results and Discussion

Clinical observation

Clinical signs in women included fever sweating, hypotension, pain, aches and bleeding most women infected with S.agalactia were aborted at first stage of pregnancy.

This result is in agreement with Young, [9] who found that Brucella transferred from mother through placenta to fetus during first stage of pregnancy and causes maternal bacteremia and spontaneous abortion

Bacterial isolation and identification

Out of the 100 aborted women, 7 (7%) were positive for culture after 2 days the S.agalactia culture recognized on the basis of colonial morphology on blood agar appeared as pinpoint, white to milky in color , colonies of 1 to 2 mm in diameter and colonies showed β-hemolysis.

Isolates from blood and placenta samples were Gram-positive, cocci, arranged in short chain or small groups stained didn't grow on macconkey agar and negative for catalase and oxidase.

Blood cytokine (IL-8) assessment

The results of this study showed a highly important increased (P<0.01) of IL-8 (93.88 ± 18.99) pg/ml in serum of aborted women infected with St. agalactiae, compared with aborted women non infected with St. agalactiae (36.69 ± 0. 95) pg/ml as shown in Table 2. The result of current study same as reported by Rana, [10] who discovered that highly significant increased (P<0.01) concentration of IL-8 (44.371+8.772) pg/ml in serum of aborted patient at first month of gestation, control groups (non-pregnant women) which were (7.423 ± 2.152) pg/ml, (6.908 ± 3.859) pg/ml respectively.

The inflammatory mediators, for instance IL-8, might show an important part in the contrivance of protease-induced neurogenic irritation leading to effort or abortions by employing neutrophils and lymphocytes in the endometrium [11].

While prior revision stated that female with natural abortions has significantly reduced plasma equal of IL-8, IL-6 and IL-11 compared to those with normal pregnancies [12]. The great equal of IL-8 in aborted female can be caused by the discharge of IL-8 from the endometrium [13].

Score			Number	Group
Maximum	Minimum	Mean ± SD		
206.94	67.63	93.88 ± 18.99	7	Patient
64.16	21.4	36.69 ± 0. 95	81	control

Table 2: The mean of IL-8 in aborted Woman infected and non and infected with st.agalactia.

Immunohistochemical assay

Histological section of placenta obtained from St. agalactiae infected women and non-infected women revealed marked placentitis associated with St. agalactiae infection. Chorionic villi showed necrotic changes of syncytial cells with extravascular accumulation of red blood cells in decidua and maternal blood spaces. There is infiltration of inflammatory cells (macrophages & neutrophils). Placentitis that occurred in placenta in the present study; necro-fibrinoid and necro-hemorrhagic type. In affected placenta, the trophoblastic epithelium lining the chorionic villi appeared necrotic with spreading of macrophage infiltration into mesynchyma of these villi Figure .

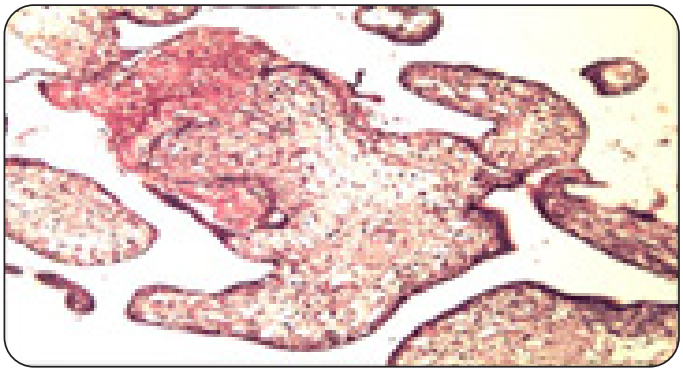

Figure 1: Placenta of women infected with St. agalactiae, showed fibrionoid deposits in the chorionic villi with degeneration and necrosis in the mesynchyma of these villi . Also there is scattered inflammatory cells (macrophage) H and E (100X).

Histopathological changes of placenta in aborted women positive for St. agalactiae infection showed conformity with clinical signs, cytokines and IHC assay results. where most of aborted placenta showed necrotic, hemorrhagic and had infiltration of macrophages this results was similar to that obtained by [14] whom reported that Preterm delivery can occur when GBS has invaded the placental membranes decreasing the membranes tensile strength and elasticity causing it to rupture, it had also been suggested that GBS produces proteases that repture placental tissue and similar mechanisms may promote membrane rupture causing miscarriage and preterm delivery. It has been shown that st. agalactiae may mostly attack the chorioamnion and amniotic fluid, improvement pass to the fetus, then start labor, lead to impulsive failure [15].

GBS successfully impasses the extracellular matrix constituents fibronectin, fibrinogen and laminin, Strangely fine modified GBS fixes to restrained fibronectin to enable mucosal colonization, but not to soluble fibronectin that may aid as an opsonin for phagocyte respect [16].

CD79 expression in placenta of aborted women infected and non-infected with St. agalactiae.

Result of imunnohistochemical analysis demonstrated positive staining for CD79 in placenta of aborted woman as showed in Figure 2. It has been shown that aborted women positive for St. agalactiae infection showed high intensity for IHC staining of CD79 compared with that of negative for St. agalactiae infection which revealed low intensity for IHC staining of CD79 as show in Figure 2.

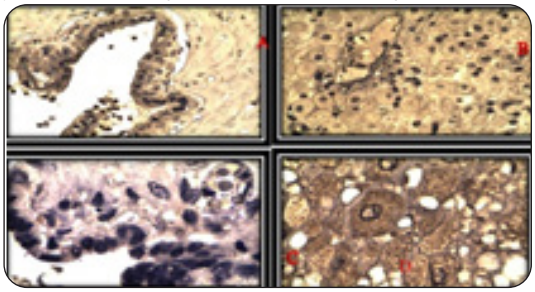

Figure 2: IHC staining results. A, B(expression of CD79) and C(expression of CD54): Women placentapostive for S.agalactia, specific staining of chorionic plate with DAB chromogen (brown) and counterstained with Hematoxylin (blue) (A:100x, B:400x). D: women placentanegative for S.agalactia; stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue) notice, non IHC reaction for 3BHSD enzyme100x.

Statical analysis with chi-square test, revealed that the total results of negative scores (1,2&3) in negative cases of St . agalactiae infection were pointedly developed ($p \leq 0.01$) than from negative cases. Meanwhile CD 79 in- testy is significantly higher in positive cases. Compared with that of negative cases. These score differences were also seen in Table 3.

Maximum	Negative for St . agalactiae infection		Positive for St . agalactiae	
	NO	%	NO	%
1	2	28.57	0	0
2				
3	206.94	206.94	206.94	206.94
206.94	*100%		14.28%	
4	0	0	2	28.57
5	0	0	4	57.14
Tatal of positive score	0%		85.71%	
*Significant ($p \leq 0.05$)				

Table 3: Occurance of CD79 molecule in placenta of aborted women. (IHC assay).

Presence of CD54 molecules in placenta of aborted women infected and non infected with St. agalactiae. According to study the CD 54 particles, stain was finished by anti-CD54 as seen in Figure 2. There is obvious rise for CD54 stain for placenta tissue through St. agalactiae infection as determined by staining of biopsies, the immune staining of CD54 were positive at high level in 85.71% (6 out of 7) in St. agalactiae infected patients, with highly statistical association ($p \leq 0.05$) between the infected and non-infected groups Table 4. Result of our study showed significantly associated between ICAM-1 expression and cytokines, CD 79 and histopathological change.

Maximum	Negative for St.agalactiae infection		Positive for St. agalactiae Infection	
	NO	%	NO	%
1	0	0	4	57.14
2	1	14.28	1	14.28
3	6	85.71	2	28.57
Score ;1< 25% ; 2(25-74)% ; (75-100)%				

Table 4: Occurrence of CD 54molecules in placenta of aborted women (IHC assay).

Functional activation molecule (ICAM-1) is a cell adhesion molecule expressed on a cell and up–regulated by inflammatory mediators [17]. Several studies reported that ICAM-1 interaction not only is required for cell adhesion and migration. But also plays a key role in the immune response, in fact it involved in leukocyte function such as antigen–specific recognization by T lymphocytes, T lymphocytes activation, and Ig production through T-dependent immune response, thus higher expression of CD54 on T cells may reflect the activation state of lymphocytes [18]. We tried to determine the pathogenic importance of ICAM-1 through comparison of its expression in aborted women that infected and non-infected with St. agalactiae. our results made obvious that the strong up- regulation of both CD79 and CD54 give a strong evidence that lymphocytes in placental tissue were with in a state of immune dysregulation, that comes together with a study done by poriadia, [19] who showed that there is increased expression of activation induced antigens (CD54) on the peripheral blood lymphocytes from patients with various type of inflammatory disorders.

Finally the high titer of cytokines (IL-8) and high expression of CD79 with profuse inflammatory cells in this study and other reports indicates that the St.agalactiae colonization in epithial of placenta causing production of cytokines lead to tissue inflammation and damage by causing the recruitment of host immunity and activation of host leukocytes, macrophage and inflammatory cells. Progression along a Th1 and Th2 passageway in united up regulation for chemokines in tissue of placenta.

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