

Immune Response in Pregnant Women Infected with Acute Vaginal Abscess Caused by *Staphylococcus aureus* and *Trichomonas vaginalis*

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ABSTRACT

Introduction: Vaginal abscess in pregnant women caused by *Staphylococcus aureus* and *Trichomonas vaginalis* is one of the most important disease. This infection is still medically uncontrolled because of poor health awareness due to the absence of personal hygiene in some women.

Aim: This study was aimed to add evaluation of immune response in pregnant women infected with acute vaginal abscess caused by *S.aureus* and *T.vaginalis* by the determination of four biomarkers level; Interleukin13 (IL-13), Prostaglandin E2 (PGE2), Transforming growth factor β 1(TGF- β 1) and Lactoferrin.

Materials and Methods: This case control study was done in AL-Zahra Hospital in AL-Najaf City, Iraq from January 2017 to November 2017. 120 pregnant women were included in this study, 30 women infected with *S. aureus*, 30 women infected with *T. vaginalis*, 30 women infected with both pathogens (inclusion criteria in this study) and 30 healthy pregnant women as control. All other cases were excluded from this study. Enzyme Linked

Immunosorbent Assay (ELISA) technique was used in this study to estimate biomarkers level in the serum of pregnant women infected with acute vaginal abscess.

Results: The results of the current study proved that there was significant increase ($P < 0.05$) in IL-3, PGE2 and TGF- β 1 levels in serum of women infected with acute vaginal abscess caused by *S.aureus*, *T.vaginalis* and both pathogens as compared with control; while, there was significant decrease ($P < 0.05$) in lactoferrin level in serum of pregnant women infected with *T.vaginalis* as compared with women infected with *S.aureus* and control.

Conclusion: The synergistically pathogenic effect of *S.aureus* and *T.vaginalis* in pregnant women infected with acute vaginal abscess lead to activation of T-cell and overproduction of IL-3, PGE2 and TGF- β 1 as a good signaling markers against infection. In contrast, this synergistic effect leads to reduction of lactoferrin level in the same women. These women may be susceptible to acute anaemia that may lead to abortion.

Keywords: IL-3, Lactoferrin, Pathogens, PGE2, TGF- β 1

INTRODUCTION

Innate and adaptive immune responses against bacterial and parasitic infections are still not fully understood, due to many complications during these infections as well as unlimited production of different immune factors in human body. *Staphylococcus aureus* and *T. vaginalis* are the most dangerous pathogens cause different infections in women such as vaginal abscess and abortion. *Staphylococcus aureus* is Gram-Positive bacteria, non-motile, coagulase positive and cause different human illnesses such as bacteremia, burn and soft tissue infections due to the high resistance to different antimicrobial agents and environmental stress and is able to produce several virulence factors. *Staphylococcus aureus* became a serious public health concern [1,2]. Recently, skin infections caused by *S. aureus* has increased rapidly [3,4]. *Trichomonas vaginalis* is a urogenital tract pathogen of humans causing a sexually transmitted diseases in the world, patients infected with skin abscess have increased colonization and infection with *S.aureus*, and *T.vaginalis* which has been responsible for decreased antimicrobial peptidase and Th2 cytokine profile such as IL-10, IL-13 and IL-4 [5-7]. Prostaglandin E2 (PGE2) is an important pro-inflammatory biomarker and there is a positive relationship between *S.aureus* growth and adherence and increase production of PGE2 level in blood of patients infected with skin abscess [8]. Transforming Growth Factor Beta 1 (TGF- β 1) is a polypeptide member which has important role in control of cell growth, cell proliferation and cell differentiation in human [9,10]. Lactoferrin is a glycoprotein with a molecular mass 80000 Dalton and has a multidimensional role in

the human body. Also known as lactoferrin, it is a part of innate immune system and has antimicrobial activity against different pathogens [11,12]. *Trichomonas vaginalis* is one of the most important sexually transmitted protozoans in the world [13], with an estimated 170 million cases occurring each year, the epidemiology of the disease is still poorly understood and some practitioners continue to question its importance, it is one of the most common causes of non-viral sexually transmitted diseases in the world [14]. *Trichomonas vaginalis* infection typically elicits aggressive local cellular immune responses with inflammation of the vaginal epithelium in women and the urethra in men [15]. It can also be transmitted to neonates during passage through an infected birth canal, but the infection is usually asymptomatic [16]. Symptomatic women with trichomoniasis usually complain of vaginal discharge, vulvovaginal soreness or irritation [17]. In Iraq, there are no studies focusing on vaginal abscess caused by *S. aureus* and *T. vaginalis* in pregnant women and immune response against these pathogens. Therefore, the aim of this study was the evaluation of four biomarker levels; IL-13, PGE2, TGF- β 1 and lactoferrin in serum of pregnant women infected with vaginal abscess and evaluation of the immune response activity of these women.

MATERIALS AND METHODS

Study Design and Pregnant Women

This is a case control study done in AL-Zahra hospital in AL-Najaf City, Iraq, during the period from January 2017 to November

2017. 120 pregnant women (age range between 20 to 40 years old) infected with acute vaginal abscess (two weeks duration of infection) were included in this study divided into three groups as follow: Group one; 30 pregnant women infected with *S.aureus*, Group two; 30 pregnant women infected with *T. vaginalis*, Group three; 30 pregnant women infected with both *S.aureus* and *T. vaginalis*. All these groups were compared with 30 healthy pregnant women as control.

Diagnosis of *Staphylococcus aureus*

Non-duplicated high vaginal swab (Oxoid, UK) were collected from pregnant women (by specialist physician) infected with acute vaginal abscess and immediately streaked onto blood agar and mannitol salt agar surface (Oxoid, UK) and incubated at 37°C overnight. All growing colonies were identified according to standard laboratory methods [18].

Diagnosis of *T. vaginalis*

Non-duplicated samples of vaginal discharge were collected from pregnant women (by specialist physician) infected with acute vaginal abscess. *T. vaginalis* was identified by wet mount smear as follows: one drop was taken from vaginal discharge and examined by microscope using 40x power object to identify *T. vaginalis* according to general characters [19].

Serum Collection

Five mL of blood was collected from healthy and infected pregnant women. Blood samples were drawn in sterile vacutainer tubes and left at room temperature for 30 min. Centrifugation was done at 3000 rpm for 5 minute (Mettler, Germany). Serum was collected and kept in sterile tubes at deep freeze at -4°C until use.

Serum Biomarkers Detection

Four human biomarkers were used in this study: IL-13, PGE2, TGF-β1 and lactoferrin. All these biomarker kits were procured from Elabscience Company, Bulgaria and the level of biomarkers in serum were determined by using ELISA device (Human reader, Germany) according to Manufacturer Company as follows: 100μL of standard or sample to each well (96 wells of micro ELISA plate) were added, all liquids were removed, 100μL Biotinylated (detection antibody specific for human IL-13, PGE2, TGF-β1 and lactoferrin) were added and incubated for 1 hour at 37°C then washed 3 times. A 100μL of Avidin-Horseradish Peroxidase (HRP) Conjugate was added and Incubated for 30 minute at 37°C then washed 5 times. A 90μL of substrate reagent was added and incubated for 15 minute at 37°C. A 50 μL of stop solution was added. Finally, the optical density was read at 450 nm immediately and the results were calculated [20].

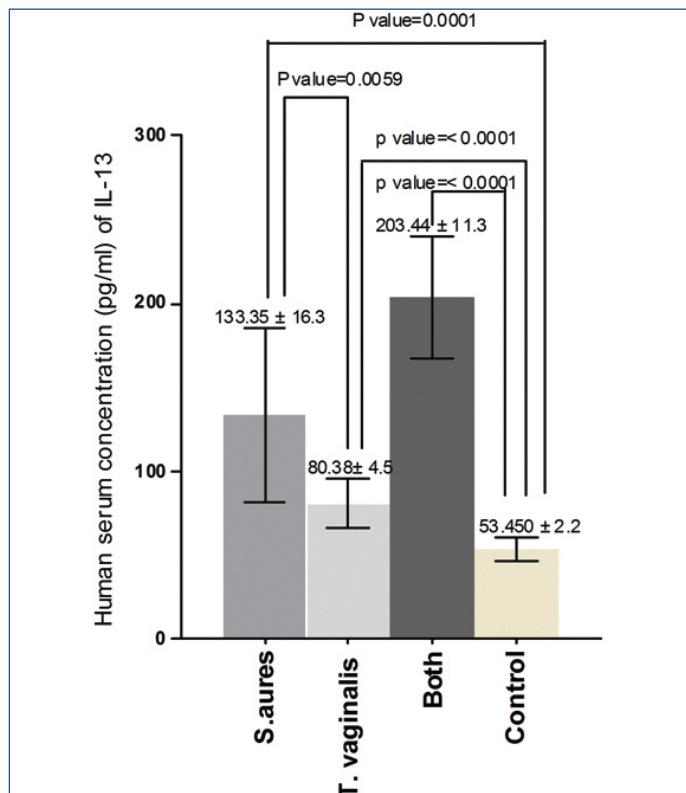
STATISTICAL ANALYSIS

T-test was used in this study for comparison between samples by using Graph-pad prism version 10 computer software. A p-value less than 0.05 considered statistically significant [21].

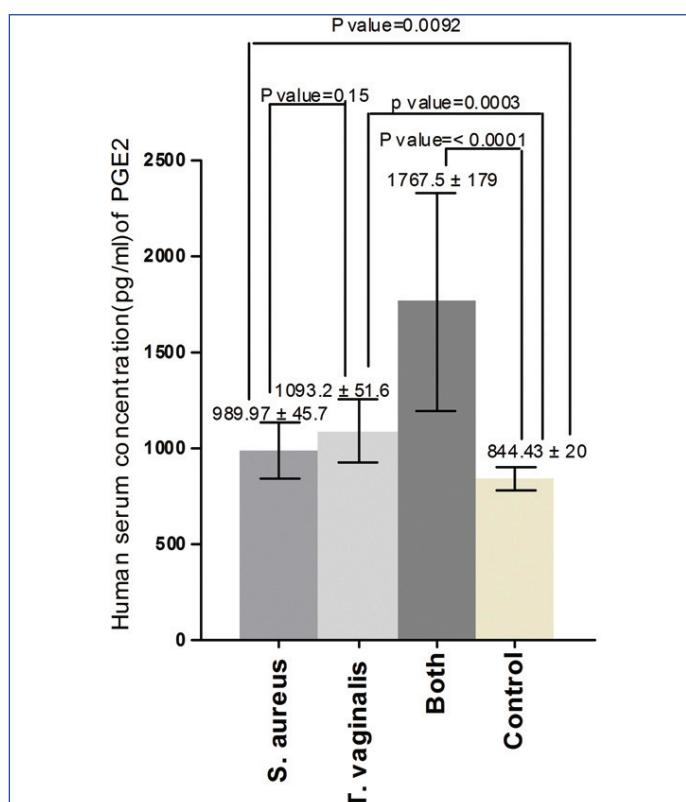
RESULTS

The results of the current study demonstrated that there was significant increase in serum concentration of IL-13 between Group one and control (p-value=0.0001), between Group two and control (p-value=<0.0001), between Group one and Group two (p-value=0.0059) and between Group three and control (p-value = <0.0001) [Table/Fig-1]. Also, the results proved that there was significant increase in serum concentration of PGE2 between Group one and control (p-value = 0.0092), between Group two and control (p-value = 0.0003) and there was no significant increase between Group one and Group two (p-value = 0.15) but there was significant increase between Group three and control (p-value = 0.0001) [Table/Fig-2]. On the other hand, the results showed that there was significant increase in human serum concentration of TGF-β1 between Group

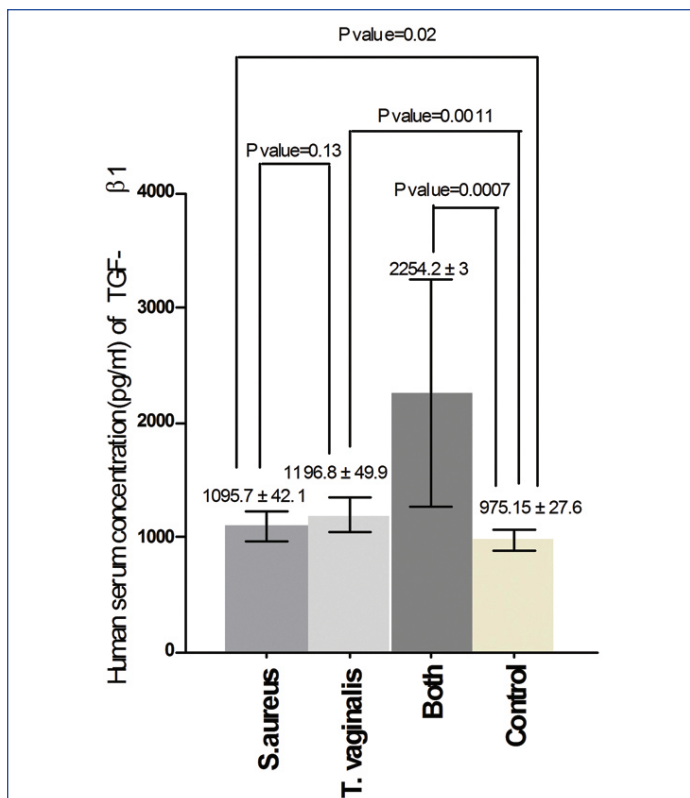
one and control (p-value=0.02), between Group two and control (p-value=0.0011) and there was no significant increase between Group one and Group two (p-value=0.13) but there was significant increase between Group three and control (p-value=0.0007) [Table/Fig-3]. Finally, the results indicated that there was no significant increase in human serum concentration of lactoferrin (p-value=0.18) between Group one and control but there was significant decrease between Group two and control and between Group one and Group two (p-value=<0.0001) also, there was significant decrease between Group three and control (p-value=0.0001) [Table/Fig-4].



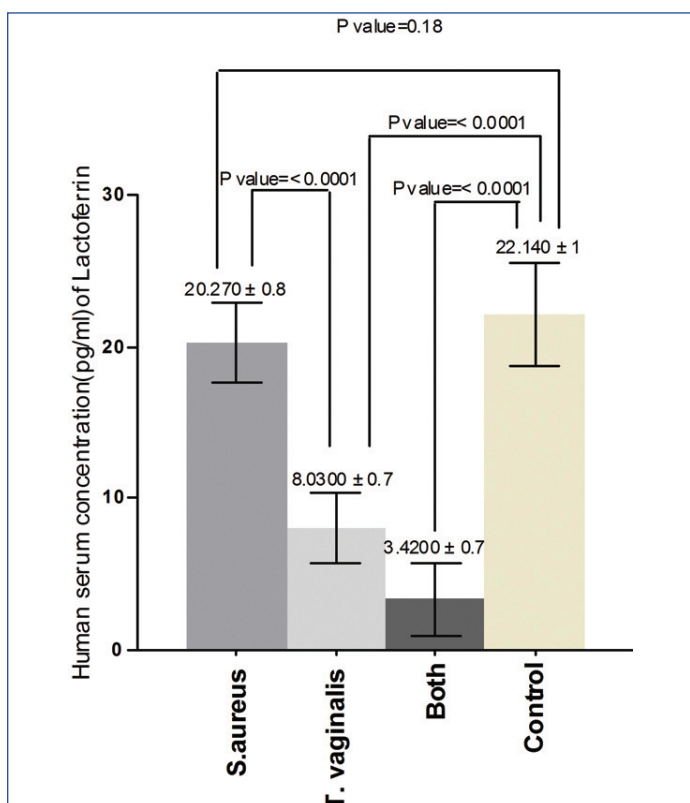
[Table/Fig-1]: Human serum concentration (pg/mL) of IL-13 in pregnant women infected with acute vaginal abscess caused by *S. aureus* and *T. vaginalis*.



[Table/Fig-2]: Human serum concentration (pg/mL) of PGE2 in pregnant women infected with acute vaginal abscess caused by *S. aureus* and *T. vaginalis*.



[Table/Fig-3]: Human serum concentration (pg/mL) of TGF-β1 in pregnant women infected with acute vaginal abscess caused by *S. aureus* and *T. vaginalis*.



[Table/Fig-4]: Human serum concentration (pg/mL) of lactoferrin in pregnant women infected with acute vaginal abscess caused by *S. aureus* and *T. vaginalis*.

DISCUSSION

This is the first study in Iraq focused on the levels of four biomarkers in serum of pregnant women infected with acute vaginal abscess caused by two different pathogens. In this study, IL-13, PGE2 and TGF-β1 levels were significantly increased (p -value < 0.05) in serum of pregnant women infected with acute vaginal abscess caused by *S.aureus* and *T.vaginalis* as compared to the control. *Staphylococcus aureus* and *T.vaginalis* are two of the most

important pathogens which produce several molecules that also contribute to the formation of abscesses in pregnant women [22-24]. IL-13 is a cytokine produced by different cell types such as T-cell and mast cell and responsible for a wide range of biology and functions in the body. Almost, all Th2 cytokines are known to mediate host defense against parasites and bacterial infections but, these cytokines can also trigger infection if their activities are deregulated [25,26]. During bacterial and parasitic infections, IL-13 is able to activate different types of immune cells such as eosinophils and basophils to eliminate bacterial cells during and post infection and activated B-cell to produce IgE (anti-parasitic antibody) [27]. Also, IL-13 has been shown to induce a potent fibrogenic program during the atopic dermatitis, therefore, it has been suggested this fibrogenic program is closely related with production of IL-13 [28]. On the other hand, IL-13 has been identified as a major pathogenic cytokine in acute and chronic disease associated with persistent type2 cytokines production such as IL-4, IL-9 and IL-5 [29,30]. These cytokines are responsible for wound healing after acute tissue injury [31,32]. Therefore, in this study, the level of IL-13 was in high significant increase in serum of pregnant women infected with acute vaginal abscess caused by *S.aureus* and *T.vaginalis*. Overuse of antimicrobials to treat of bacterial infections lead to the growing incidence of antibiotic resistant *S.aureus* and other pathogens. Therefore, that leads to over production of most immune biomarkers such as PGE2, is an oxygenated metabolite of arachidonic acid, PGE2 can be produced by all cell types of the body that have epithelia, fibroblast, and infiltrating inflammatory cell that represent the major source of PGE2 in the immune response as a response to infection and has different functional versatility [33,34]. In addition, PGE2 act as a pro-inflammatory mediator activating mast cell, macrophages and neutrophils in acute infection stage [35,36]. Some previous studies suggested that PGE2 has a strong suppressor action against innate and adaptive immunity at the molecular and cellular levels [37,38]. While, in other separate studies proved that PGE2 is facilitated the growth and adherence of *S.aureus* and *T.vaginalis* and was responsible for colonization of these pathogens, this results are in agreement with result of this study when demonstrated that there was high significant increase of PGE2 in serum of pregnant women infected with *S.aureus* and *T.vaginalis* as compare with control. TGF-β1 is a protein, first discovered in platelets of human containing and 390 amino acids with molecular mass 25,000 Dalton and has an important role in wound healing, controlling of immune system, proliferation, differentiation and activation of different cell types [39,40]. TGF-β1 is secreted by leukocytes and most immune cells such as T-cell [41,42]. In the present study, TGF-β1 was in high significant increase in serum of pregnant women infected with *S.aureus* and *T.vaginalis*. Transforming growth factor β1 is able to promote *S.aureus* adhesion to and invasion into mammary fibroblasts by increasing collagen I and Alpha-Smooth Muscle Actin (β-SMA) expression and provide a novel target for controlling of infection [43]. Previous studies showed that *T. vaginalis* is able to activate caspase protein-3 and reduce expression of Mcl-1 gene lead to neutrophilic apoptosis during vaginal infection in women [44,45]. As a proven scientific fact, the uptake of apoptotic cells activates the produce of anti-inflammatory biomarkers such as PGE2, TGF-β and Interlukine-10; on the other hand, the uptake of apoptotic cells inhibits the secretion of pro-inflammatory mediators such as Tumour necrotic factor-β from phagocytic cells [46-48]. Lactoferrin is one of the most important components of human immune system serve as antibacterial and anti-parasitic and belongs to iron-binding system [49,50]. Iron is an essential element which has a significant effect on the virulence of *T. vaginalis* [51]. In this study, the decrease of lactoferrin level in women with *T. vaginalis* may be due to the pathogenicity of this parasite dependent on the positive relationship between iron concentration and adhesion of parasite on epithelial cell [52,53]. The decrease in lactoferrin levels may be due to an increase in consuming iron

by this parasite and this leads to decrease in the storage of iron as ferritin or increased utilization by parasite [54]. The result of the current study is in agreement with the study by Weinberg [55] who suggested that the *T. vaginalis* uses the ferritin as source of iron which leads to decrease in the ferritin level in serum of women infected with *T. vaginalis* compared with healthy control. Enzymatic degradation is one of the important mechanisms was used by the parasite to proteases secretion and release iron from lactoferrin as a source of nutrition [56].

LIMITATION

We think this study has reached its aim, but there were some unavailable limitations such as study the level of other biomarkers such as CD4+ and CD8+ in the same patients.

CONCLUSION

The personal hygiene plays an important role to prevent wide range of sexually transmitted disease caused by different pathogens. The synergistically pathogenic effect of *S.aureus* and *T.vaginalis* in vagina of pregnant women infected with acute vaginal abscess lead to activation of T-cell and overproduction of IL-3, PGE2 and TGF- β 1 as a good signaling markers against infection. In contrast, this synergistic effect leads to reduction of lactoferrin levels in the same women. These women may be susceptible to acute anemia that may be lead to abortion. Therefore, the individuals can protect themselves from different sexually transmitted diseases by paying attention to personal hygiene and use a condom.

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