

Level of eosinophil cationic protein in *Strongyloides stercoralis* infection in Najaf province/Iraq

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Abstract

The eosinophil cationic protein (ECP), which is stored in the secretory granules of eosinophils, has a cytolytic actions against parasites and some other pathogens. Infection with *Strongyloides stercoralis* (thread worm) was diagnosed by direct fecal smear and formal-ether concentration. Serum ECP levels were measured by ECP assay kit (Mesacup ECP test) for 40 patients infected with thread worm and compared with same numbers of healthy patients. There was a significant increase of ECP sera levels of patients (66.358 ± 0.861 ng/ml) ($p < 0.05$) when compared to that of control group (10.962 ± 0.635 ng/ml). There were no significant differences of ECP in relation to sex and age ($p > 0.05$). This study aimed to assess serum ECP in relation to threadworm infection and their potential activity as a biological marker for infection.

Keywords: ECP, threadworm infection.

1. Introduction

Strongyloides stercoralis is an intestinal nematode, endemicity is mostly in tropical and sub-tropical areas where it chronically infects more than thirty million individuals (1). Infections in humans may be extremely long lived, and this chronic parasitism is probably maintained by autoinfection as the worm has the capacity to replicate within the human host (2). *Strongyloides stercoralis* can cause significant disease in the setting of acquired immunosuppression (3). Granulocyte was activated through infection of helminthes and behave as immune active cells, and antibody will be attached to the surface and triggering degranulation of toxic granules against the helminth. When active parasitic infection occurs, the eosinophilic fraction in the host blood will be increased to more than 39% (4). ECP is a single-chain, zinc-containing protein which is stored in the secretory granules of eosinophils and have a cytolytic actions against parasites (5). ECP is secreted locally when there is a helminth or allergen (6). The increased parasitic intensity was associated with increasing ECP levels (7). Some researchers have suspected that ECP granulocytes act only on the larval stages of parasites

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without effects on adult (8). One of the types of immune response is separating of pathological agent and the infected area as granulomatous state (9). ECP mediates the modeling of the cellular matrix by onset of chemotactic view of ECP on the fibroblasts, and also ECP stimulates the tumor growth factor β (TGF- β) (10). Serious morbidity can be caused by *Strongyloides stercoralis* so identification of biomarkers is critical for the prevention of severe infection (11). This research measured serum level of ECP in relation to threadworm infection in Najaf province / Iraq and considered as a biomarker for threadworm infection.

2. Materials and Methods

2.1 Patient's stool samples

Eighty-three stool samples were collected in labeled stool containers from different localities of hospitals and private laboratories in Najaf province / Iraq. Stool samples were kept at room temperature, then transported to parasites laboratory in medical laboratories techniques department where general stool examination was done to detect the presence of helminthes larvae and using formal-ether concentration method (12) during year 2019.

2.2 Serum samples

Venous blood samples (about 5 ml) were collected by venipuncture vacutainer (Becton Dickson Company). The prepared sera were immediately stored at -20°C until the performance of analysis to avoid repeated freezing and thawing during assay procedure for ECP measurement.

2.3 ECP Kit

The Mesacup ECP test is quantitative kit for human ECP in serum by sandwich ELISA (MBL. Japan) with high sensitivity; this ELISA detects human ECP with a minimum detection limit of 0.125 ng/ml.

2.4 Measuring ECP

The serum levels of ECP were measured by means of sandwich ELISA as recommended by the manufacturer.

2.5 Calculation of results

This was done by calculation of the mean absorbance value of each standard and plot on the semi-log graph to construct a standard curve [Absorbance on the vertical axis. Concentration (ng/ml) on horizontal axis. (fig. 1). Reported ECP concentration of samples were then multiplied by the value read from the standard curve by dilution.

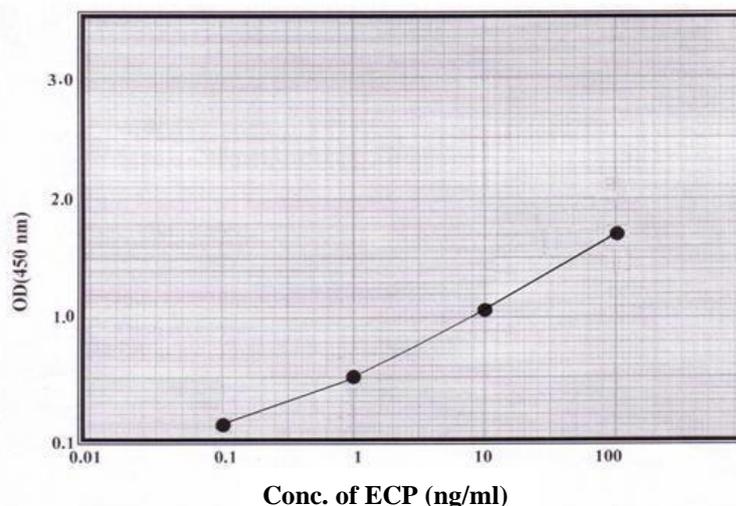


Figure 1: Standard curve for the estimation of ECP.

3. Results

Table 1: Mean concentration \pm SE(ng/ml) of ECP in the sera of 40 patients infected with threadworm and control.

Control	Patients	P-value
10.962 \pm 0.635	66.358 \pm 0.861	<0.05

Table 2: Mean concentration SE \pm (ng/ml) of ECP in the sera of 40 patients infected with threadworm in relation to sex and age.

Sex		P-value	Age (year)			P-value
Male	Female		10-20	20-30	30->50	
62.434 \pm 1.820	64.427 \pm 1.63	>0.05	65.721 \pm 2.614	64.641 \pm 1.28	60.213 \pm 4.23	>0.05

There was an increase of ECP levels in the sera of patients with threadworm infection (66.358 \pm 0.861 ng/ml) when compared to that of the control group (10.962 \pm 0.635 ng/ml) (P<0.05) (tab.1). There were no significant differences (p>0.05) in relation to sex and age (tab.2).

3.1 Statistical analysis

The data were analyzed using the available software package. The results were presented as number percentage and mean \pm SD whenever possible. The data were analyzed by using analysis of variance (ANOVA) test taking P<0.05 as the lowest limit significance. These manipulations were carried out according to Statistical Analysis System (13).

4. Discussion

This study was done to measure the level of ECP during threadworm infections and to investigate it as a considerable biomarker. The ECP levels were increased in threadworm and shared in a large number of biological reactions (14). The role of ECP in allergic response is clear, especially in the pathological inflammation of bowel disease (11). ECP and EDN have active roles that make puncture into the membrane of certain cells, leading the entry of toxic molecules into the target cells with gradual degeneration (15). The protective immune response uses different granular molecules of neutrophil and eosinophil in innate adaptive immunity and are needed in the innate response (16). The ECP secreted by eosinophil could escape to the protoscoleces of hydatid cyst and considered as a molecular event that may directed as a response for inflammation against the hydatidosis cyst disease (17). ECP might be considered as a good biomarker for inflammation activity of female schistosomal genital tract (18). The biomarker of schistosomal male genital tract infection was ECP which is important in healthy reproduction (19). In Ghana, researchers emphasized that the mean ECP which is higher in Schistosoma haematobium infection than the negative samples (20). In this study, there was no obvious differences between male and female ECP values in Strongyloides stercoralis infection, this may be due to the small sample of patients upon time of study, or the two genders have the same activity of ECP of granulation, this agrees with previous studies (21), while others found no such similarity (22), and also there was no change in ECP activity with age, but in contrast there was a significant relationship between sex and Schistosoma haematobium infections in the treated children (8). ECP levels persisted in a significant proportion indicating continuous eosinophil activity in the bladder wall (23). In conclusion, this research exhibits that serum ECP levels were tightly correlated with threadworm infection and more studies to explore its development as a threadworm diagnostical tool are sanctified.

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