

## Immunohistopathological Study for Patients with Appendicitis due to *Enterobius vermicularis* worm

Ali A. Mohy<sup>1</sup>, Saleem Khteer Al-Hadrawy\*<sup>1</sup>, Kais Khudhair ALhadrawi<sup>2</sup>

<sup>1</sup>Department of Biology, Faculty of Science, University of Kufa, Iraq

<sup>2</sup>Radiology Techniques Department, Collage of Medical Technology, Islamic University, Najaf, Iraq

\*Corresponding author E-mail: [saleem.alhadrawi@uokufa.edu.iq](mailto:saleem.alhadrawi@uokufa.edu.iq), Mobile: 009647803171807

### ABSTRACT

**Background:** Intestinal *E. vermicularis* worm infection is asymptomatic and endemic in Iraq, but it can cause appendicitis; symptoms vary depending on the stage of the gastrointestinal disease. The *E. vermicularis* worm has caused several cases of appendicitis. **Objective:** This study aimed to find out how common *E. vermicularis* was in appendectomy samples and whether it played a role in appendicitis development. **Patients and Methods:** Just 30 (3.15 %) of the 950 surgical specimens removed from patients with clinical appendicitis tested positive for *E. vermicularis* worms. Two ml blood was drawn from positive samples to measure cytokines (prostaglandin two and Transforming growth factor-beta 1), with 30 healthy people serving as a control group. From January to August 2019, all samples were taken from patients who visited AL-Zahra maternity and paediatrics and AL-Hakeem hospital in AL-Najaf province. Patients' appendixes were biopsies, stained with eosin hematoxylin, and microscopically diagnosed. **Results:** The findings of this study showed a substantial increase (P0.05) in serum levels of PGE2 and TGF-1 $\beta$  as compared to the control group, as well as a histological spectrum of appendicitis associated with a large number of *E. vermicularis* eggs, the lymphoid follicle reactive germinal center, prominent eosinophilic infiltration. **Conclusion:** Based on this research's findings, we believe that *E. vermicularis* worm infection can cause appendicitis and an immune response manifested by elevated PGE2 and TGF-1 $\beta$  levels in the blood.

**Keywords:** Immunohistopathological Study, *Enterobius vermicularis*, appendicitis, worm, Al-Najaf, Iraq.

### INTRODUCTION

In the appendix, *E. vermicularis* is the most common parasite. It is rarely linked to acute appendicitis cases, but it can play a role in appendiceal pain and chronic inflammation. Inflammation of the appendix with *E. vermicularis* causes "appendiceal syndrome," characterized by recurrent intermittent pain in the right iliac fossa. Histologically, the appendix can tend to be regular. Nonetheless, it also shows several pathologic changes. Patients sometimes undergo an emergency appendectomy, but the definitive diagnosis can only be made after a pathologic examination of the appendix. To avoid re-infection, all patients diagnosed with *E. vermicularis* appendix infestation should receive antihelminthic treatment <sup>(1)</sup>. The most popular reason for emergency surgery is acute appendicitis <sup>(2)</sup>.

In developing countries, parasitic and gastrointestinal infection are rarely observed in the pathogenicity of acute appendicitis <sup>(3)</sup>. Parasitic infections, on the other hand, often cause acute appendicitis symptoms. Even though several parasites were found in the appendix cavity, the enigmatic association between parasitic infection and acute appendicitis is still unknown, and conversely, these parasites cause appendicitis <sup>(4)</sup>. *E. vermicularis* infection in humans has been documented for around 10,000 years, and the parasite was introduced to the supplement <sup>(5)</sup>.

*E. vermicularis* mature worms can be found in the ascending colon's proximal region and the cecum, appendix and ileum. The male worm dies after fertilization, while the female worm migrates to the anal canal, where the eggs are laid <sup>(6)</sup>. This study aimed to find out how common *E. vermicularis* was in appendectomy

samples and whether it played a role in appendicitis development or not.

### PATIENTS AND METHODS

#### *Samples collection:*

Two milliliters of blood were drawn from each healthy and infected person and stored in sterile straight tubes at room temperature for 30 minutes. For 5 minutes, centrifugation was carried out at 3000 rpm (Memmert, Germany). Serum was collected and stored in sterile tubes at -20°C until it was required.

A total of 250 surgical specimens were examined from fully consenting patients undergoing surgical operations for clinical appendicitis. The faculty of Science at the University of Kufa's institutional ethics committee approved the collection of samples, and all participants signed informed consent documents. From January to August 2019, all samples were obtained from patients who visited the surgical department at AL-Zahra maternity and paediatrics and AL-Hakeem hospital in AL-Najaf province. Before surgery, both patients were tested in clinical consultations.

#### *Serum biomarkers detection:*

The TGF-1 $\beta$  and PGE2 human biomarkers kits offered by Elabscience Company were used in this research. According to Manufacturer Company, Bulgaria and the level of biomarkers in serum were calculated using an ELISA system (Human reader, Germany).

#### *Histological processing:*

The appendixes were fixed in a 10% formaldehyde

solution. As shown by (7,8). All blocks were stained with hematoxylin and eosin and examined for the presence of *E. vermicularis* and the form of inflammation. A traditional light microscope with a digital camera was used to view the slides.

**Ethical approval:**

**An approval of this study was obtained from University of Kufa Academic and Ethical Committee. Informed consent of all the patients and healthy individuals was obtained. The study was conducted in accordance with the Declaration of Helsinki for studies on humans established by World Medical Association.**

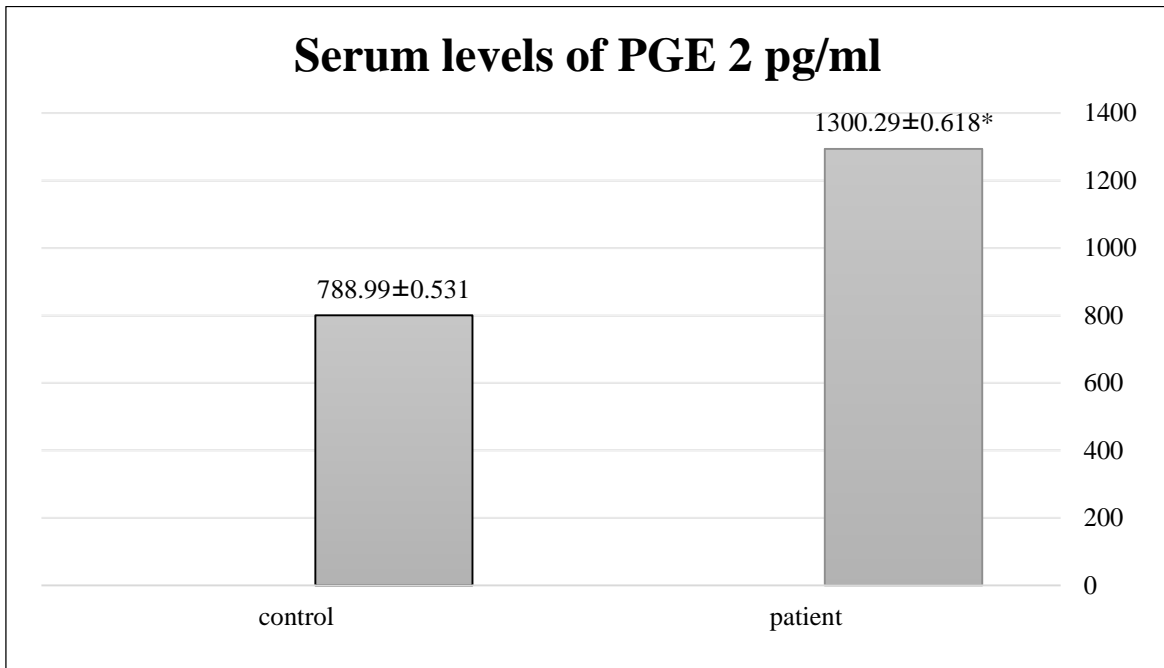
**Statistical analysis**

A P-value of less than 0.05 is considered statistically significant in this study based on a T-test using Graph-pad prism version 10 software (9,10).

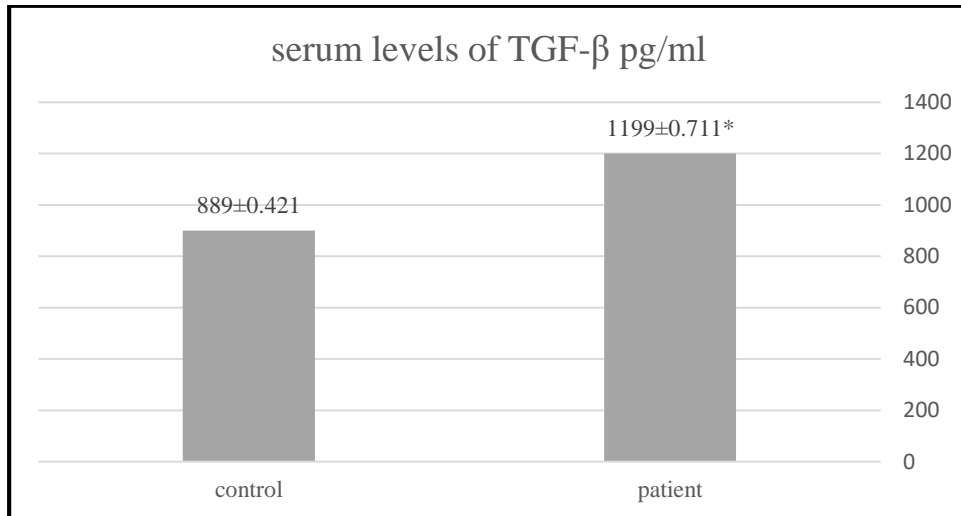
**RESULTS**

We found adult or eggs of *Enterobius vermicularis* in appendix biopsy samples from patients with appendicitis in thirty (3.15 percent) positive samples. The current research also discovered that serum PGE2 and TGF-1 $\beta$  levels were significantly higher (P = 0.05). PGE2 concentrations in patients and controls were (788.99 0.531 pg /ml), (1300.29 0.618 pg /ml), and (8890.421 pg /ml), (1199 0.711 pg /ml), respectively, as shown in figure (1, 2).

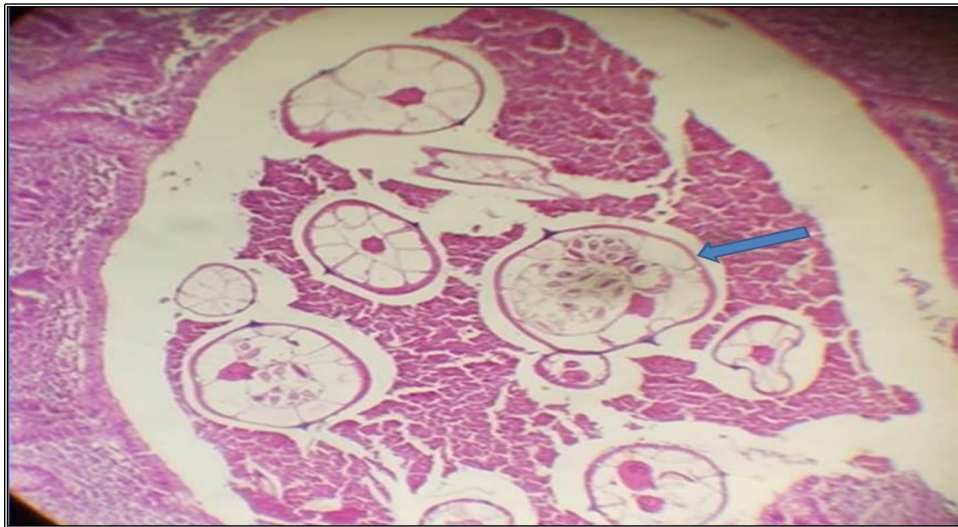
While the findings of the histological analysis indicated that the histological spectrum of appendicitis was associated with a large number of *E. vermicularis* larvae, the lymphoid follicle reactive germinal center, prominent eosinophilic infiltration, and a somatic segment of worm in the appendix lumen as shown in Figures 3, 4, 5, and 6, the histological spectrum of appendicitis was associated with a large number of *E. vermicularis*.



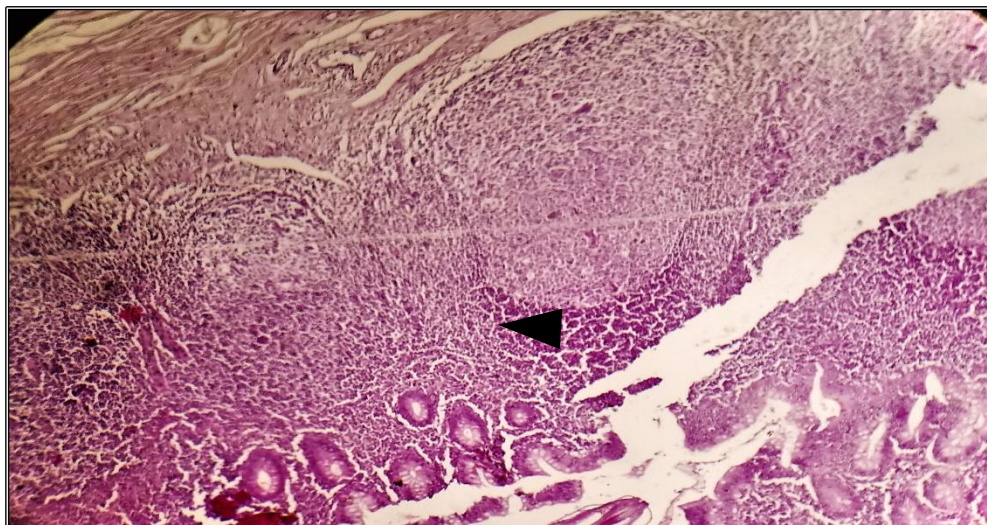
**Figure (1):** Serum concentration of PGE2 pg /ml cytokine in patients and control group



**Figure (2):** Serum concentration of TGF-β1 pg /ml cytokine in patients and control group

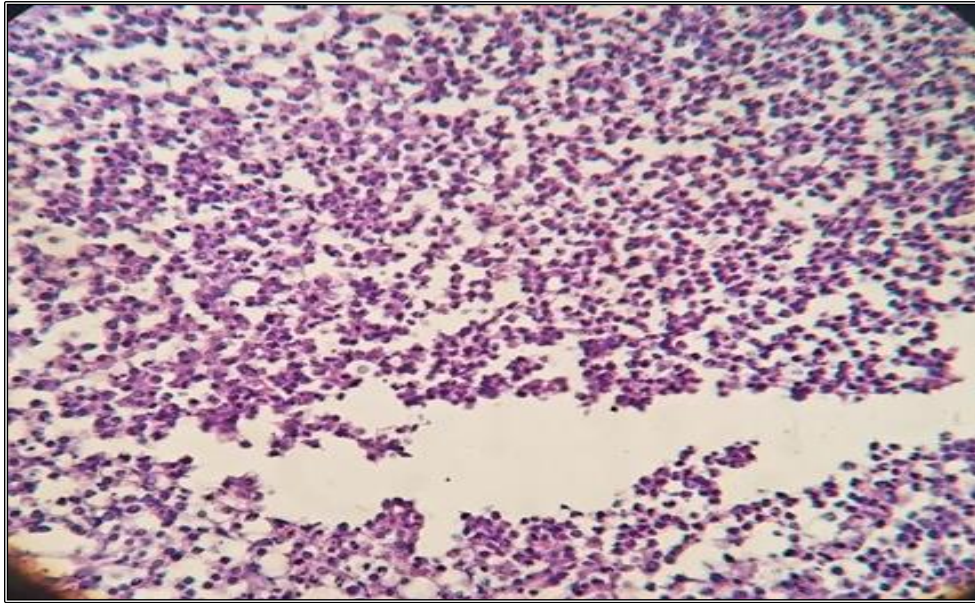


**Figure (3):** This figure show the egg of *Enterobius vermicularis* (under pointer) staining with Hematoxylin-eosin (400 X).

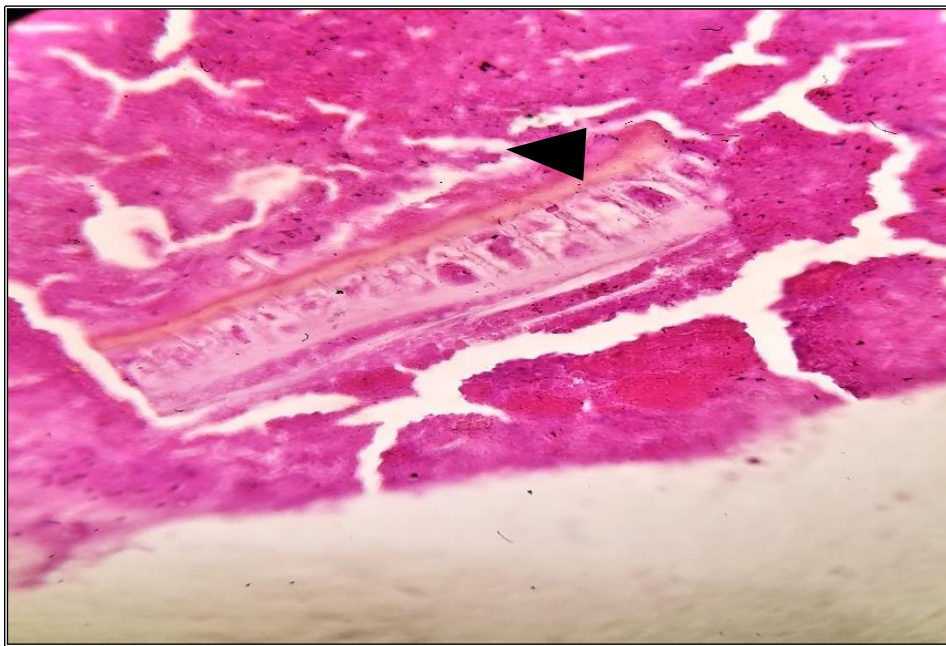


**Figure (4):** This figure show the lymphoid follicle reactive germinal center (under pointer) staining with Hematoxylin-eosin (400 X).





**Figure (5):** This figure show the infiltration staining with Hematoxylin-eosin (400 X).



**Figure (6):** This figure show the somatic segment of worm in the lumen of appendix (under pointer) staining with Hematoxylin-eosin (400 X).

## DISCUSSION

The current study's findings showed a substantial rise ( $P = 0.05$ ) in serum concentration. TGF- $1\beta$  and prostaglandin 2 (PGE2) levels were higher in patients with *E. vermicularis* infection than in the control group, which may be attributed to *E. vermicularis* induced inflammation allergenicity and immunogenicity. TGF- $1\beta$  is present at higher inflammatory sites, whether during acute or chronic stages of inflammation<sup>(11,12,13)</sup>.

PGE2 is developed by all body cell types with epithelial, fibroblast, and infiltrating inflammatory cells, which are the primary sources of PGE2 in the immune response. The presence of four PGE2 receptors, EP1, EP2, EP3 and EP4, reflects the effects of heterogeneous PGE2, with an additional degree of functional variability arising from multiple splice variants of EP3 occur in at least eight forms in humans and three forms in mice<sup>(14)</sup>.

**Ramaswamy et al.**<sup>(15)</sup> backs up a *Schistosoma mansoni* infection study, which observed a substantial rise in PGE2 levels after the parasite infects the skin. As the parasite migrated out of the skin, the levels of PGE2 decreased.

When neutrophils take up the parasite, it stimulates the development of TGF- $\beta$ 1, an immunosuppressive cytokine beneficial for parasite persistence within neutrophils and suppresses the T-helper 1 form (Th1) response, according to a study on *Leishmania* infection. They also discovered a significant increase in TNF-production and a decrease in TGF-concentration in rHNP-1-treated neutrophils, a response that should potentiate the immune system against parasite invasion<sup>(16)</sup>, which contradicts the current finding. The histological spectrum of appendicitis associated with a large number of eggs from *E. vermicularis*, the lymphoid follicle reactive germinal center, with prominent eosinophilic infiltration and a somatic segment of worm in the lumen of the appendix was found to be 30 (3.15 %) in the current study.

The presence of parasites in the appendectomy samples, which were usually excluded, was discovered by chance. *E. vermicularis* infection caused gastroenteritis all over the world. Two factors can explain this infection. First, the parasite's spread was influenced by its geographical location and social circumstances, which resulted in widespread changes in the parasite's spread. The second factor may be due to minor variations in pathologists' techniques. If the natural tissue attachment is examined extensively, evidence indicates that it can show molecular proof of inflammation<sup>(17)</sup>.

*Enterobius vermicularis* infection may lead to a variety of pathological changes in the appendix, ranging from lymphatic hypertrophy to potentially fatal

complications, including gingivitis and perforation with peritonitis, the parasite's presence may cause lymphocyte inflammation, which is the first tissue reaction that leads to clinical signs of excess colic<sup>(18)</sup>.

While *E. vermicularis* may play a role in appendiceal pain and chronic inflammation due to obstructive phenomena, the vast majority of cases do not involve acute inflammation. Pinworms in the appendix can cause a clinical "appendiceal syndrome", even though no acute inflammation is present<sup>(19)</sup>. Leukocytosis can be observed in laboratory tests, but in most cases, WBCs are common, and the diagnosis is made by eosinophilia and a positive stool analysis.

In the case of acute appendicitis, the condition is less clear. It's likely that in the few cases where *E. vermicularis* is found in conjunction with acute inflammation, the worm was the one who initiated the inflammatory response. However, this and other research indicate that its appearance is purely coincidental<sup>(18, 20)</sup>. *E. vermicularis* is rarely associated with the histological changes of acute appendicitis when it comes to histopathology.

The inflammatory process can be triggered by the obstructive effect of the prominent lymphoid tissue<sup>(3)</sup>. The number of granulomas in *E. vermicularis*-infested appendices far outnumbered those in a control group of non-infested appendices. *E. vermicularis*, according to **Budd and Armstrong**<sup>(21)</sup>, can invade the appendix wall and cause inflammation in addition to granuloma formation.

A search of the literature revealed a single case of *E. vermicularis* invasion and intramural position in a patient with acute appendicitis symptoms. In their report, **Sah and Bhadani**<sup>(19)</sup> identified two acute appendicitis cases in which parasite eggs were attached to the mucosa in one case and *E. vermicularis* had invaded the mucosa and was lying intramurally in the other. Another study by **al Hadraawy et al.**<sup>(22)</sup> found a strong connection between hydatid cyst infection and serum lipid levels, especially cholesterol and triglycerides, as well as LDL, suggesting that infection may affect serum parameters.

## CONCLUSION

According to the findings, infection with the *E. vermicularis* worm can affect immune response by increasing serum concentration of PGE2 and TGF- $1\beta$  and cause acute appendicitis due to histological changes in cellular infiltration. Enterobiasis plays a significant role in the transformation of contaminated human tissues.

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