

Full Length Research Paper

Hematological response in human toxocariasis patients

Zubair Ahmad Dar^{1*}, Syed Tanveer, G. N. Yattoo², Bashir Ahmad Sofi³, Showkat Ahmad Wani³
and Perviz Ahmad Dar³

¹P. G. Department of Zoology, University of Kashmir Srinagar – 190006, J&K, India.

²Department of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar – 190011, J&K, India.

³Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar – 190011 J&K, India.

Accepted 10 June, 2013

The present study was carried out on the human population in Kashmir valley to study the status of hematological parameters of *Toxocara* infection. Blood samples were collected from 514 individuals; 298(57.97%) males and 216 (42.02%) females; 187 (36.38%) tested seropositive for human toxocariasis. In the present study, *Toxocara* infection and hemoglobin value were correlated. It was found that infected persons had less mean values of hemoglobin (10.23±1.5 g/dl) than uninfected individuals (10.51±1.5 g/dl). The mean erythrocyte value of infected and uninfected individuals was 4.82±0.39 × 10⁶/mm³ (4.0-6.2, 95% CI 4.76-4.88) and 4.84±0.44 × 10⁶/mm³ (3.4-6.7, 95% CI, 4.79-4.89) respectively. The mean total leukocyte count in *Toxocara* positive individuals was 8.73±1.45 × 10⁴ and in uninfected individuals was 7.70±1.23 × 10⁴, respectively. *Toxocara* infection considerably increased the total number of leukocytes. Individuals with *Toxocara* infection had higher values of eosinophil in their blood, 9.0±4.30% (range 1-23; 95% CI 8.36-9.63), where as the mean values of eosinophil in uninfected individuals was 3.22±2.47% (range 1-17.00; 95% CI 2.77-3.67).

Key words: Toxocariasis, humans, hematological study.

INTRODUCTION

Toxocariasis is a zoonotic disease caused by the ascarid of dogs and cats, the main representative of which is *Toxocara canis* (Glickman and Schantz, 1981). The eggs of *Toxocara canis* are unembryonated when passed out in the faeces of dogs into the environment. Under optimal temperature and humidity the eggs develop into embryonated eggs that are infective to both final and paratenic hosts. Infective eggs are reported to survive optimal circumstances for at least one year. Humans may acquire the infection by oral ingestion of infective *Toxocara* eggs from contaminated soil (sapro-zoonosis), unwashed hands or consumption of raw vegetables (Ahmad et al., 2002).

The disease manifests itself in three distinct forms; visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis. The signs and symptoms of VLM

vary from an asymptomatic state with mild eosinophilia to a severe and potentially fatal disorder including hepatomegaly, hyperglobulinemia, pneumonitis and neurological disorders (Gillespie, 1993). The disease has a chronic state and the symptoms can even persist for more than a year. Patients with OLM also show variable clinical signs varying from asymptomatic state to acute lesions including endophthalmitis accompanying loss of vision and mass similar to retinoblastoma (Mirdha and Kokhar.,2002; Shimizu et al., 2005; Fomda et al., 2007).

Distribution of the disease is world wide. There is no definitive method in diagnosing *Toxocara* infection. As the larvae of *T. canis* are arrested in the paratenic host-larvae during migration and do not mature into adults, a stool examination of the patient will not give any clue about the infection. However, numerous studies have

*Corresponding author. E-mail: zubair_mun@yahoo.co.in. Tel: 91-9469080593.

shown that immunoassay for detection of antibodies using a purified excretory-secretory antigen from the larval stage significantly improves sensitivity and specificity compared to assays using crude antigens (Fenoy et al., 1996; Kenny et al., 1995). The most widely used test, because of its high sensitivity and specificity is the enzyme linked immunosorbent assay (ELISA) in which antibodies to *T. canis* larval excretory-secretory antigens (De Savigny et al., 1979; Glickman and Schantz, 1981) or to larval extracts are measured (Fan and Suke, 2004; Glickman et al., 1985).

In India cases of human toxocariasis have been reported (Ahmad et al., 2002; Mirdha and Khokar, 2002) but there are limited studies from the Kashmir valley (Ahmad et al., 2002). The present study was conducted to determine the effect of *Toxocara* infection on hematological parameters in the human population of Kashmir valley.

MATERIALS AND METHODS

Strategically located, Jammu and Kashmir (J&K) State constitutes the Northern most extremity of India. J&K is situated between 32.17° and 36.58° North latitude and 37.26° and 80.30° East longitude. The projected population of the state is 76.77 lacs. The state with its summer and winter capital at Srinagar and Jammu respectively is divided into 14 districts. For this study, samples were randomly selected from six districts of Kashmir valley. Blood samples were collected from 514 individuals consisting of 298 males and 216 females.

Before taking a blood sample, each individual's consent was sought and obtained. Blood was collected by commercially available 5 ml disposable syringes. The blood samples collected were stored in two separate bottles; one with anticoagulant EDTA (ethylene diamine tetra acetic acid) and another without anticoagulant from which serum was later separated. The bottles with blood samples were simultaneously labeled to prevent intermixing after which the samples were transported to the laboratory for further investigation.

The samples were stored at -20°C until tested. ELISA was used for the qualitative screening of serum IgG antibodies to *Toxocara* infection. Commercially available ELISA kit which uses an inactivated purified specific excretory secretory antigen (*Toxocara* Micro well serum, ELISA, IVD, Research, Inc., Carlsbad, CA_92008) was used to carry out tests on microtitre plates for the detection of Anti-*Toxocara canis* antibodies IgG. The whole procedure was carried out according to the manufacturer's instructions. Optical density (OD) value was recorded in an automatic ELISA reader (Anthos) at 450 nm. The samples were considered positive if absorbance reading was equal to or greater than 0.3 OD units and negative if the absorbance value was less than 0.3 OD units.

Estimation of blood hemoglobin was done by Sahli's acid hematin method. Counting of the total leukocyte count is clinically significant when accompanied by a differential leukocyte count. Improved Neubauer chamber was used to obtain total leukocyte count. The differential leukocyte count calculates the relative proportion of 5 types of leukocytes. It is expressed as a percentage of each type of 100 leukocytes counted in a suitable area of the smear. Red blood cell count was done by improved Neubauer chamber. Simple Interactive Statistical Analysis (SISA) software was used for data analysis. The descriptive data were given as mean \pm standard deviation (SD). The differences were considered to be significant when the p-value obtained was less than 0.05.

RESULTS

Blood samples were collected from 514 individuals, 187(36.38%) tested seropositive for human toxocariasis. *Toxocara* infection and hemoglobin, erythrocyte, leukocyte and eosinophil values were correlated. It was found that infected persons had less mean values of hemoglobin (10.23 \pm 1.5 g/dl) than uninfected individuals (10.51 \pm 1.5 g/dl) ($P < 0.05$). The Hb value in infected persons ranged between 5-14 g/dl (95% CI 10.0-10.4) and 5.4-14.6 g/dl (95% CI 10.34-10.68) in uninfected persons. The mean erythrocyte values of infected and uninfected individuals were 4.82 \pm 0.39 $\times 10^6$ /mm³ (4.0-6.2, 95%CI 4.76-4.88) and 4.84 \pm 0.44 $\times 10^6$ /mm³ (3.4-6.7, 4.79-4.89), respectively.

The mean total leukocyte count in *Toxocara* positive individuals was 8.73 \pm 1.45 $\times 10^4$ and in uninfected individuals was 7.72 \pm 1.23 $\times 10^4$, respectively. *Toxocara* infection considerably increased the total number of leukocytes. Individuals with *Toxocara* infection had higher values of eosinophil (9.0 \pm 4.30%; range 1-23; 95% CI 8.36-9.63) in their blood, whereas the mean values of eosinophil in uninfected individuals was 3.22 \pm 2.47% (range 1-17.00; 95% CI 2.77-3.67) ($P < 0.05$). This shows a threefold increase in eosinophil count on infection with *Toxocara* as shown in Table 1.

DISCUSSION

In the present study, *Toxocara* infection and hemoglobin (Hb) value were correlated and it was observed that infected individuals had lower mean Hb values as compared to uninfected ones. The reasons for this difference are numerous. Due to poverty, people are already at risk of having low Hb value and when infected by *Toxocara* conditions get aggravated. The present results are supported by many other studies such as Rayes et al. (2001) who reported Hb value of <12.5 g/dl in 88% of toxocariasis patients studied; Sharma et al. (1984) while conducting experimental work on chickens infected with *T. canis* found a significant decrease in hemoglobin; Thakur et al. (1998) and Baldisserotto et al. (1999) found hemoglobin levels of below normal values in toxocariasis patients; Arango and Fla (1998) in visceral larva migrans case also found low Hb value. Similarly, in other studies like Singh et al. (1992) and Alonso *et al.* (2000) found hemoglobin level falls below the normal value in toxocariasis patients. From the above discussion it is clear that toxocariasis is associated with a condition leading to anemia.

Leukocytes play an important role against various types of infectious diseases by lessening their effect on the human body. During any type of infection in the human body, leukocytes increase in number so as to quickly fight against the infecting pathogenic organism. In the present study, it was found that *Toxocara* infected individuals had high numbers of leukocytes compared to uninfected

Table 1. Hematological parameters in *Toxocara* seropositive and seronegative individuals.

Parameter	Type	Mean \pm SD	Range	95% CI	p
Hemoglobin (g/dl)	+	10.23 \pm 1.5	5.0-14.0	10.0-10.4	0.040
	-	10.51 \pm 1.5	5.4-14.6	10.34-10.68	
Erythrocyte 10 ⁶ /mm ³	+	4.82 \pm 0.39	4.0 -6.2	4.76-4.88	0.62
	-	4.84 \pm 0.44	3.4 - 6.7	4.79-4.89	
Leukocyte 10 ⁴ /mm ³	+	8.73 \pm 1.45	4.50-1.35	8.73-8.83	0.00
	-	7.72 \pm 1.23	4.40-1.24	7.5-7.86	
Eosinophil %	+	9.0 \pm 4.30	1-23	8.36 -9.63	0.001
	-	3.22 \pm 2.47	1-17	2.77-3.67	

(+): Seropositive, (-): Seronegative.

individuals. Arango and Fla (1998) reported visceral larva migrans displaying a white blood cell count of 42,000 cells per mm³. The other studies that are in agreement with the present study include Baldisserotto et al. (1999); Singh et al. (1992); Ashwath et al. (2004); Vidal et al. (2003); Xinou et al. (2003); Sommerfelt et al. (2006) and Sharma et al. (1984). Yarsan et al. (2003) while conducting an experimental work on mice infected with *Toxocara* found significant increases in leukocyte counts occurring only after 8 days of *Toxocara* infection. In the present study eosinophils levels were found to be raised in individuals who were *Toxocara* seropositive and the difference was significant. These results are supported by other authors like Sommerfelt et al. (2001) who in an experimental work found that eosinophils were significantly higher in pigs inoculated with *Toxocara* eggs compared to control groups. Figueiredo et al. (2005) observed extremely significant association between seropositivity and eosinophilia. Tonz et al. (1983) found eosinophilia as an excessive and sustained symptom in 6 clinical observations. Giacometti et al. (2001) found all *Toxocara* seropositive individuals, with the exception of the subject in the control group, showed an increase above normal in the number of eosinophils per unit volume of peripheral blood. Alonso et al. (2000) found very high values of total eosinophilia in *Toxocara* seropositive children. Marmor et al. (1987) found that all cases of *Toxocara* infection had higher mean percentages of eosinophils than controls (2.6 \pm 4.3% in cases vs. 1.3 \pm 2.8% cells/mm³) in controls; mean difference = 1.3% and higher absolute number of eosinophils (211 \pm 36.2 cells/mm³ in cases vs. 121 \pm 290 cells/mm³ in controls; mean difference = 90 cells/mm³). Havasiava et al. (1993) found that clinical manifestations of *Toxocara* in the studied group of patients were highly variable. The most frequent were leukocytosis and eosinophilia (46%). Berrocal (1980) reported that in many cases of toxocariasis eosinophilia was predominantly high and acted as a diagnostic feature in these cases. Hayashi et

al. (2005) reported that 24 of the 34 subjects (70.6%) had hypereosinophilia with five of these showing extreme hypereosinophilia. Santos et al. (2004) found that individuals with higher eosinophil counts presented a greater frequency of anti-*Toxocara* antibodies and the relation between eosinophilia and *Toxocara* infection was found to be statistically significant. Sommerfelt et al. (2001) reported a significant relation between eosinophil count and groups inoculated by *T. canis* as compared to control. Various other studies that report a correlation between eosinophil count and *Toxocara* infection include Ashwath et al. (2004), Arango and Fla (1998), Yarsan et al. (2003), Xinou et al. (2003), Vidal et al. (2003), Sharma et al. (1984), Sugane and Oshuma (1984), Inan et al. (2006), Alonso et al. (2000), Yokoi et al. (2003), Shimizu et al. (2005), Thakur et al. (1998), Singh et al. (1992), Baldisserotto et al. (1999), Azuma et al. (2002) and Taranto et al. (2003). Thus from the present study and the above discussion it is concluded that while going for the *Toxocara* serological examination in humans, patients should be advised for the eosinophil count so as to get the proper diagnosis of the disease easier.

In the present study, the effect of *Toxocara* infection on the total erythrocyte count showed no significant difference between infected and uninfected individuals. Similar results have been reported by other workers such as Sommerfelt et al. (2001) who found that in *T. canis* infected pigs there were no significant changes in RBC count. Similarly various other studies supporting the present observation include Xinou et al. (2003), Inan et al. (2006), Ashwath et al. (2004) and Alonso et al. (2000). Therefore it was found that in the case of *Toxocara* infection in humans there is no effect on RBC count.

ACKNOWLEDGEMENTS

The authors are indebted to all the study population for their unfailed cooperation.

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