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Epidemiological Study on Trichomonas Vaginalis Parasite and Estimation of Some Elements and Antioxidants in the Serum of Infected Women in Samarra City

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Abstract. The current study investigates the prevalence of Trichomonas vaginalis in women visiting Samarra General Hospital between August 2023 and June 2024. A total of 325 samples were examined from women aged 19 to 51 years, with an infection rate of 2.34%. The highest infection rate was observed in the 30-40 age group, among women experiencing itching during menstruation (4.16%), those with white vaginal discharge (2.97%), and infertile women (4.16%). The infection rate was higher among rural women (3.57%) compared to urban residents.
Regarding trace elements, the study recorded a significant decrease in serum zinc and iron levels in infected women ($p < 0.01$) compared to the control group. However, no significant difference was observed in lead concentrations, with levels recorded as 2.83 µg/dl and 2.62 µg/dl in infected and control groups, respectively.
Additionally, antioxidant levels showed a notable decline in infected women:
• Catalase (CAT): 6.88 pg/ml
• Superoxide dismutase (SOD): 15.31 pg/ml
• Glutathione peroxidase (GPx): 112.9 pg/ml

Highlights:

1. Prevalence: 2.34% infection in 325 women, highest in 30-40 age group.
2. Trace Elements: Decreased zinc/iron, no significant lead difference.
3. Antioxidants: Reduced CAT (6.88), SOD (15.31), GPx (112.9) pg/ml.

Keywords: Trichomonas vaginalis, prevalence, trace elements, antioxidants, infection rate

Introduction

Trichomoniasis is one of the most common sexually transmitted infections (STIs), caused by the anaerobic protozoan Trichomonas vaginalis (T. vaginalis) (1). The parasite is classified as an atrial flagellate, inhabiting the genito-urinary tract of both sexes. In females, it causes trichomoniasis, which is often associated with vaginal discharge, while in males, it is typically asymptomatic or may cause urethritis (2). This infection is prevalent worldwide, especially in developed countries, due to its mode of transmission (3).

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The role of trace elements such as zinc and iron in parasitic infections has gained increasing attention. These elements are essential micronutrients required in small amounts to support immune function and prevent various diseases (23).

Zinc plays a crucial role in enzyme synthesis, collagen production, DNA and RNA metabolism, and immune system regulation (24). It is also vital for human reproduction and acts as a defensive mechanism in both humans and animals.

Iron is a key transitional metal in biological systems, essential for oxygen transport, DNA synthesis, and electron transfer (5). In T. vaginalis, iron regulates parasite adhesion (cytoadherence) to epithelial cells, enhances cytotoxicity, and activates proteolytic enzymes (protenase activity).

Researchers suggest that the exacerbation of menstrual symptoms in T. vaginalis-infected women is linked to elevated vaginal iron levels and increased vaginal acidity (4).

Methods

Sample Collection

Samples were collected from August 2023 to June 2024, with a total of 325 specimens obtained from women visiting the outpatient clinic at Samarra General Hospital. The direct wet mount examination was performed as follows:

Wet Swab Test

A sterile cotton swab was used along with a medical speculum to collect vaginal secretions. The sample was mixed with 2 ml of physiological saline solution (normal saline), shaken thoroughly, and a drop was placed on a glass slide for examination under a light microscope at 10× and 40× magnification. T. vaginalis was identified based on its jerky motility, flagellar movement, and undulating membrane among epithelial cells.

Serological Tests

Measurement of Serum Iron Levels

The Iron Liquicolor Test from Human GmbH, Germany, was used for photometric colorimetric determination of serum iron levels.

Procedure:

1. Preparation of reagents (RGT, STD) before use.
2. Blank reagent (Rb) preparation:

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- Mix 1000 µl of RGT with 50 µl of distilled water.

3. Sample preparation:
   - 50 µl of serum was mixed with 1000 µl of RGT, thoroughly mixed, and incubated at 20-25°C for 15 minutes.
   - The absorbance of the sample and standard was measured against the blank reagent within 60 minutes at a wavelength of 623 nm.
4. Iron concentration calculation using the following equation:

Iron concentration=Absorbance of sampleAbsorbance of standard×Standard concentration\text{Iron concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}Iron concentration=Absorbance of standardAbsorbance of sample×Standard concentration.

Measurement of Zinc Levels in Serum

The Colorimetric Determination of Zinc in Serum test, manufactured by LTA s.r.l. (Italy), Via Milano, 15/F, was used to measure zinc concentration.

Procedure:

1. Preparation of Reagents:
   - To prepare the work reagent, add 2 ml of reagent B to the glass vial containing reagent A and shake well for uniform mixing.
   - This solution is then used with the serum sample for zinc concentration measurement.
2. Preparation of Blank, Standard, and Sample Solutions

Reagent	Blank Solution	Standard Solution	Sample Solution
Work Reagent	1 ml	1 ml	1 ml
Distilled Water	50 µl	—	—
Standard Solution	—	50 µl	—
Sample Solution	—	—	50 µl

3. Measurement:
   - The absorbance of the blank solution was recorded at 578 nm using a spectrophotometer after 30 minutes to allow color stabilization.

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4. Calculation of Zinc Concentration:
    - Zinc concentration in serum was determined using the following formula:

$$C(Zn) = \frac{\Delta Sample}{\Delta STD} \times 200 \quad (\mu g/dl)$$

Lead Measurement Method

The lead concentration in serum was determined using the Kirchhoff and Bunsen method (2018).

Procedure:

1. Sample Preparation:
    - Serum sample was treated with concentrated nitric acid (HNO_3).
    - After acid digestion, the sample was dried in an oven at below 100°C.
    - Once dried, 5 ml of distilled water was added to dissolve the sample.
    - The sample was filtered using filter paper to remove any impurities.
2. Measurement:
    - The lead concentration was determined using an atomic absorption spectrophotometer (AAS).

Measurement of Antioxidant Levels

The levels of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in serum were measured using ELISA-based detection kits.

Procedure:

1. Microplate Coating:
    - A 96-well microplate was pre-coated with a specific antibody for each test (CAT, GPx, SOD).
2. Sample Incubation:
    - Serum samples were added to the wells, followed by a biotin-labeled specific antibody.

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3. Enzyme Conjugation:
    - HRP-conjugated secondary antibodies were added to each well and incubated.
4. Substrate Reaction:
    - A TMB substrate solution was added, leading to a color change in wells containing GPx, CAT, or SOD.
5. Reaction Termination:
    - The reaction was stopped by adding sulfuric acid solution, and absorbance was measured at 450 nm using a microplate reader.
6. Concentration Determination:
    - The concentration of GPx, CAT, and SOD in serum samples was determined by comparing the sample absorbance values against a standard calibration curve.

Result and Discussion

The prevalence of Trichomonas vaginalis infection among the examined women was 3.38% out of 325 samples. This finding aligns with Suleiman (9) in Kirkuk, who reported an infection rate of 2.4%, and Hamada (7) in Tikrit, who recorded 1.6%. In neighboring countries, the infection rates were 1% in Jordan (13), 3.8% in Iran (14), and 2.5% in Turkey (16). The relatively low infection rate may be attributed to the frequent prescription of metronidazole (Flagyl) by gynecologists for vaginal infections, as it is considered the most effective treatment against the parasite.

The highest infection rate was observed in women experiencing itching during menstruation (5.10%). This increase is likely due to the enhanced activity of the parasite, characterized by its rapid jerky movement and flagellar activity during menstruation, as iron ions from menstrual blood provide optimal conditions for its growth (17). This finding is consistent with study (27), which reported that iron availability during menstruation is essential for the parasite's survival in the vagina.

T. vaginalis Infection Based on Symptoms

Symptom Type	Total Samples Examined	Number of Infections	Infection Rate (%)
White Discharge	148	3	2.02

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Green Discharge	79	3	3.79
Itching During Menstruation	98	5	5.1

T. vaginalis Infection Among Infertile and Non-Infertile Women

Group	Total Samples Examined	Number of Infections	Infection Rate (%)
Infertile	136	7	5.14
Non-Infertile	189	4	2.11

T. vaginalis Infection by Age Group

Age Group	Total Samples Examined	Number of Infections	Infection Rate (%)
19-29	167	3	1.7
30-40	100	8	8.0
41-51	58	0	0.0

T. vaginalis Infection by Residential Location

Residence	Number of Infected Women	Infection Rate (%)
Peripheral Areas	7	63.6
City Center	4	36.36

Serum Zinc, Iron, and Lead Concentrations

Test	Infected Women	Control Group
Serum Zinc Concentration	147.3 µm/dl	193.1 µm/dl
Serum Iron Concentration	88.9 µm/dl	130.2 µm/dl
Serum Lead Concentration	2.83 µg/dl	2.62 µg/dl

Antioxidant Levels in Infected and Control Groups

Test	Infected Women	Control Group
CAT	6.88 pg/ml	13.54 pg/ml
SOD	15.31 pg/ml	36.47 pg/ml
GPxs	122.31 pg/ml	298.7 pg/ml

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