

Role IL6,IL8,Hu MIF, NF-κB-P65 in the development of trichomoniasis

¹NADIA JAFFER KADHUM, ^{2*}SHAYMAA JABBAR RAISAN, ²SABEEH H. AL-MAYAH

¹Educational Supervisor, General Directorate of Education, Basra Governorate/ Basrah /Iraq

²University of Basrah, College of Education for Pure Sciences, Biology Department,

*Corresponding Author:

Email:Shaymaajabbar2003@yahoo.co.uk

Received: 10.07.20, Revised: 03.08.20, Accepted: 14.09.20

ABSTRACT

The present study aimed to assess level of IL6,IL8,Hu MIF, NF-κB-P65 in the serum of women . Collected 80 sample women suffered from vaginal infections, as well as samples were from 10 healthy women as a control group. Results showed a significant increase (P <0.05) in the immunological parameters in the women who were infected with Trichomoniasis parasite versus the women who were suspected and the control group ,there was an increase in the level of IL-6 in the infected women with a concentration rate of 16.19 pg / ml compared to its concentration in the control and suspected women groups and whom their concentration rates were 2.20 and 2.83 pg / ml respectively. When comparing the IL-8 concentration among women infected with T. vaginalis its concentration rate was 9.024 pg / ml, while the concentration rates in the other two groups were 4.306 and 1.957 pg / ml respectively. the concentration of Hu MIF was higher in women infected with T. vaginalis and those who were suspected of being infected , reaching 7.229 and 7.169 ng / ml respectively, compared with its low concentration in women in the control group where it was 1.468 ng / ml. Finally, the highest rate of the NF-P65 concentration was recorded at 2.982 ng / ml in the infected women compared to control and suspected groups, as the concentration rates in these two groups were 2.681 and 2.701 ng / mL, respectively.

Keywords: NF-κB-P65, Hu MIF, Trichomoniasis, ELISA, Immune

INTRODUCTION

Trichomoniasis is the most common non-viral sexually transmitted pathogen. It is caused by an anaerobic ,flagellated protozoan parasite called Trichomonas vaginalis that is usually found in the female lower reproductive tract and the male urethra [1]. The WHO has estimated that about 160-180 million cases of infection are acquired annually worldwide [2,3]. It infects mostly reproductive age women and cause a number of different and serious issues such is vaginal discharge, vulval irritations , endometritis ,vaginitis, cervicitis ,infertility and cervical erosion [4]. In pregnant women, it can lead to premature rapture of membrane, preterm deliveries and low-birth-weight infants [5]. And it risk might reach to causing cervical cancer,PV and HIV infection(6). While in men ,its complications include urethritis, prostatitis, epididymitis, and infertility through inflammatory damage. As noticed infections in women are usually symptomatic while in men are asymptomatic [7]. Trichomonas vaginalis can be transmitted mainly through direct vaginal sexual intercourse with an infected individual [8]. Mucosal epithelial cells are the first immune is formed of defense against

pathogenic organisms in the female reproductive tract [9] capable of producing Pro-inflammatory chemokines that attract and cytokines that activate immune cells, contribute to normal physiological homeostasis in the female reproductive tract [10]. It involves both innate and adaptive immune responses including humeral and cell-mediated immunity, and evokes lymphocyte effectors functions including cytokines production, cytotoxic effects, and antibodies produced after presentation by antigen presenting cells [11] a cell-mediated immune response includes immune cells and cytokines serve as key factors in the regulation of mucosal responses in various parasitic infections [12]. The present study aimed to assess level of IL6,IL8,Hu MIF, NF-κB-P65 in the serum of women ELISA assay.

MATERIALS AND METHODS

Collected 80 blood sample from women with vaginitis disease when ,all these cases were examined and defined as suspected with T.vaginalis by obstetrician when attended to Basrah Teaching Hospital in Basrah Province ,addition10 women of healthy as control group.

Blood sample collection

Blood samples were collected from women who had previously had vaginal swabs for the assessment of immunological parameters IL-6,IL-8,Hu MIF, NF- κ B p65, take 5 ml of blood was collected from each women by disposable syringe , blood samples was drawn in sterile plain tubes and remains for 15 minutes at room temperature after which they were centrifuged at 3000 rpm for 15 minutes to obtain serum and collected in another sterile tube. ,

Wet mount and direct microscopy

Wet mount preparations were obtained by mixing of the vaginal fluid with a normal saline drop and directly examined microscopically at 40x to the observation of trophozoite movement. Results obtained by direct microscopic examination from 90 samples of vaginal secretions showed that the number of positive cases were only 8 and 72 suspected cases of trichomoniasis, in addition to 10 women who appeared to be in good health and did not have a history or clinical evidence of other diseases, Microscopy results were negative for trichomoniasis as a control group [13].

Quantitative Estimation of Human IL-6, IL-8, MIF, NF- κ B p65, by Enzyme-linked Immunosorbent Assay

cytokines concentration (IL-6, IL-8)were estimated in the sera of the studied groups using ELISA kit (KOMABIOTECH, Korea) and cytokines (MIF, NF- κ B p65) using ELISA kit(Technology Laboratory Bioassay,China) . The procedure were done according with the leaflet provided by the manufacturer.

RESULTS

Results obtained by direct microscopic examination from 90 samples of vaginal

secretions showed that the number of positive cases were only 8 and 72 suspected cases of trichomoniasis, in addition to 10 women who appeared to be in good health and did not have a history or clinical evidence of other diseases, Microscopy results were negative for trichomoniasis as a control group . The present study showed in table (1) a significant increase in the concentration of IL-6 in serum of women who are infected with *Trichomonas vaginalis*(P <0.05)with a concentration rate of 16.19 pg / ml compared to its concentration in the two groups of controlled and suspected women where its rate in the two groups was 2.20 and 2.83 pg / mL respectively.as well as In the present study showed in table (2) that was determined in the serum of the women under consideration and there was a significant increase level of IL-8concentration in the serum of the women infected with *T. vaginalis* (P <0.05) with a concentration rate of 9.024 pg / ml compared to its concentration in the two groups of controlled and suspected women as it reached 1.957 and 4.306 pg / ml respectively.Where measuring the level of HuMIF factor in the serum of the infected women, the present study noted in table(3) there was a significant increase in the concentration of this factor in women infected with *Trichomonas vaginal* parasite and who were suspected to be infected (P <0.05) as the concentration rate was 7.229 and 7.169 ng / ml respectively, compared to its low concentration in the women of the control group 1.468 ng / ml. The present study also showed that the highest concentration of NF- B was recorded in women infected with *T. vaginalis* parasite with a significant increase (P <0.05) in the infected women compared to the other two groups table(4).

Table (1): Cytokine (IL-6) concentration in women with trichomoniasis , Suspected women and control groups.

groups	Mean ± SE	No. of samples
Control group	2.20 ± 0.611	10
Infected women	16.19 ± 7.89	8
Suspected women	2.83 ± 0.80	72
Total	90	

) $X^2 = 22$, df = 2 , p = 0.000(

Table(2): Cytokine (IL-8) concentration in women with trichomoniasis , Suspected women and control groups.

groups	Mean ± SE	No. of samples
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Control group	1.957 ± 0.274	10
Infected women	9.024 ± 0.881	8
Suspected women	4.306 ± 0.691	72
Total	90	

) $\chi^2 = 14$, df. 2, p = 0.001 (

Table(3): Cytokine (MIF) concentration in women with trichomoniasis , Suspected women and control groups.

groups	Mean ± SE	No. of samples
Control group	1.468 ± 0.29	10
Infected women	7.229 ± 0.73	8
Suspected women	7.169 ± 0.41	72
Total	90	

) $\chi^2 = 14$, df = 2 , p = 0.001(

Table(4):NF- KB-P65 concentration in women with trichomoniasis , Suspected women and control groups.

groups	Mean ± SE	No. of samples
Control group	2.681 ± 0.120	10
Infected women	2.982 ± 0.241	8
Suspected women	2.701 ± 0.156	72
Total	90	

= 8 , df = 2 , p = 0.018) χ^2 (

DISCUSSION

This study showed increase in the concentration of IL-6 in the serum of women who are infected with *Trichomonas vaginalis* (P <0.05) . The reason of the high level of IL-6 may be its production in women infected with Trichomoniasis from more than one source of inflammatory cells such as vaginal epithelial cells and immune cells such as neutrophils and macrophages indicating that infection with *Trichomonas vaginalis* parasite generated an inflammatory response as a defensive immune method to the host against the parasite. The results of the current study are consistent with the results of the [14] study in Baghdad, in which it indicated a higher concentration of IL-6 in the infected women compared to healthy women and linked this through the increase of the concentration of antibodies in the serum of the infected women, as well as the change of the physiological state that may be the caused by the change in the immune system and the immune cells .and [15] study in Najaf governorate conducted on the level of IL-6 in the serum of men infected with trichomoniasis was measured ,this study showed a significant

increase in the concentration of IL-6 . Also Interleukin-8 (IL-8) is a cytokine that has inflammatory effects as it is considered a chemical attractant and it plays a major role in the migration and recruitment of neutrophils from the blood to the site of infection. In the present study ,the level of IL-8 was a significant increase in its concentration in the serum of the women infected with *T. vaginalis* (P <0.05) . The reason for the high concentration of IL8 in the serum of the infected women may be attributed to its role in generating an inflammatory response against *T.vaginalis* as it has the ability to regulate the migration and the recruitment of neutrophils , and it may be secreted from other cells such as monocytes and macrophages. And these results were in agreement with a lot of studies and one of them is the study of Kim and Haynes,(2012), in which they confirmed that infection with *T. vaginalis* leads to an inflammatory response that includes the release of the chemical attractant IL-8 and pro-inflammatory cytokines such as TNF, IL-1 and IL-6 that are responsible for initiating the immune response and recruiting the immune cells such as monocytes, macrophages and

neutrophils. Often, the vaginal secretions in women contain neutrophils that respond to the presence of IL-8 ,however, the immune response may fail to get rid of *T. vaginalis* , and there is no long-term immune memory to prevent the recurrence of infection. Although IL-8 is considered a powerful chemical attractant that recruits the immune cells to the location required to eliminate the infection .And the [17] study has showed through the laboratory experiments that they conducted to evaluate the interaction between the *Trichomonas vaginalis* parasite and the cells of the host's immune system that the cells of the immune system respond to the invasion of the parasite through the secretion of various inflammatory cytokines of which are IL-6,IL-1 and IL-8, and they also found that *M. hominis* bacteria (symbionts with the parasite) had asignificant influence on cytokines response , as the *M. hominis* bacteria enabled the secretion of various inflammatory cytokines in response to the parasite .And they also found that strains of the parasite that were free from *M. hominis* caused only the secretion of IL-8 from monocytes, while the parasite strains that harbor the bacteria secrete cytokines from different cells, especially Tcells and B cells, therefore likely to be influenced the adaptive immune response of the organisms symbiosis with the parasite and the associated infections. The human macrophage migration inhibitory factor (HuMIF) is a widely expressed and multidirectional cytokine that acts as a major mediator for innate immunity and enhances many pathophysiological processes (Lin et al., 2000 Bozza et al., 2004). When measuring the level of HuMIF factor in the serum of the infected women, the study noted that there was a significant increase in the concentration of this factor in women infected with *Trichomonas vaginalis* parasite and those who were suspected to be infected (P <0.05) , and that the high level of HuMIF was associated with the infections that occur due to trichomoniasis or other associated infections, the results were in agreement with the study of Kerschbaumer et al. [18] , it indicated that HuMIF is an inflammatory cytokine that regulates innate immunity in inflammatory responses. It also promotes the production of other inflammatory mediators such as TNF-α , NO and IL-6.In Calandra and Bucala [19] study they showed that Hu MIF is the first mediator to be identified that can regulate the inhibitory effects of glucocorticoids as MIF Hu exerts inflammatory activities and thus plays an important role in controlling inflammations and the host's immune.Human nuclear factor-κB p65 NF B-p65 It is responsible for regulating

cell's growth, differentiation, inflammatory responses and apoptosis [20].The present study also showed that the highest concentration of NF- B was recorded in women infected with *T. vaginalis* parasite with a significant increase (P <0.05) in the infected women compared to the other two groups. And that the reason for the increase may be attributed to the binding of the factor NF-B as a marker of the activity of the inflammatory process that occurs in women when they are infected with the parasite which is the catalyst for its activation, as well as the increase in the level of cytokines under consideration is considered as an evidence of the activation of the factor and its contribution to the production of cytokines that cause inflammation, as the current study agreed with the study [21] They investigated whether the macrophages in humans could be causing the inflammation caused by *T. vaginalis* , by culturing the macrophages that were derived from the monocytes of the human (HMDM), with jointly Whether the living trophozoite or its combinations, this increased the production of inflammatory-stimulating cytokines IL-6 TNF-α and IL-1β by HMDM. The participation of the NF-B signaling pathway in cytokine production by phosphorylation and nuclear transport of p65 NF-B was also confirmed. The inflammation which caused by *T. vaginalis* stimulates macrophages in humans to produce inflammatory stimulant such as IL-1 , IL. -6, TNF-α, and NO [22,23].

CONCLUSION

The revealed a significant increase IL-6,IL-8,and NF- κ B p65 in women with trichomoniasis ,so this factors play role important in diagnosis this infections.

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