

Study the Role of Eosinophilic Cationic Protein, Copper and Iron in Women Infected with *Trichomonas vaginalis* Parasite

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Abstract

Since *Trichomonas vaginalis* (*T. vaginalis*) only manifests as a trophozoite stage during sex, it is typically transmitted during sex as a trophozoite. The purpose of this study was to determine the concentrations of copper, iron, and Eosinophil cationic protein (ECP) in vaginitis patients. Between July and November 2021, 355 suspected patients who visited the AL-Zahra maternity and pediatrics laboratory in the AL-Najaf region (Iraq) and 30 healthy respondents of the same age participated in the study. According to the manufacturer's instructions, the ELISA technique was used to measure the levels of three biomarkers in serum (copper, iron, and ECP). On the other hand, the colorimetric approach was used to measure the copper concentration. The investigation showed that in the blood sample of *T. vaginalis*-infected patients, the amounts of copper and iron considerably decreased ($P > 0.05$). In contrast, the serum levels of ECP considerably increased ($P > 0.05$) when compared to the control group. As a result of the *T. vaginalis* infection, copper levels in the blood are dramatically impacted, according to the current study's findings. The current study results suggested that the *T. vaginalis* infection alters serum iron and ECP concentrations, significantly affecting copper levels in the blood.

Keywords: Eosinophilic Cationic Protein, Copper, Iron, Women, *Trichomonas vaginalis*.

INTRODUCTION

Copper and iron levels in the blood sample considerably dropped ($P > 0.05$). *T. vaginalis* is only visible as a trophozoite stage and lacks a cystic stage; hence it is primarily transmitted during sexual activity as a trophozoite (1). Women can spread illnesses to one another through direct contact, contact with lately depositions of moist secretions on toilet seats, splashing in restrooms, or contact with borrowed towels or clothing. Rubber gloves, douche nozzles, and inadequately sterilized medical exam equipment are among additional potential sources of infection (2). The virus is most likely spread by clothing and bath tubes in young girls and women. It occasionally affects female infants and is transmitted by infected moms (3). The main transmission of *T. vaginalis* infection is male (4,5). Children's growth and development depend on minerals like magnesium, iron, copper, and zinc (6). Early detection and treatment of intestinal parasite infections are essential for children's healthy growth and mental development (7). According to Olivares et al.(8), trace minerals decrease numerous illnesses by influencing metabolic pathways and the immune system. The most important essential elements in the human body are iron, copper, magnesium, and vitamin B12. Lack of zinc affects lymphocyte and thymus function and is necessary for immune system functions. Neonatal zinc deficiency results in severe diarrhea because it affects immune system functions (9). Red blood cells, hemoglobin, iron absorption, and the activity of many enzymes all require copper for development (10). As cofactors for the enzyme cytosolic superoxide dismutase, copper and zinc deficiency impairs the activity of this enzyme (11). In this way, zinc is a crucial cofactor for a variety of biological processes.

PATIENTS AND METHODS

Ethical approval:

The academic and ethical committee at the University of Kufa approved this work. All patients and healthy persons provided their informed permission. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work.

Diagnosis of *Trichomonas vaginalis*:

A specialist doctor took non-duplicate samples of vaginal discharge from pregnant patients who had acute vaginal abscesses. Wet mount smear was used to identify *T. vaginalis*. One drop of vaginal discharge was obtained, and it was viewed under a microscope at a 40x magnification using an item to help with identification (12).

Serum collection:

Blood samples totaling 5 ml were taken from both healthy and infected pregnant women. In sterile vacutainer tubes, blood samples were taken and left at room temperature for 30 minutes. For five minutes, centrifugation was carried out at 3000 rpm (Mettler, Germany). Before usage, serum was collected and stored in sterile tubes in a deep freezer at -4°C.

Serum Biomarkers detection:

This study used three human biomarkers: copper, iron, and ECP. All of these biomarker kits were purchased from the Elabscience Company in Bulgaria, and, in accordance with the manufacturer company's instructions, the amount of Copper and ECP in serum was assessed using an ELISA equipment (Human Reader, Germany) as follows: 100 mL of a standard or sample was added to each well of a 96-well micro ELISA plate. After all liquids had been removed, 100 mL of biotinylated detection antibodies for human Copper and ECP were added. These antibodies were then incubated for an hour at 37 °C before being thoroughly washed three times. After being added, 100 mL of the Avidin-Horseradish Peroxidase (HRP) Conjugate was incubated for 30 minutes at 37 °C before being five times rinsed. Substrate reagent A90L was added, and it was then incubated at 37°C for 15 minutes. The stop solution was increased by 50 L. The optical density was instantly read at 450 nm, and the outcomes were calculated (13). While the iron concentration was measured in accordance with the accepted methodology (14,15,16) using a colourimetric method.

Statistical analysis:

In this investigation, samples were compared using the T-test with Graph-Pad Prism version 10 software. A p-value of 0.05 or less is regarded as statistically significant (14,15,16).

RESULTS

Table 1 results from this study show a substantial rise in ECP concentrations (ng/ml) ($P > 0.05$). The levels of copper (mg/dL) and iron (g/ml) were significantly lower ($P > 0.05$) in the *T. vaginalis*-infected individuals than in the healthy control group. Furthermore, the current investigation found no conclusive evidence of a gender effect. ECP, iron, and copper values are comparable in males and females. In the total patients infected with *T. vaginalis*, the concentrations of ECP, iron, and copper were reported to be 32.49 (ng/ml), 63.22 (g/ml), and 91.27 (mg/dl), respectively, while this value was reported to be 11.12 (ng/ml), 89.71 (g/ml), and 130.39 (mcg/dl) in the healthy control.

Table 1: Trichomonas vaginalis infection effect on three biological markers

Respondents	Mean±SE		
	Iron (µg/ml)	ECP (ng/ml)	Copper (mg/dL)
Male (Patients)	64.14±0.71	31.57±0.18	88.82±0.82
Male (Control)	89.23±0.15	1.32 ±0.22	131.33±1.24
Female (Patients)	63.17±0.84	33.59±1.62	91.36±1.23
Female (Control)	85.93±1.28	1.83±0.08	128.56±1.31
Total Patients	63.22±0.42	32.49±0.28	91.27±0.79
Total Control	89.71±1.62	11.12±0.88	130.39±1.44

SE: Slandered error, ECP: Eosinophilic Cationic Protein

DISCUSSION

As compared to those in the control group, patients with *T.vaginalis* infections had significantly lower copper levels. Copper, zinc, and magnesium are crucial for growth and development in growing children and play a crucial part in the metabolism and physiology of the human body, so these results may be caused by the virulence factors of this parasite (16, 17). These results are in line with those of Uwah et al. (18), who found that patients with parasite infections had lower serum levels of zinc, copper, and magnesium than did the control group. This may be linked to intestinal parasites that transform the host's nutritional sources of carbohydrates, fats, minerals, vitamins, and other nutrients into the life-essential cycle's fuel. Females with *T. vaginalis* infection had considerably higher concentrations of (ECP) than the healthy group in the current study. In the present study, eosinophil cationic protein (ECP) may have contributed to the results, as an extremely basic, single-chain, strong cytotoxic protein released by granulocytes in response to parasitic infections (19).

Activated eosinophils, neutrophils, basophils, and other immune cells produce large amounts of cytokines during allergic inflammation infections. They might be caused by interactions between the human immune response and *T.vaginalis* antigens or by products from *T.vaginalis*. Studies have suggested that measuring ECP by serum monitoring many inflammatory diseases, such as parasitism, allergic inflammatory diseases, and other inflammatory disorders. ECP is one of the body's natural defenses against invading parasites, allergic inflammatory diseases, and other inflammatory disorders. There are four essential proteins in granular cells, and one of them is eosinophil cationic protein, which stimulates the production of amino acid granules in the surrounding tissue (20). These findings are consistent with Tischendorf et al. (21) who discovered that eosinophilic illnesses were the only conditions where ECP levels were raised.

According to the findings, patients with *T. vaginalis* infections had considerably lower serum iron levels than those in the control group. Patients with *T. vaginalis* may have a reduction in iron levels due to malabsorption or the parasite's pathogenicity, which depends on the link between iron levels and parasite adhesion to epithelial cells (22). While the source of iron may come from the hemolysis of red blood cells caused by parasite lesion or from menstrual blood that is increased by infection.

T. vaginalis use of iron may result in a drop in iron levels. The drop in ferritin levels could be brought on by this parasite's increased consumption of iron. As ferritin or more parasites use them, this results in diminished iron storage; some research list

T. vaginalis as an iron source (7). Ferritin diminishes when an infectious process takes place because *T. vaginalis* uses ferritin as an iron source, as shown by the results from Fernando et al. (9). According to Weinberg's study (21) *T. vaginalis* uses ferritin as a source of iron, which results in a drop in ferritin levels in the serum of men who have the infection compared to the control group (22). This finding is supported by the current study.

CONCLUSION:

According to the current research, the *T. vaginalis* infection significantly reduces the blood copper level by altering serum iron and ECP concentrations.

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