

Contested role of procalcitonin and C-reactive protein in the diagnosis of fascioliasis

Original
Article

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ABSTRACT

Background: Procalcitonin (PCT) and C-reactive protein (CRP) are acute phase reactants indicating inflammation, particularly in bacterial infections. High PCT or CRP levels were proposed in malaria, invasive amoebiasis, babesiosis and schistosomiasis. Since liver is the sole habitat, it was hypothesized that PCT and CRP may have a role in the diagnosis of fascioliasis.

Objective: The study aimed to investigate whether PCT and CRP have a role in the diagnosis of fascioliasis.

Subjects and Methods: Fifty fascioliasis patients and 50 healthy blood donors in the same age range, as the control group, were included in the study. Fascioliasis was diagnosed by anti-*Fasciola* IgG ELISA, in addition, direct stool examinations (fresh smear and after sedimentation) were performed in both groups. Serum PCT and CRP levels were determined by I-chroma kits on I- CHROMA™ II device, and NFL BN-II immunonephelometry system, respectively.

Results: There was no statistically significant difference of PCT and CRP values between the fascioliasis patient group and the control group ($P > 0.05$).

Conclusion: It was concluded that PCT and CRP were not relevant parameters for diagnosis of fascioliasis.

Keywords: diagnosis, fascioliasis, procalcitonin, C-reactive protein.

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INTRODUCTION

Fascioliasis is a zoonotic disease caused by two species of liver trematodes, *Fasciola hepatica* and *Fasciola gigantica*^[1]. The World Health Organization (WHO) included fascioliasis in the list of NTDs (Neglected Tropical Diseases) in the foodborne trematodiasis group^[2,3]. According to the WHO, the number of people afflicted by this disease is around 17 million annually^[4]. Fever, hepatomegaly, abdominal pain, weight loss, anemia and eosinophilia are seen in the acute phase of the disease while biliary colic, cholestasis, cholangitis, jaundice and even cirrhosis may occur in untreated chronic cases^[5]. It was reported that the disease can cause permanent neurological and ophthalmological sequelae and even death in infected individuals^[6].

Fascioliasis is frequently overlooked particularly in non-endemic regions because the disease is rare and has nonspecific symptoms. Since the sensitivity of current laboratory methods varies according to the stage of the disease, it becomes difficult to diagnose^[7]. Diagnosis of fascioliasis is routinely made by detecting the eggs of the parasite in the stool using the sedimentation method. In addition, Ultrasonography (USG) and computed tomography (CT) are radiological supporting diagnostic methods. However, in the early stages of the disease, the parasites are not yet mature, so eggs cannot be detected in the stool and radiological findings are insufficient^[8]. Thus, serological tests were introduced for the diagnosis of fascioliasis. Their high specificity and sensitivity of positivity long before egg

detection in the stool samples increase the importance of serological methods. However, serological methods are also inadequate for distinguishing acute from chronic stages of infection^[9]. Increased levels of immunoglobulin E (IgE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and hypereosinophilia were observed in patients with fascioliasis. However, these laboratory findings are not specific and often ignored. Therefore, new biomarkers that can detect the infection in the acute phase are needed^[9].

Accordingly, PCT, the precursor of calcitonin and one of the hormones that regulate calcium homeostasis was considered. This peptide precursor is synthesized by different cells in response to pro-inflammatory stimuli^[10]. Its plasma levels are undetectable (<0.05 ng/ml) in healthy individuals, but increase in severe viral, bacterial, parasitic, and fungal infections, sepsis, and non-infectious inflammatory diseases such as autoimmune diseases and pancreatitis^[11-15]. Additionally, CRP is a polymeric acute phase protein synthesized in the liver^[16]. It is found at very low levels in the serum under normal conditions but rises in infection, inflammation, malignancy and autoimmune diseases^[11]. Notably, PCT and CRP levels are elevated mainly in bacterial and in some viral infections. Although they are not used in the diagnosis of parasitic infections, it was reported that PCT or CRP levels were high, especially in invasive parasitic infections involving the liver^[16-21]. It was suspected that PCT and CRP levels would increase in the pathogenesis of fascioliasis, which mainly involves

the liver and biliary tract. The aim of our study was to investigate if *Fasciola* worms that settle in the liver may affect PCT and CRP values as nonspecific markers.

SUBJECTS AND METHODS

This case-control study was carried out in the Microbiology laboratory of Dicle University Hospital, a tertiary level hospital in Diyarbakır, between September 2021 and February 2022.

Study population: A total number of 50 patients (15 males and 35 females) diagnosed with fascioliasis in various polyclinics of Dicle University Hospital. The diagnosis of fascioliasis was made by anti-*Fasciola* IgG ELISA (DRG International Inc., USA) and stool analysis (fresh smear and after sedimentation) in patients with clinical findings. A total of 30 males, and 20 females apparently healthy blood donors of both sexes and same age range, were included as the control group. The age range of patient group was 18-88 and of the control group was 18-62 years. Antibody testing and stool analysis were also performed in the control group to exclude fascioliasis.

Samples collection: Approximately 5 ml venous blood samples were withdrawn from each individual of both groups. Blood samples were allowed to clot and centrifuged for 10 minutes to obtain serum. Collected serum samples were stored at -80°C and thawed at room temperature on the day of analysis. Besides, stool samples were collected from each individual of both groups for three consecutive days. None of the subjects had a history of eating animal liver for 3 days before sampling.

Stool examination: Native-Lugol and sedimentation (in formalin-ether solution) methods were used to detect eggs of *Fasciola* spp.^[22].

***Fasciola* IgG antibody ELISA test:** It was performed and evaluated according to manufacturer instructions^[23]. The cut-off value of the *Fasciola* IgG antibody ELISA test was set to 10 DRG Units (DU); values of >11 DU were considered positive, <9 DU as negative and 9-11 as grey zone.

Parameters assessment: The PCT values were studied on the I-CHROMA™ II device (Boditech Med Inc., Korea) using I-Chroma kits by immunofluorescence measurement technique with a reference range of 0-0.05 ng/ml^[24]. CRP values were measured by the NFL BN-II immunonephelometry system (Siemens, Dade Behring, Germany), and values of < 6 mg/L were considered normal^[25].

Statistical analysis: The SPSS 12 (SPSS Inc., Chicago, IL) package software was used for data analysis. The PCT and CRP values obtained from the two groups

were statistically compared by Person chi-square and McNemar tests. Significance was considered when *P* values were < 0.05.

Ethical consideration: The current study was approved by Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (21.09.2021/430). Informed consent forms were obtained from patients and the control group to participate in the investigation and withdrawal of blood samples.

RESULTS

Among the patient and the control groups, the rate of men to women was determined as 15/35 and 30/20 respectively. The relationship between fascioliasis and gender was statistically significant (*P*=0.022). The mean age of patient group was 41.5±16.6, whereas the mean age of control group was 36.26±12.32 old. There was no statistical significant difference between the two groups in terms of age (*P*=0.134).

The mean value of anti-*Fasciola* IgG antibodies of patient group was 24.62±0.99 (range: 11-47) DRG Units (DU). Of the control group, the mean value of anti-*Fasciola* IgG antibodies was 2.61±0.41 (range: 0.5-8). The difference in anti-*Fasciola* IgG antibody levels between the patient and control groups were statistically significant (*P*<0,001) (Figure 1). Anti-*Fasciola* IgG antibodies were negative in the control group, also *Fasciola* spp. eggs were not detected in stool analysis. *Fasciola* antibodies were positive in the entire patient group, while only 8 patients had *Fasciola* eggs in the stool.

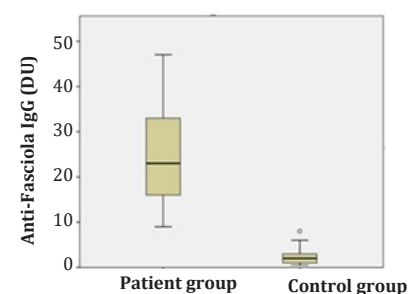


Fig. 1. Box plot of Anti-*Fasciola* IgG values by groups

A total of 43 patients (86%) had a history of eating watercress. The recorded symptoms were abdominal pain, fever, nausea, weight loss and urticaria. Hyper-eosinophilia was observed in 34 (68%) of the patients. The USG and CT reports were suggestive of fascioliasis in all patients including fluke worms in the bile duct and canaliculi, hepatomegaly, abscess-like lesions and heterogeneous liver with hypodense nodular lesions. The differences in PCT and CRP levels between the patient and control groups were statistically insignificant (*P*> 0.05) (Table 1).

Table 1. Comparison of fascioliasis patients and control group in terms of age, gender, CRP and PCT values.

	Fascioliasis	Control	P value
Age	41.5±16.6	36.26±2.32	0.134
Gender	Male (n)	15	0.022*
	Female (n)	35	
CRP	2.16 ± 1.07	1.12 ± 0.89	0.250
Anti-Fasciola IgG	24.62±0.99	2.61±0.41	<0.001*
PCT	0.28 ± 0.23	0.24±0.21	0.524

DISCUSSION

Various diagnostic methods such as direct stool examination, serological and radiological imaging are used in fascioliasis diagnosis. Concentration methods of three successive stool samples plus serological methods using specific *Fasciola* antigens are conclusive. However, the difficulty of the process, the intermittent excretion of parasite eggs, and the need for examination by experienced eyes are the handicaps of the concentration method. The absence of *Fasciola* antibodies in the early stages of the disease has led to the search for additional diagnostic methods^[26,27]. Therefore, additional biomarkers that can be used in the diagnosis of fascioliasis are required.

In the current study, regarding the prevalence of parasites by gender, fascioliasis was found to be higher in women. While there was no difference in the prevalence of parasites by gender in developed countries, it was reportedly more common in women in countries where the parasite was hyperendemic, such as Egypt and Bolivia^[4,28-31]. Dicle University Hospital is a well-established hospital serving patients from other provinces in the southeast of Turkey. Due to the frequent consumption of wild plants by women and traditional food culture, infection is more common in women in our region^[32]. Previous studies reported that fascioliasis was most common between the ages of 5-15 years in hyperendemic regions^[2,27,28]. No statistical correlation was found between age and fascioliasis in the current study ($P > 0.05$).

Elevated PCT and CRP levels were reported to be associated with bacterial infections as well as various invasive infections such as malaria, invasive amebiasis, babesiosis and schistosomiasis^[14,15-21]. In two different studies investigating malaria and PCT levels, a significant correlation was found between elevated PCT levels and parasitemia in the first stage of malaria^[14,33].

The level of PCT was even found to be more helpful than IL-6 in the diagnosis and monitoring of treatment in a study conducted by Carannante *et al.*^[33] on hospitalized children with malaria. In the same study, a strong correlation was found between the number of parasites and PCT levels^[33]. In another

study, it was stated that the level of CRP was high in malaria, but CRP alone was not sufficient for diagnosis, and it must be confirmed by other diagnostic tests such as microscopy and serological tests^[34]. In a study conducted by Chimponda *et al.*^[16] the CRP level was shown to increase significantly in schistosomiasis. In a study involving *S. japonicum* infected patients aged 7-20 years in the Philippines, Coutinho *et al.*^[19] reported that the severity of the disease was correlated with the elevation of CRP. In another study conducted with patients aged 7-12 years infected with *S. haematobium*, high CRP was found to be correlated with the disease and it was stated that it could be useful for schistosomiasis screening in children with anemia^[20].

In the present study, no statistically significant difference was observed in PCT and CRP values in the fascioliasis patient group and control group. Al Ezzy *et al.*^[21] reported that CRP level was increased in chronic diarrhea caused by *E. histolytica* and *G. lamblia*, therefore it can be used as a non-specific immunological marker in the clinical follow-up of these two parasites.

In conclusion, the current study revealed that PCT and CRP values did not increase in fascioliasis, and these two parameters were not appropriate for the early diagnosis of fascioliasis. Further studies are recommended to reveal with more precise data how PCT and CRP values were affected in invasive helminth infections.

Author contribution: Çicek, M proposed the study design, analyzed the results. Bilden, A analyzed and interpreted the study results. Çakir, F drafted, and revised the article. Özcan, N interpreted the data, drafted and revised the article. All authors revised and approved final draft.

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