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Effect of *Enterobius vermicularis* parasite on IgE antibody levels among children in Al- Diwaniyah City, middle Iraq.

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ABSTRACT:

The aim of this study was to detect the concentration of IgE antibody in children infected with *Enterobius vermicularis* and healthy children as a control group by using Enzyme linked immunosorbent assay (ELISA) technique. The results indicated that there was an increase in the concentration of IgE antibody levels in people with *E. vermicularis* parasite with an average of (227.08 IU) compared to the control group, and the study of elevation ratios for age, where the results showed that the lowest increase in IgE antibody was recorded in the age group 1-3 years. The highest increase was recorded in the age group between 7-9 years with an average of (306.84 IU). The results of the statistical analysis showed very significant differences indicating a higher level of IgE among people with *E. vermicularis* parasite compared to the control group at a probability level of 0.05. The results of the statistical analysis through the use of the F-test showed no significant differences between the high antibody IgE in children with *E. vermicularis* parasite depending on age groups.

KEYWORDS: Immunoglobulin IgE , cellular immunity, Intestinal helminths, immune response .

1. INTRODUCTION

The immune system is one of the most complex organisms and shows many signs of co-evolution with parasites, which are well-tuned for its task. Multicellular organisms that do not last long may not survive and outperform their short-lived and multiple parasites. Immune responses among host species vary with many other species such as the environment and the life cycle phase (Al-Kabee, 2014). Similarly, parasites cannot always exercise maximum virulence due to the costs involved. For example, a host may be killed before being fully exploited by the parasite and result in a pattern of host and parasite genotypes. Since only certain parasite genotypes are capable of infecting a particular host genotype and vice versa, only some host genotypes resist a particular set of parasite genotypes (Schmid-Hempel, 2009).

The immune response caused by parasitic infection is complex and multiple. In parasitic worms, antiparasitic stimuli stimulate an intense response of Th2 with the production of Interleukin-4 and Interleukin-5 leading to the synthesis of IgE and activate eosinophil cells (Medeiros *et al.*, 2006). Acid cell hypertrophy and elevated IgE levels are characteristic of many parasitic worm infections, and when they are not interpreted, hidden worm disease (Yazdanbakhsh, 2002). should be sought. Previous studies have shown that IgE antibodies and eosinophil cells play an important role in the immune response to helminths (Meeusen & Balic, 2000). Interleukin-4 and Interleukin-5

Th2 responses also increase in IL-9, IL13 and IL21 levels, activating and enlarging CD4 + TH2 cells, mast cells and basophils, all of which can produce several types of TH2 cytokines (Robert *et al.*, 2009).

Immunoglobulin IgE

IgE antibody is one of the widely known antibodies due to its involvement in all types of hypersensitivity reactions as well as it can also participate in protecting the body from parasites (Al-Hashemey, 2019). Y-shaped and composed of four chains form the basic units of the construction of the antibody (Monomer). These chains are classified into two similar heavy chains called (Heavy chain) and two similar light chains called (Light chains). The type of heavy chain is Epsilon on the surface of IgE cells is known to be FcεRI, which is high in IgE and CD23 FcεRII, which have a low affinity for IgE. These receptors are found on the surface of mast cells, Eosinophils and Basophils (Kelly & Grayson, 2016; Oettgen, 2016). IgE antibody is excreted from B and plasma cells (Mukai *et al.*, 2016). Despite its half-life of less than a day in the plasma, it can last for several weeks or months when it is bound to the cell surface by the FcεRI receptor, making it a long gatekeeper (Oettgen, 2016). The main function of IgE is to defend against parasites such as helminths (Fitzsimmons *et al.*, 2014). Many of these parasitic infections are defended by activating T2-assisted T cells, which produce antibodies to IgE. IgE binding to its FcεRI receptors on the surface of MC and Basophils is

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likely to produce and release substances that play a role in the parasite (Mukai *et al.*, 2016).

Immunoglobulin IgE, which is found naturally in small amounts in the serum and excreted in less than 0.001% of all immunoglobulin in the serum. The neonatal level of the total IgE is less than 1IU / ML. IgE levels show a slow increase during childhood to adult levels in the second decade and are generally responsible for allergic reactions, however, it is necessary to mean that about 30% of patients with allergic manifestations can have a normal level of the total IgE, in contrast, high levels of IgE can be detected in anyone without any sensitivity. These high antibodies cause a smooth muscle contraction and eventually lead to allergic conditions such as skin reactions, allergic reactions, chills, dermatitis, rhinitis, hay fever, asthma and allergic shock (Al-kape, 2014). Worm allergens are potent inducers of IgE production and stimulate strong IgE responses in humans but may contribute to host immunity against parasitic worms or life-cycle phases. It has been suggested that high levels of polyclonal IgE are a defense mechanism against worms against IgE antiparasitic effects (Villarreal *et al.*, 1999). The immune response to IgE began with B-lymphocytes produced by TH-2 lymphocytes (IL-4 + IL-5). IgE production is a response to initial exposure to an antigen or an allergen (Rosario *et al.*, 2007).

The relationship of pinworms in high IgE antibody level

As a result of the impact of intestinal parasites on some immune indicators, including IgE antibody has been conducted several studies on this subject, the most important of which came in a study in Nigerian Okada where 334 children under the age of 15 years were examined, the results of the study recorded that the total infection rate of parasites 50% of children aged 5-11 years had the highest rate of intestinal parasites and in both sexes, and in contrast, children under 5 years of age had the lowest rate of intestinal parasites. IgE in sucking Blood of children with intestinal parasites compared with uninfected children (Ehiaghe *et al.*, 2013).

In Northwest Ethiopia, (Amare *et al.*, 2013) conducted a study to determine the nutritional status, intestinal parasite infestation, and allergy among primary school children. With no subjects with an average concentration of (344 IU / ml), it was concluded that there was no association with IgE concentration with a parasitic infection or an allergy history.

In Iraq, specifically Karbala Governorate, the results of the study showed that the concentration of antibody IgE and interleukin-5 and histamine in the serum of children with intestinal parasites reached an average (56.63 IU / ml, 22.08 pg/ml and 76.27 ng/ml respectively) A comparison with the group of uninfected children (42.76 IU / ml, 15.43 pg/ml and 65.35 ng/ml respectively), the statistical analysis showed that there were significant differences in the concentrations of these criteria in children with and without intestinal parasites (Al-Hashemey, 2019).

As for Al-Diwaniya Governorate, an immunological study was conducted for people infected with pinworm parasites, where the results of the study showed a noticeable increase

in the levels of IgE among people with parasite with a mean (377.4481 IU / ml) and by 36% compared to the control group and with an average (29.28175 units) International/ml) (Al-Kabee, 2014).

2- Materials and methods of work

Collect the blood samples.

About (cc2) of venous blood was collected for the 90-pinworm patients after confirming the diagnosis of infection by microscopic examination of stool samples for children aged 1-12 years, and was allowed to coagulate in 37°C using centrifugation and then stored in -20°C until Use for the purpose of immunological examination.

The ELISA kit used in this research was prepared by the Dutch company CALBIOTECH with following the publication steps provided by this company.

Detection of IgE antibody levels in the serum of children with *E.vermicularis* infection

Test principle:

Total IgE is a single, immune, step-by-step basis of the sandwich method. Based on the principle of Streptavidin-biotin. This is done by fixing the antigens or antibodies in the plate's plastic holes where the serum and standard samples are placed in the Streptavidin-coated Microwell drill. The internal IgE of the patient's serum is associated with the antibody-antigen of the antibiotic IgE. Simultaneously the antibiotic is frozen into the pits by a high Streptavidin-Biotin reaction, the non-bound protein and excess biotin antibodies are washed by the Wash buffer. Then add an enzyme linked to the anti-molecule Enzyme Conjugate and incubate for half an hour and then wash again, then add the substrate to the base material and incubate the plate, which leads to the development of blue color and then add a stop solution to stop the reaction where the color becomes yellow in the holes, the concentration of IgE is directly proportional to the color intensity in the test sample. Absorption is measured using a 450 nm wavelength spectrometer (Al-Kabee, 2014).

3- Results and discussion

The study included measuring the levels of serum IgE by the IgE ELISA kit group, and 90 blood serum samples were tested, 70 of which were infected with the *E.vermicularis* parasite (positive in fecal tests) belonging to 35 males, 35 of whom are females, and 20 serum were healthy children as a Control group includes 10 males and 10 females. These results show that among those with the *E.vermicularis* 39 parasite, that is, 55.71% had a positive examination of IgE with a mean (227.09 IU), while 31, 44.28% of patients with a negative examination (IgE (37.87 IU) compared with the results of the control group negativity for IgE examination with mean (48.66 IU).

The results of the statistical analysis showed the presence of very clear significant differences indicating the high level of IgE among those with the *E.vermicularis* parasite compared with the control group at the probability level 0.05, as shown in Table (1). These results are higher and are consistent with the results of (Al-Kabee, 2014). That observed a 36% increase in IgE levels in children with pinworms in Al-Diwaniyah governorate. It also agreed with (Durmaz *et al.*, 1996). Who

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observed that levels of IgE decreased significantly after anti-parasite treatment. The result is also consistent with the result of (Al-Hashemey , 2019) That showed high levels of IgE in people with an average score of 56.63 IU compared to the control group with an average score of 42.76 IU. The ability of helminthes to manipulate the immune system of their host to ensure their survival and the host often has an immune response to other pathogens (Fibey *et al .*, 2019). The main function of IgE is to immune parasites such as helminths, and important levels of IgE have been studied in people with helminths or parasites infection. Although higher IgE was higher in worm infection than that of parasites, Bengul *et al .* (1996) showed that children with the *E.vermicularis* parasite had higher IgE levels than found in *G.lambli*a and Perlmann *et al .* (1994) who demonstrated importance during immune defense against some primary parasites such as *Plasmodium falciparum*.

Table (1): Immunological Variables represented by Examination of IgE Levels in People with *E. vermicularis* Parasite Compared to the Healthy Group (Control Group).

The group	Rate±Standard deviation
Healthy	48.67±38.24
Infected	227.08±156.08
T-test	8.690
P value	0*

* There are significant differences at the probability level 0.05.



Fig. (1): IgE screening results for *E. vermicularis* and healthy individuals using ELISA

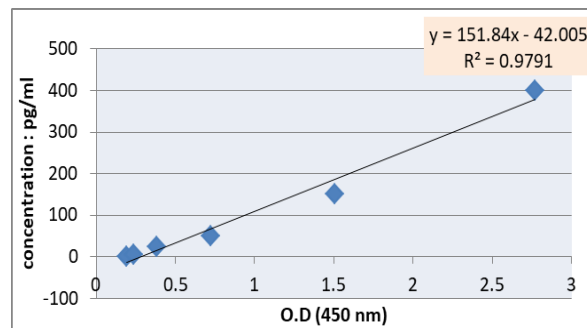


Fig (2): Curve for IgE ELISA screening.

Table (2) shows the concentrations of IgE antibody among children infected with pinworm parasites in Al-Qadisiyah governorate depending on the age groups. The highest serum mean in the age group 7-9 years was 306.68 IU. The results of the statistical analysis using the F-test showed no significant differences between the high antibody IgE in children with pinworm parasites depending on age groups. IgE antibody levels were divided based on age groups and not on the sex of children depending on (Rezaeipoor & Zadeh , 1993).

Table (2): IgE antibody concentrations for *E. vermicularis* parasites for age groups.

Age groups	Numbers	Mean± SE
1-3	14	196.85±42.71
4-6	27	207.2±27.83
7-9	11	306.84±50.92
10-12	18	231.68±37.36
Total	70	227.08±18.66
F test	1.299	
P-value	0.282(NS)	

Parasitic infections not only stimulate the production of IgE antibodies to the parasite but also stimulate the nonspecific polyclonal IgE synthesis (Nagaraji *et al .*,2004). The significant increase in IgE antibody concentration in children with intestinal parasites compared to non-infected children may be due to the immune response and host defense mechanism against the parasite and its toxins, which may vary depending on the type of parasite that causes the infection (Mukai *et al .*, 2016). Many parasitic infections are defended by activating T2-assisted T-cells, leading to the production of IgE antibodies and activating the role of eosinophil cells (Amâncio *et al .*, 2012). Since the increase in IgE levels with its high receptor FcεRI on the surface of cells such as MC cells and Basophils and the

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activation that follows to these influential cells to produce and release biological mediators such as histamine and some other active amines that have an important role in the expulsion of the parasite, there is another mechanism in the expulsion of the parasite is an antibody-dependent cell-mediated cytotoxicity via IgE or IgG receptors (Mukai *et al* ., 2016).

As for the potential roles of IgE in host defense against toxins, tissue injury appears to be the main cause of immune responses to T2 cells (Kelly & Grayson , 2016 ; Mukai *et al* ., 2016). In mammals including humans, Th2 responses may lead to a general function in host defense, including as a protective mechanism against toxins and other harmful substances as well as identification of worm-acquired immunity and possibly other pathogens (Al-Hashemey , 2019 ; Al-Ibrahimi , 2019). Most toxins are a complex mixture of biologically active amines, peptides, and enzymes and often have toxic or neurotoxic activity. However, many toxins also contain compounds that cause tissue damage, so toxin-induced tissue damage can generate major risk signals. It is sensed by the immune system that initiates type 2 immunity and directs the development of IgE antibodies (Mukai *et al* ., 2016).

4- Conclusion

The immune response caused by parasitic infection is complex and multiple. In parasitic worms, especially *E.vermicularis*, antiparasitic stimuli stimulate an intense response of Th2 with the production of Interleukin-4 and Interleukin-5 leading to the synthesis of IgE immunoglobulin.

The results of this study conclude that the antibody level of IgE is higher in people with *E.vermicularis* parasite, and its height is related to age and not sex.

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