



## Immunological study for infected women with *T.vaginalis* in AL-Najaf AL-Ashraf province

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### Abstract

The study was conducted on 450 out patients and 30 healthy women, whom have visited the department of infertility at Al-Sadder medical city, Al-Zahra Hospital and in Najaf Province during the period from January till August, 2012. The infection with *T.vaginalis* in clinical suspected women determine by using the wet amount microscope, the infection women numbers and percent by wet mount microscope gave 49 and 10.88% respectively. The results showed significant elevation ( $P<0.01$ ) in serum concentration of IgA, IgG, IgM and IgE in serum of *T.vaginalis* infection patients in comparison to healthy control group.

### Introduction

*Trichomonas vaginalis* is pathogenic protozoan flagellate, it is similar to other species of genus *Trichomonas* (*T.Tenax*, *T.hominis*) in shape, but it is somewhat larger, its size ranged from 7 to 30  $\mu\text{m}$  in length, 5 to 12  $\mu\text{m}$  in width its undulating membrane is shorter and has costa, size and morphological shape of *T.vaginalis* have been shown to vary depending upon the condition under which the organism is maintained (Al-Mahdawy, 2006).

The nucleus of *T.vaginalis* is located in anterior portion and surrounded by a porous nuclear envelope. A slender hyaline, rod-like structure and called an axostyle, commences at the nucleus and bisects the protozoan longitudinally, protruding through the parasite's posterior end, and terminating in a sharp point (Muluneh, 2011). This structure is thought to anchor the parasite to vaginal epithelial cells. (Upcroft *et al.*, 2001).

The organism is able to maintain energy requirements by the use of a small amount of enzymes to provide energy via glycolysis of glucose to glycerol and succinate in the cytoplasm (Singh *et al.*, 2010). It is one of etiological agent of vaginitis, more than half of end cervical infections do not cause sufficient inflammation to result in clinical signs and symptoms, however, symptoms can include dysuria, vaginal discharge, dyspareunia, perineal itching and pelvic discomfort or pain, erythema multiform and swelling of the vulva or labia suggest trichomonal infection. (Margaret *et al.*, 2004).

The parasite is commonly associated with other STDs, and is marker of high-risk sexual behavior. Due to the side effects of the drugs used to treat the infection, it is suggested that the treatment be performed after definite diagnosis using a diagnostic with a higher sensitivity. (Zarrintaj *et al.*, 2010). Like many protozoan parasite, the ability of it to evade the host immune system is an important aspect of pathogenesis. Avoidance of complement is one such tactic that is used by *T.vaginalis*. It has long been known that *T.vaginalis* activate, the alternative pathway of complement but it has taken advantage of niche in which there is little complement present (Jane and Donald, 2004).

*T.vaginalis* can coat itself with host plasma proteins, thus hosts immune system does not recognize the parasite as foreign and continuous release of antigens may neutralize antibody or cytotoxic T-lymphocytes, thus short-circuiting specific anti-*T.vaginalis* defense mechanisms.



The influence of *T.vaginalis* lysate and excretory-secretory (ESP) on the fate of neutrophils has been repeated. *T.vaginalis* lysate inhibits apoptosis of human neutrophils (Song *et al.*, 2010). The current study aimed to estimate the prevalence of *T.vaginalis* in AL-Najaf AL-Ashraf province by used wet mount smear test and determined the concentration of IgA, IgG, IgM and IgE by immunoradial assay in women infected with *T.vaginalis*.

## Materials and Methods

### The subjects:

The study was conducted on 450 women clinical suspected woman with *T.vaginalis* infection and 30 of healthy women as control groups, when all these cases were examined and defined as suspected with *T.vaginalis* by obstetrician when attended to AL-Zahra, maternity and paediatric, AL-Sadder teaching hospital in AL-Najaf province from January to August 2012. A careful history was taken from each patient according to the following Questionnaire sheet:

Case No:	Date:
Name :	Age :
Address:	
Economic status:	Type of contraception:
Education Level:	Vaginal discharge:
Colour: Oder:	
Cervical ulcer :	
History of vaginal infection:	
*Treatment use:	
*Any disease :	

**\*Any women using drug or undergo disease removal from current study.**

### Specimen collection

From suspected women vaginal discharge was carefully collected from the posterior vaginal fornix after putting the patient at a lithotomic position and taking swab after opening the vagina by a sterile speculum, the swab are immersed in a tube with ml of a sterile normal saline (Shaio and Lin, 1997). The swab was examined in wet mount preparation and two ml of blood was collected from each clinical suspected woman with *T.vaginalis* infection and non-suspected women ( as control group) by disposable syringe , blood samples was drawn in sterile plain tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Back man/counter, Germany) to separate the serum and collected in another sterile tubes, each sample of serum was divided into 4 parts.



### Wet mount Examination:

Immediately, 1 drop from one of the tubes was applied to a glass slide, covered with a cover slip, and examined under the microscope by using the high power objective (X40) for the presence of *T.vaginalis*. The wet mounts were examined for at least 10 minutes (Philip *et al.*, 1987). Positive results were defined as the presence of one or more Trichomonads with characteristic motility (jerky movement) and morphology (Demoe *et al.*, 1996). The Trichomonads may be inactive and non-motile as in chronic or asymptomatic condition (Wolner *et al.*, 1989). The wet amount is also used to demonstrate the presence of clue cells in vaginal secretions, these cells were epithelial cells covered by masses of bacteria of varying morphology (Priestly & King, 1996).

### Detection of Immunoglobulin IgA, AgM and IgG by used single Radial Immuno diffusion Plate.

- 1- Open the plate, if moisture is present, allow evaporating.
- 2- Apply 5  $\mu$ l of sample or control use human serum as sample.
- 3- Close the lid firmly. Incubate the plate at room temperature if possible inverted.
- 4- Measure the diameter accurately to within 0.1 mm with a suitable device.
- 5- Evaluate result using the table of reference or standard curve

Procedure by (Mancini *et al.*, 1965):

### Detection of IgE

- 1- Sample preparation:

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum sample without additives only.

- 2- Reagent preparation:

A-All reagent should be brought to room temperature (18-22C°) before use.

B- Dilute 1 volume of wash buffer (50 x) with 49 volumes of distilled water. For example Dilute 15 ml of wash buffer (50 x) into distilled water to prepare 750 ml of washing buffer (1x). Mix before use.

- 3- Secure the desired number of coated wells in the holder.
- 4-Dispense 20  $\mu$ l of standard, specimens and controls into appropriate wells.
- 5- Dispense 100  $\mu$ l of zero Buffers into each well.
- 6- Through mix for 10 seconds. It is very important to have completed mixing in this setup.
- 7- Incubate at room temperature (18-22C°) for 30 minutes.
- 8 -Remove the incubation mixture by flicking plate content into a waste container.
- 9- Rinse and flick the microtiter wells 5 times with washing buffer (1x) strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- 10- Dispense 150  $\mu$ l of enzyme conjugate Reagent into each well. Gently mix for 5 seconds.
- 11- Incubate at room temperature for 30 minutes.
- 12- Remove the incubation mixture by flicking plate contents into sink.
- 13- Rinse and flick the microtiter wells 5 times with washing buffer (1x).



14- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

15- Dispense 100  $\mu$ l of TMB solution into each well. Gently mix for 5 seconds.

16- Incubate at room temperature in the dark for 20 minutes.

17- Stop reaction by adding 100  $\mu$ l stop solution to each well.

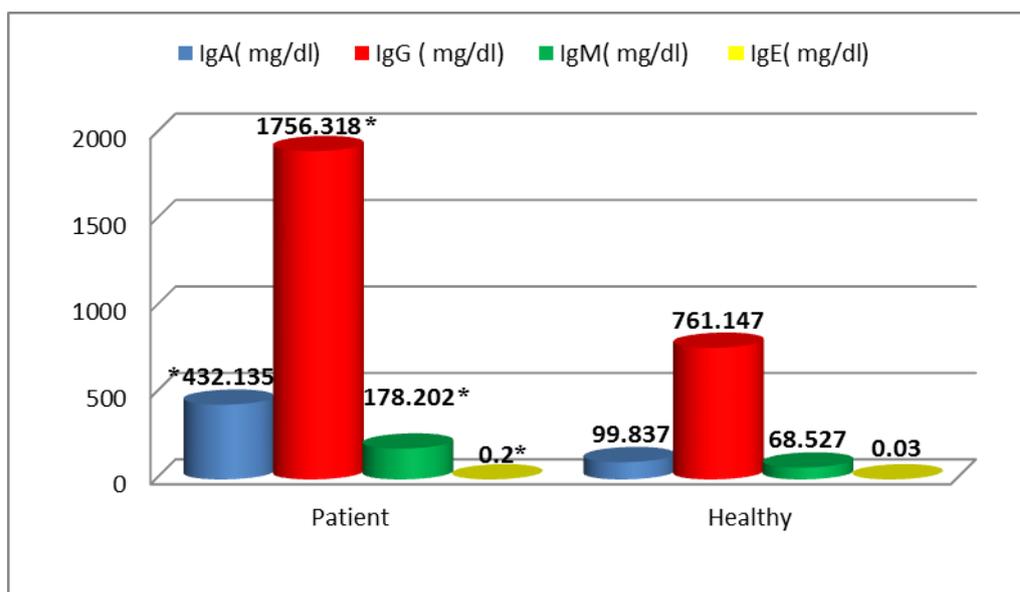
18- Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour completely.

19- The absorbance on bio Elisa reader EL x 800 was read at a wave length of 450 nm immediately Results were provided within 1 minute on the LCD display and printed out in the printer.

## Results

### The Immunoglobulin status in patient and control group:

The result of present study as shown in figure (1) revealed that the concentration of Immunoglobulin A, G, M and E were highly significant increase ( $P < 0.01$ ) in compared to healthy control group.



\*Significant difference ( $P < 0.01$ ) between control group and patients.

**Figure (1): IgA, IgG, IgM and IgE comparison between patients suffering from *T. vaginalis* infection and healthy control group.**

## Discussion

### Immunoglobulin E, A, G and M.

The present study showed significant increase in the concentration of IgE, IgA, IgG, and IgM in serum of infected with *T vaginalis* patients compared to control group.

This is agreement with the experimental trichomoniasis conducted by paintlia *et al.*, (2001) on mice infected with symptomatic and asymptomatic isolates of *T.vaginalis* alone. This increase in the concentration of IgE, IgA and IgM cooperate with increase in the B-lymphocyte which generate IgE, IgA, IgG and IgM responses (Finkelman *et al.*, 1990). Another study was



done by Simernject *et al.*, (2008) proved that the concentration of IgM, IgA and IgG significantly increase in serum of infected with *T.vaginalis* patient in compared to control group. From this study concluded that there is a significant increase in the concentration of IgE, IgM, IgA and IgG due to increase in the percentage of B-lymphocyte in peripheral blood in women infected with *T.vaginalis* when compared to the controls. This indicates a stimulation of the humeral immune response during the infection with *T.vaginalis*.

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