Alkaline Phosphatase in Serum is a Marker of Human Toxocariasis

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Abstract
Background: The present study was carried out on the human population of Kashmir valley to evaluate the status of the biochemical parameters of infected population.

Materials and Methods: Blood samples were collected from 514 individuals, 298 (57.97%) males and 216 (42.02%) females, 187 (36.38%) were found sero-positive for human toxocariasis.

Results: Alkaline phosphatase level was found higher in infected children and adults than in uninfected population. Serum bilirubin concentration was not affected by Toxocara infection. The mean value of serum creatinine in uninfected persons was similar to that of infected persons. There was no effect of Toxocara infection on blood urea level in infected persons. The mean value of blood urea in uninfected persons and infected persons was normal. The blood glucose level was not affected by Toxocara infection.

Conclusion: From above results alkaline phosphatase level was only found to be affected in Toxocara infection.

Keywords: alkaline phosphatase, serum, biological markers, toxocariasis

Introduction
The larvae of Toxocara canis have been reported in different organs like intestine, liver, lungs, kidneys, skeletal muscles, and nervous tissue of rat and mice. The prevalence of the infection throughout the world shows different infection rates of Toxocara canis in dogs; it is 18.3% in Italy, 25.7% in France and 18.3% in Indiana. The life cycles of Toxocara canis and Toxocara cati are complex. Adult worms in the intestinal tract of infected dogs and cats shed large number of eggs. These eggs find their way into the environment, where their hosts defecate. Once into the field, these may be ingested by natural hosts as well as paratenic host. The larvae then hatch and migrate through blood vessels all over the body and are referred as visceral larva migrans (VLM). After predation of Toxocara infected paratenic hosts by dogs or cats, larvae are released and develop into adult worms in the intestinal tract. After their complete development, they start shedding a large number of eggs along with their faeces. In the pregnant bitch and queen, dormant tissue larvae are reactivated and migrate across the placenta to infect the fetus. New born puppies and kittens also acquire infection through ingestion of larvae in milk. Toxocariasis is a public health problem. Humans act as an unnatural host in whom Toxocara larvae will not develop but migrate and survive for a long time. The mode of transmission to humans is by oral ingestion of infective Toxocara eggs from contaminated soil (sapro-zoonoses), unwashed hands, or consumption of raw vegetables. While most people infected by Toxocara canis do not develop covert clinical disease. Three clinical syndromes have been associated with Toxocara infection in humans; visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis.
humans, ingested Toxocara canis larvae penetrate the intestinal wall and disperse to other organs via lymphatic and venous pathways without undergoing further development. In Kashmir valley little work has been conducted on toxocariasis. So keeping in view the aforesaid comments, the present study was taken into consideration to know the effect of Toxocara infection on the blood biochemical parameters of humans.

Materials and Methods
Strategically located Jammu and Kashmir State constitutes the northern most extremity of India. It is situated between 32.17 degree and 36.58 degree north latitude and 37.26 degree and 80.30 degree 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 during years 2005 and 2006. Blood sample was taken from each individual to be studied. Before taking a sample each individual was made mentally prepared for it and only after obtaining consent, blood was taken. Blood was collected by commercially available 5 ml disposable syringes. The collected blood was stored in two separate bottles, one with anticoagulant EDTA (ethylene diamine tetra acetic acid) and another without anticoagulant from which later serum was separated. The bottles with blood samples were simultaneously labeled to prevent intermixing. Then the samples were transported to the laboratory for further investigation. The samples were stored at –20 0C before test. Antibody (IgG) specific to Toxocara purified excretory secretory (ES) antigen was detected by ELISA in all serum samples using kit obtained from IVD research Inc. Carlsbad, CA 92008. The test was performed according to the manufacturer 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 absorbance reading was less than 0.3 OD units. Fisher’s exact test was used for statistical analysis. Various types of biochemical parameters examined in the present study are blood urea, blood glucose, serum bilirubin, serum creatinine, and alkaline phoshatase. END POINT DAM method was employed for the quantitative determination of urea in serum/plasma. End point O-TOLUIDINE method was employed for the quantitative determination of glucose in blood. The total serum bilirubin was estimated by the method described by Jendrassik and Graf’s (1938). Estimation of serum creatinine was done by alkaline picrate method. Serum alkaline phosphatase activity was done by Kind and King’s method.

Results
The present study was carried out on the human population of Kashmir valley to study the status of the biochemical parameters of infected human population. Blood samples were collected from 514 individuals, 298 (57.97%) males and 216 (42.02%) females, 187 (36.38%) were found seropositive for human toxocariasis. Various biochemical parameters were examined.

The alkaline phosphatase level was found to be higher in infected children (622.84±168.85 U/L) and adults (510.06±185.69 U/L) than in uninfected children (389.37±152.15 U/L) and adults (308.79±138.96 U/L) (Table 1) (Normal values: Children 300-650, Adults 70-320). The serum bilirubin concentration was not affected by

<table>
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<th>Type</th>
<th>Mean±SD</th>
<th>Range</th>
<th>95% CI</th>
<th>P-value</th>
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<td>Infected children</td>
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<td>96-940</td>
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<tr>
<td>Uninfected adults</td>
<td>308.79±138.96</td>
<td>80-656</td>
<td>285.13-332.44</td>
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Toxocara infection. The mean value of serum bilirubin in uninfected persons was 1.0±0.50 mg/dl and in infected persons was 1.0±0.40 mg/dl (Normal value <1.5 mg/dl) (Table 2). The mean value of serum creatinine in uninfected persons was 0.83±0.34 mg/dl and in infected persons was 0.89±0.32 mg/dl, as shown in Table 2, (Normal value 0.8-1.5 mg/dl). There was no effect of Toxocara infection on blood urea level in infected persons. The mean value of blood urea in uninfected persons was 34.66±8.27 mg/dl and in infected persons was 33.85±8.08 mg/dl (Table 2) (Normal value 10-40 mg/dl). The blood glucose level was not affected by Toxocara infection. The mean value of blood glucose in uninfected persons was 103.73±18.51 mg/dl and in infected persons was 105.80±18.95 mg/dl (Normal value 80-120 mg/dl) (Table 2).

Discussion

In the current study, various blood biochemical parameters were studied to evaluate the effect of Toxocara infection. Most of the blood biochemical parameters were found unaffected by the Toxocara infection except alkaline phosphatase which was significantly raised. The serum creatinine levels were found to be in the normal range in Toxocara infected individuals. Similar results have been reported by Singh et al. who observed the level of serum creatinine in normal range in patients with visceral larval migrans. The other studies that are in agreement with the present study are Inan et al. Eberhard and Alfano; Vidal et al. and Xinou et al. Thus it was concluded that serum creatinine was not one of the Toxocara infection determining factors.

The Toxocara infection had not any effect on blood glucose level and there was no significant difference between the infected and uninfected persons. This observation is supported by other studies. Vidal et al. and Xinou et al. found no change in blood glucose level in Toxocara infected patients. Inan et al. and Singh et al. observed that the level of blood glucose was in the normal range in adults with visceral larva migrans. Several other studies are in agreement with the present work. Hence, there is no effect of Toxocara infection on blood glucose.

Serum bilirubin is a byproduct of bile juice secreted by liver. This blood biochemical parameter acts as an indicator for various types of hepatic infections. In our study, individuals infected with Toxocara infection were found to have no significant change in their serum bilirubin level compared to uninfected individuals. This indicates that serum bilirubin level does not get altered by Toxocara infection. Our study is supported by Inan et al. who observed no change in serum bilirubin level in a case of visceral larva migrans, similarly Sommerfelt et al. observed no significant change in serum bilirubin in Toxocara infected pigs compared to uninfected pigs. Some other studies are in agreement with the present observation. Thus from the present findings and literature it was concluded that in case of Toxocara infection the serum bilirubin level remains in the normal range.

Individuals infected with Toxocara showed no significant change in their blood urea. The present study results are supported by Inan et al. who migrans observed no change in the level of blood urea by Toxocara infection in a case with visceral...
larva. Similarly Singh et al.\textsuperscript{11} observed no significant change in blood urea level by Toxocara infection in a case with visceral larva migrans. Other studies are also in agreement with this study.\textsuperscript{14,15,18} Thus it could be concluded that Toxocara infection does not have an effect on blood urea level in humans.

People who were positive for Toxocara infection had higher mean alkaline phosphatase levels compared to uninfected individuals and the difference was statistically significant. These results are in agreement with a study by Azuma et al.\textsuperscript{19} who reported a case of hepatic involvement of visceral larva migrans due to Toxocara canis and found that the patient had high level of alkaline phosphatase. So, it can be interpreted from the present study that at the time of screening patients for Toxocara infection, alkaline phosphatase level should also be determined.

References