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Plasma Concentration of Platelet Factor 4: As an Evidence of Platelet Activation in Parasitic Infections

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ABSTRACT

Platelets number in the circulation largely exceeds that needed for haemostasis. There is increasing evidences that platelets have an immunological role against parasites. Assessment of platelet factor 4 concentrations in patients with parasitic infections can be used as an indicator for platelet activation. This study aims to evaluate the *in vivo* platelet activation in parasitic infections through measuring the plasma level of platelet factor 4 in a protozoal and a helminthic infection both before and after treatment. Thus 30 patients, 22 diagnosed to have giardiasis and 8 diagnosed to have hydrated disease, were subjected to serum samples collection before (Ag1 and Ah1) and after treatment (Ag2 and Ah2), respectively. The study also included 20 healthy adult as a control group. Both platelet counts and plasma levels of PF4 were measured. Platelet counts in both giardiasis and hydatid patients were significantly elevated after treatment compared to their counts before treatment. Plasma level of PF4 was reduced with a statistically significant difference in both diseases after treatment. Also there was a statistically significant difference between the mean values of PF4 of the control group (C-PF4) and in the tested groups of both diseases before and after treatment ($p<0.5$). Thus parasitic infections lead to platelets activation with increase in platelet count although within normal range for platelets. Plasma level of platelet factor 4 is significantly increased in both infections and decreased after treatment, thus can be used as an indicator for parasitic infection and for prediction of succession of recovery after treatment.

Key words: Platelets, platelet factor 4, hydatid disease, giardiasis, parasitic infections

INTRODUCTION

Human internal environment can be evaluated by many means of which haematological indices are of utmost importance (Uboh *et al*., 2012). Each of blood components has other functions in addition to its main known function. Platelets are partially responsible for haemostasis and for maintenance of vascular system. Normal platelet count is about 150.000 to 350.000 platelets μL^{-1}, of which only (<10.000 platelets μL^{-1}) can prevent bleeding normally, although a bigger of platelets may be consumed in surgeries and in severe injuries (Clemeston, 2010). Also the primitive cells, haemocytes from which platelets are evolved, are said to have a protective role preventing infection in addition to their role in haemostasis (Saeed *et al*., 2004).

There are increasing evidences that platelets have an important role in the body's defence against different pathogens e.g., antiparasitic immunity. This immunological role is mainly due to the receptors they carry and their storage granules that have a wide variety of immunologically active substances (Blockmans *et al*., 1995; Weyrich and Zimmerman, 2004).

Many factors as direct contact with parasite, increase IgG and/or IgE concentration, the presence of complement components, CRP or lymphokines may cause stimulation of platelets. This results in cytotoxic activity of the stimulated platelets via release of various inflammatory mediators, phagocytic activity and cooperation with other cells of immunologic system (Matowicka-Karna, 2006).

During platelet aggregation, alpha-granules of activated platelets release a small cytokine that belongs to the CXC chemokine family which is called Platelet Factor 4 (PF4). The major physiologic role of PF4 is to neutralize the heparin-like molecules on the endothelial surface of blood vessels, resulting in inhibition of local antithrombin III activity thus promoting coagulation (Maurer *et al*., 2007; Matowicka-Karna *et al*., 2006).

PF4 also has a chemotactic effect on neutrophils, fibroblasts and monocytes. In addition it interacts with CXCR3B which is a splice variant of the chemokine receptor (Elzey *et al*., 2003).

Such evidenced roles suggest an important role of platelets in wound repair and inflammation (Bikfalvi and Gimenez-Gallego, 2004; Elshamaa *et al*., 2007).

Parasitic infections may cause platelets activation within few seconds with consequent change in their discoidal shape into irregular, resulting in lesions in the actin-containing microfilaments. Elevated concentration of platelet factor 4 could be used as an indicator for platelet activation (Matowicka-Karna and Kemona, 2001).

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Hydatid disease is a helminthic zoonotic infection prevalent in many parts of the world especially in rural communities, where *E. granulosus* larvae dwell the viscera of the man (intermediate host) (Kojouri and Moshtaghi, 2008).

Giardiasis is a global disease. It infects nearly 33% of people in developing countries, passed person-to-person or even animal-to-person and inhabiting the small intestine causing diarrhoea (Robertson *et al*., 2010).

This research aimed to study the *in vivo* platelet activation in parasitic infections. PF4 was the indicator used to reflect platelet activation. Thus plasma concentration of PF4 was evaluated in *E. granulosus* and *Giardia lamblia* infections, as examples for parasitic infections, both before and after treatment.

MATERIALS AND METHODS

The present study was done over the period from January 2011 to January 2012. The study included 30 patients (16 males and 14 females), aged (18-54), 22 had giardiasis and 8 had hydatid disease, retrieved from the outpatient clinic and department of tropical medicine, Cairo University.

The patients with hydatid disease were diagnosed by abdominal ultrasound together with indirect haemagglutination. After sample collection from these patients (Ah1), PAIR technique (puncture, aspiration, injection and re-aspiration) was done combined with albendazole.

On the other hand, giardiasis was diagnosed by stool examination where *Giardia* cysts and/or trophozoites were detected in stool. After sample collection from these patients (Ag1), metronidazole was given.

Other samples were collected from hydatid patients (Ah2) and from giardiasis patients (Ag2) after treatment. The study also included 20 healthy adult volunteers aged (18-50) who had not received any medication for the last 7 days as a control group. Both groups had given an informed consent.

Plasma sample preparation: Two drops of each of the collected blood samples were used for making blood films for platelet count, then rest of samples were anticoagulated by adding to 3 mg mL^{-1} of ethylenediamine tetraacetic acid dipotassium salt $2H_2O$ (EDTA), mixed with 10% vol. of Citrate-theophylline-adenosine-dipyridamole (CTAD) (Kucukbayrak *et al*., 2010). The anti-coagulated samples were centrifuged at 2500 g for 30 min to separate the plasma.

Levels of PF4 in the plasma samples were determined in accordance with manufacturer's instructions by an enzyme immunoassay using ZYMUTEST PF4 (Hyphen Biomed. "Eragny 95000 Neuvile Sur-Oise-France" RK0064). The measuring limits for PF4 1-100 ng mL^{-1}; assay CVs were within 15%. When concentrations exceeded the upper measuring limits, samples were diluted with supplied diluents.

Statistics: Data were statistically described in terms of range, Mean±Standard Deviation (±SD), median, frequencies (number of cases) and percentages when appropriate. A probability value (p-value) less than 0.05 was considered statistically significant. Comparison of quantitative variables between the study groups was done using Kruskal-Wallis analysis of variance (ANOVA) test with Mann Whitney U test for independent samples as *post hoc* multiple 2-group comparisons. Within group, comparison of quantitative variables was done using paired t-test in comparing 2 groups when normally distributed and Wilcoxon signed rank test for paired (matched) samples when not normally distributed. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non normal variables. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Prior to antiparasitic therapy, the mean count of blood platelets in the hydatid patients (Ah1-P) was 161.25+40.9 μL^{-3} and in giardiasis patients (Ag1-P) was 191.01+52.7 $\times 10^{-3}$ μL^{-1}. Following treatment their number increased to 184.5+44.3 μL^{-3} (Ah2-P) and 202.27+49.2$\times 10^{-3}$ μL^{-1} (Ag2-P) in hydatid and giardiasis patients, respectively (Table 1). The difference between the mean values in the groups (Ah1-P) and (Ah2-P) was statistically significant ($p<0.05$). The same was noticed in giardiasis patients where there was significant difference between (Ag1-P) and (Ag2-P), ($p<0.05$).

In the group of healthy individuals (C-P) the mean platelet count was 342.0+57.7$\times 10^{-3}$ μL^{-1} (Table 1).

The mean concentration of Platelet Factor 4 (PF4) was 59.8+3.7 ng mL^{-1} in the hydatid patients prior to therapy (Ah1-PF4), being reduced to 22.56+0.9 ng mL^{-1} after treatment (Ah2-PF4). The difference between these values was statistically significant ($p<0.5$) (Table 2).

Table 1: Platelet count in patients with hydatid disease and with giardiasis, before and after treatment and in control group

Parameter	Platelet count/$\times 10^{-3}$ μL^{-1}	p-value	r
Ah1-P	161.25±40.9	<0.05*	0.984**
Ah2-P	184.5±44.30		
Ag1-P	191.01±52.7	<0.05*	0.989**
Ag2-P	202.27±49.2		
C-P	342.0±57.70		
Ah1-P:C-P, Ah2-P:C-P		<0.05*	
Ag1-P:C-P, Ag2-P:C-P		<0.05*	

Mean values are Mean±SD, *Significant p-value, **Strong positive correlation

Table 2: Platelet factor 4 in patients with hydatid disease and with giardiasis, before and after treatment and in control group

Parameter	Platelet factor 4 (ng mL^{-1})	p-value	r
Ah1-PF4	59.8±3.70	<0.05*	-0.158**
Ah2-PF4	22.56±0.9		
Ag1-PF4	53.08±3.2	<0.05*	-0.077**
Ag2-PF4	21.11±1.1		
C-PF4	17.07±2.8		
Ah1-PF4:C-PF4, Ah2-PF4:C-PF4		<0.05*	
Ag1-PF4:C-PF4, Ag2-PF4:C-PF4		<0.05*	

Mean values are Mean±SD, *Significant p-value, **Weak correlation

Regarding giardiasis patients mean concentration of Platelet Factor 4 (PF4) was 53.08+3.2 ng mL^{-1} prior to therapy (Ag1-PF4) and decreased to 21.11+1.1 ng mL^{-1} after treatment (Ag2-PF4) and the difference between these values was also statistically significant ($p<0.5$) (Table 2). In the control group (C) the mean (PF4) concentration (C-PF4) was 17.07+2.8 ng mL^{-1}. The difference between the mean values obtained in the control group (C-PF4) and in the tested groups (Ah1-PF4 and Ah2-PF4), also the difference between (C-PF4) and (Ag1-PF4 and Ag2-PF4) were statistically significant ($p<0.5$).

Thus the net conclusion of the present results was that the mean count of blood platelets in the hydatid patients and in giardiasis was found to be statistically elevated after antiparasitic treatment compared to its mean count prior to treatment. Also it was found that plasma level of Platelet factor 4 (PF4) was elevated in these patients and was statistically decreased after therapy.

DISCUSSION

Platelets help to fight parasitic infections, by means that clearly exceed an exclusive function as mere players in the primary physiological process. They are capable of killing parasites either with or independent of leukocytes (Elzey *et al*., 2003). This has strengthened the view of platelets as participants in host immune defense (Flad and Brandt, 2010).

Recent analysis of the secreted platelet proteome, upon platelet activation, has detected numerous chemokines including ligand 4 (CXCL4) known as Platelet factor 4 which has an important role in the phased arrival of lymphocytes and other cells of the immune system immune at site of infection (Xia and Kao, 2003).

Thus it was a reasonable objective to evaluate the platelet count and the plasma concentration of platelet factor 4 (PF4) as practical indicators of platelet activation and secretory activity in hydatid disease patients and in giardiasis both before and after treatment. Such parameters may be used as follow up markers in parasitic diseases.

In the present study blood platelet counts were significantly elevated after treatment in the studied parasitic diseases compared to levels in both diseases prior to therapy. In both hydatid disease and giardiasis, platelet counts were low normal being 161.25+40.9×10-3 μL^{-1} in (Ah1-P) and 191.01+52.7×10-3 μL^{-1} in (Ag1-P) although still within normal range for platelet count. The platelet count levels were significantly elevated after treatment to 184.5+44.3 (Ah2-P) and 202.27+49.2 (Ag2-P).

In a research done by Matowicka-Karna *et al.* (1996) on 35 patients infected *G. intestinalis*, a decrease of blood platelets count and an increase the phagocytic activity of platelets were recorded in these patients. The researchers concluded that *Giardia* is able to stimulate the phagocytic activity of blood platelets resulting in thrombocytopenia (Matowicka-Karna *et al.*, 1996).

Another study was in accordance to the results of the present study where the researchers recorded a considerable decrease in the number of platelets with the course of parasitic infections (hydatid disease and giardiasis). The mean platelet count was 134.2+63.9×10-3 μL^{-1} before treatment and was elevated to 165.3+45.3×10-3 μL^{-1} after treatment. They referred the reduction of platelet count prior to therapy to the consumption of platelets as a result of platelet activation in the course of parasitic disease. They also stated that thrombocytopenia may also be due to the cytotoxic effect of parasites on megakaryocytes (Matowicka-Karna and Kemona, 2001).

However, (Kucukbayrak *et al.*, 2010) reported in their research that platelet count didn't show significant difference in preoperative and postoperative states in patients with pulmonary hydatid cysts being 320.48+98.42×10-3 μL^{-1} and 307.29+96.45×10-3 μL^{-1}, respectively. However, they recorded a significant difference in mean platelet volume, being significantly higher in preoperative states compared to postoperative ones.

The increase in platelet volume may be due to peripheral platelet destruction with early release of large immature platelets from the bone marrow (Heldin and Westermark, 1999).

Following antiparasitic treatment, the elimination of parasitic cytotoxic effect on thrombopoiesis leads to stimulation of bone marrow megakaryocytes with elevated platelet count. This was also observed in other parasitic infections as schistosomiasis.

The authors referred thrombocytopenia to platelet interaction with circulating immune complexes (Blockmans *et al.*, 1995; El-Bassioni *et al.*, 1997).

Affected platelet count with other parasitic infections was reported also by Jeremiah and Uko (2007) who recorded that thrombocytopenia is not only a feature of acute malaria infection but also that of asymptomatic malaria infection in the tropics and might be a useful indicator of malaria in children. They noticed also that an inverse relationship was established between parasite density and platelet count.

Regarding PF4 there was a significant increase in PF4 in parasitic infections prior to therapy; being (Ah1-PF4: 59.8+3.7) and (Ag1-PF4: 53.08+3.2) compared to both post-treatment states (Ah2-PF4: 22.56+0.9), (Ag2-PF4: 21.11+1.1), respectively and also compared to control group, (C-PF4: 17.07+2.8). This reduction of PF4 after treatment may be due to elimination of the cytotoxic agents secreted by parasites.

This was supported by Matowicka-Karna and Kemona (2001) who studied PF4 levels in both hydatid disease and giardiasis. Although they used different, they recorded significantly elevated mean PF4 levels in both diseases preoperatively being (20.3+9.4 UL), compared to postoperative state (6.0+3.0 UL). They suggested the increased level is a result of platelet activation and increase in platelet release reaction in both acute and chronic conditions which lead to more inhibition of megakaryopoiesis. It also lead to chemotaxis of neutrophils, monocytes and eosinophils.

Similar results were reported by Matowicka-Karna *et al.* (2005) who stated that, although parasitic stages in hydatid disease do not get into a direct contact with platelets, yet an increase was observed in the plasma concentration of platelet factor 4 which may indicates the involvement of platelets in the hydatid disease defense mechanism.

Others recorded that the increase in PF4 level could be referred to the ability of hydatid disease, giardiasis also other parasitic diseases as *Ascaris lumbricoides*, *Entamoeba histolytical*/*Entamoeba dispar* to secrete and expose numerous immunomodulatory molecules to the host's immune system. (Siracusano *et al.*, 2008; Matowicka-Karna and Kemona, 2002). This leads to platelet release reaction which is connected with the appearance of P-selectin (from α granules) on the surface of platelets that result in the recruitment of leukocytes and platelets (Doyle *et al.*, 2011) resulting in more platelet activation and in release of PF4.

Another study agreed with such changes of PF4 in parasitic infections. They studied PF4 level in 50 patients with hepatosplenic schistosomiasis. A significant increase in PF4 was noted in ascetic patients or hematemesis compared to control group reflecting platelet alpha-granule release and consequently increased in vivo platelet activation (El-Bassioni *et al.*, 1997).

Thus specific platelet responses as Platelet factor 4 against parasites may be a useful indicator for parasitic infection and for prediction of succession of recovery after treatment with antiparasitic drugs (Zaki, 2011).

CONCLUSION

The present study directs the attention of those working in the parasitic field to measure plasma level of Platelet factor 4 if parasitic diseases are suspected and can't be diagnosed by direct methods. Also PF4 can be measured in diagnosed parasitic infections prior and after therapy for assessing the succession of treatment. Future researches can be directed to changes in other platelets' parameters or changes in hematological parameters in and how to benefit from them in assessing parasitic diseases and their therapies.

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