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Assessment of Prothrombin Time Activated Partial Thromboplastin Time, and Platelets Count among Children with Schistosomiasis at Alhajalej School, Assalay Locality, White Nile State, Sudan

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Abstract
Schistosomiasis is playing critical role to increase the risk for several diseases worldwide particular in developing country, also are related to hematologic changes by disturbing blood flow and endothelial function, which leads to hypercoagulability. This study aimed to estimate a Prothrombin time [PT], and international normalization ratio [INR], Activated Partial Thromboplastin Time [APTT], and Platelets count among school Children infected and non-infected Schistosomiasis. A Cross – section study included 149 school children students [101 cases, and 48 controls] were enrolled in this study, all were matched aged and sex. Direct sampling technique was used for bloody stool and urine specimens; the venous blood sample was collected to perform the coagulation tests, Prothrombin time [PT], international normalization ratio [INR], Activated partial thromboplastin time [APTT], and platelet count as well as Hb g/dl and PCV%. The data were analyzed using SPSS version 26; *P. value* less than 0.05 were considered as statistically significant. The results showed that there was statistically higher mean ± standard deviation of PT [16.80 ± 4.18 Sec], INR [1.58 ± 0.27], APTT[35.45± 3.85 Sec], while the

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platelet count [261000 ± 6500 count/ cumm] was significantly lower in schistosomiasis infected than non - infected students, PT [11.50 ± 1.32 Sec], INR [0.90 ± 0.19], APTT[29.67 ±1.4 Sec], were significantly higher, while the platelet count [280000 ±12000 count/cumm]with *P. value* <0.001.In conclusion Prothrombin time, international normalization ratio, and Activated partial thromboplastin time were significantly higher**,** while the platelet count was significantly lower in schistosomiasis infected than non - infected students. The coagulation tests PT, INR, APTT, and platelet count should be used to monitor and manage schistosomiasis-related complications.

Keywords: *Schistosomiasis, PT, INR, APTT, platelet count, Alhajalej School Children.*

Introduction

Blood coagulation is host defense system closed to maintain the integrity of high circulation system after blood vessel injury [**1**]. Hemostasis is functional process to maintain blood in the fluid state and minimize blood loss via the arrest of bleeding at sites of vascular injury [**2**]. The coagulation system is involved in the conversion of soluble fibrinogen to fibrin clot and consists of various protein factors produced by the liver [**3**]. Platelets play a critical role in the process of stopping bleeding at the site of interrupted endothelium through platelet adhesion, aggregation, and activation of the coagulation system [**4, 5**]. Activated platelets release adenosine diphosphate (ADP), which induce vasoconstriction, stimulate secondary coagulation, and promote further platelet activation and aggregation [**6, 7**]. The sequential activation of certain plasma, proenzyme that proceeds of blood through the intrinsic or extrinsic pathway [**8**]. Schistosomiasis is a significant health parasitic infection problem increasing risk for several diseases worldwide particular in developing country [**9**]. Globally, around 207 million people are infected with schistosome and 120 million of these suffer chronic symptoms [**10**]. Schistosomiasis is grades second only to malaria in standing among parasitic diseases [**11**], which can cause abdominal pain, portal hypertension, hepatic and intestinal fibrosis in chronically infected patients, as well as nutritional Iron deficiency anemia [**12**]. Patients with hepatosplenic Schistosomiasis are disposed to develop complex potential risk of bleeding in those patients which changes in procoagulant-anticoagulant balance, associated with low level of vitamin K-dependent and contact factor proteins were prominent in hepatosplenic Schistosomiasis, main while state of low-grade association chronic disseminated intravascular coagulation [DIC], likewise reduced to presence of immunological and / or inflammatory stimulus [**13, 14**]. Schistosomiasis usages various mechanisms to prevent primary hemostasis. The schistosome tegument contains several enzymatic activities that lead to the degradation of ADP, resulting in inhibition of ADP-mediated platelet activation and aggregation [**15, 16**].

Materials and Methods

This is cross-sectional was included 149 students [101 infected schistosomiasis students were classified into two groups [65 infected with Schistosoma mansoni [S. mansoni], and 36 students infected with urinary Schistosomiasis [S.haematobium], all are male aged 10-15 years; compare

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with 48 students non-infected schistosomiasis used as controls. from Alhajalej, Assalay Locality, White Nile State, Sudan, during the period of September 2022 through to March 2023.

Inclusion Criteria

All students attending Alhajalej children schools in Assalay locality; and who agreement to participate during the study period.

Exclusion Criteria

Multiple parasite infections, students who were on anticoagulant therapy, students having history hypertension, cardiac disease, and diabetes mellitus, chronic renal disease, bleeding disorders, liver disease, Student who received treatment of parasitic infections during the last two weeks before the study were excluded, and/ or who refused to give their consent.

Ethical consideration

The study agreement received ethical permission from the Ministry of Health and Ministry of Education, then administration of the schools. Objective of study were well explained to school student and their parents, then asked for verbally.

Data collection:

Data was collected by using a questionnaire which includes personal, clinical information and laboratory investigation.

Data Analysis:

Data was exported into the statistical package for social sciences (SPSS) software, version 26 [Chicago, IL, USA] from Microsoft Excel 7. Descriptive statistics were used to describe the variables of the study samples, the P. value of < 0.05 was considered statistically significant

Sample collection

Four milliliter (ml) of venous blood was collected by venipuncture, and then divided into two tubes (one with 2.25 ml of blood in 0.25ml 3.2% tri-sodium citrate for coagulation tests PT, INR, APTT and other tube with Ethylene Demine Tetra-Acetic Acid (EDTA) (1.75 ml of blood for Hb, PCV, platelet count.

Methods

Prothrombin Time (PT)

Preparation of Platelet Poor Plasma (PPP)

Platelet Poor Plasma (PPP) is prepared by centrifugation of citrate blood at 2000g for 15 minutes at 4 **ºC**, and the test was performed immediately after samples were prepared.

Method

Deliver 0.1ml of PPP into small test tube (65x10mm) placed in water bath at **37ºC.** Added 0.1 ml of Thromboplastin, wait for 1-2 min to allow the mixture to worm (Thromboplastin without calcium); then add 0.1 ml of warmed $cacl_2$ and mixed well, start stop watch until the $cacl_2$ was added. Expressed the PT in second as the mean of duplicated for control and test plasma [**17**].

Activated Partial Thromboplastin Time (APTT):

APTT test measures the clotting time of plasma after the activation of contact
Factors, calcification by adding of $CaCl_2$ to phospholipids.

Method

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Pre-warmed $CaCl_2$ reagent in the water bath at 37℃ for at least 10 min.
In clean test tube, 0.1 ml of plasma from test or control samples was added in water bath at 37℃, mixed with equal volumes of APTT reagents (phospholipids reagent and the kaolin), wait for 1-3 min, after that, 0.1 ml of pre-warmed $CaCl_2$ was added and start a stopwatch immediately. The time for clot formation was observed and recorded; the results of APTT were expressed in second as the mean of duplicated for control and test plasma [17].

Results:

Table 1: General characteristic of student infected, and non-infected schistosomiasis in Alhajalej School.

Variables	**infected Schistosomiasis**	**Non- infected [control]**
Male	101 [68%]	48[32%]
Age /years	13.11 ±1.56	13±2.00
Hb g/dl	11.46 ± 1.85	14.96 ± 1.5
PCV %	35.51 ± 5.80	45.00 ± 1.90
APTT/ Sec	35.45 ± 3.85	29.67± 1.4
PT/ Sec	16.80 ± 4.18	11.50 ±1.32
INR	1.58 ± 0.27	0.90 ± 0.19
Platelet count/cumm	261000 ± 6500	280000± 12000

Hb: Hemoglobin; **g/dl**: gram per disliter; **PCV**: Packed Cell Volume; **APTT:** Activated Partial Thromboplastin Time**; Sec:** Second; **PT:** Prothrombin Time**; INR,** and **cumm**: Cell per cubic millimeter.

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Table 2: Compression of Age, Hb, PCV, APTT, PT, INR and Platelet count among infected Schistosomiasis student with non- infected in Alhajalej school children.

Variables	**infected- Schistosomiasis**	**Non- infected [control]**	***P-value***
Male	101 [68%]	48[32%]	0.003
Age /years	13.11 ±1.56	13±2.00	0.71
Hb g/dl	11.46 ± 1.85	14.96 ± 1.5	0.001
PCV %	35.51 ± 5.80	45.00 ± 1.90	0.001
APTT/ Sec	35.45 ± 3.85	29.67± 1.4	0.001
PT/ Sec	16.80 ± 4.18	11.50 ±1.32	0.001
INR	1.55 ± 0.27	0.90 ± 0.19	0.001
Platelet count/cumm	261000 ± 6500	280000 ± 12000	0.001

Hb: Hemoglobin, **g/dl**: gram per disliter, **PCV**: Packed Cell Volume, **APTT**: Activated Partial Thromboplastin Time, PT: Prothrombin Time, **Sec**: Second, and **cumm:** Cell per cubic millimeter.

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Table 3: Compression of Hb, PCV, APTT, PT, INR and Platelet count among infected Schistosomiasis students according to Schistosoma type in Alhajalej school children

Variables	**S. *mansoni***	**S.*haematobium***	***P. value***
Infected students	65 [64%]	36[36%]	0.01
Hb g/dl	11.7 ± 2.3	13.03 ±1.06	0.001
PCV %	35 ± 7.00	36.01 ± 4.68	0.001
APTT/ Sec	37.82 ± 3.78	33.08 ± 3.92	0.001
PT/ Sec	17.03± 4.99	13.07± 3.36	0.004
INR	1.57 ± 0.26	1.32 ± 0.27	0.001
Platelet count/cumm	257000 ± 7100	266000 ± 5900	0.001

Mansoni: Schistosoma Mansoni, **S. *haematobium***: Schistosoma ***Haematobium***, **Hb**: Hemoglobin, **g/dl**: gram per disliter, **PCV**: Packed Cell Volume, **APTT**: Activated Partial Thromboplastin Time, PT: Prothrombin Time, **Sec**: Second, and **cumm:** Cell per cubic millimeter.

Table 4: Correlation of Hb, PCV, APTT, PT, INR and Platelet count among infected with non infected Schistosomiasis students according to Schistosoma type in Alhajalej school children.

Variables	**infected Schistosomiasis**		**non infected [control]**
	S. *mansoni*	**S.*haematobium***	
Male	65 [43%]	36[24%]	48[32%]
Age /years	13.18 ±1.71	13.03 ±1.06	13±2.00
Hb g/dl	11.7 ± 2.3	11.21 ± 1.41	14.96 ± 1.5 *P
PCV %	35 ± 7.00	36.01 ± 4.6.8	29.00 ± 1.90 *P
APTT/ Sec	33.82 ± 3.78	33.08 ± 3.92	28.67± 1.4 *P
PT/ Sec	17.03± 4.99	13.07± 3.36	10.50±.50 *P
INR	1.57 ± 0.26	1.32 ± 0.27	0.90 ± 0.10 *P
Platelet count/cumm	257000 ± 7100	266000 ± 5900	280.0 0± 20000 *P

S. *mansoni*: Schistosoma Mansoni, **S. *haematobium***: Schistosoma ***Haematobium***, **Hb**: Hemoglobin, **g/dl**: gram per disliter, **PCV**: Packed Cell Volume, **APTT**: Activated Partial Thromboplastin Time, PT: Prothrombin Time, **Sec**: Second, and **cumm:** Cell per cubic millimeter.
**P*. value consider statistically significant < 0.05

Discussion

Globally schistosomiasis increases mortality and morbidity rate of death among school children, the prevalence of intestinal and urinary schistosomiasis is major public health problem **[9, 18, 19].** Schistosome mansoni parasites can live for years within human blood vessels and appear to be

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refractory to intravascular thrombus formation deaths per year [**20, 21**]. Urinary schistosomiasis causes chronic infection with negatively affect all aspects of health, nutrition, learning, considerable growth obstruction and anemia, hematuria, as well as coagulation changes in countries where the disease is endemic [**22-24**].

A total of 149 students 101[68%] infected schistosomiasis, 48 [32%] students non-infected], were matched age and sex [**25-26**]. Our results revealed that from 101students 65[43%] infected S. *mansoni*, and 36 [24%] infected S. *haematobium,* and APTT, PT, INR, and platelet count [35.45± 3.85 Sec, 16.80 ± 4.18 Sec, 1.58 ± 0.27, and 261000 ± 6500 count/ cumm],in effected schistosomiasis students in relation to non- infected [29.67 ± 1.4 Sec, 11.50 ±1.32 Sec, 0.90 ± 0.19, and platelet count [280000 ±12000 count/ cumm] respectively , table 2, and 3 were statistically different *p. value* = 0.001. This results comparable by Eyayu *et al* 2020 both, intrinsic [APTT] and extrinsic [PT, INR), among an infected patients ; could possibly indicate is either inhibition of both intrinsic and extrinsic coagulation pathways, or an inhibition of the common pathway [**27**], and apparently , S. mansoni was found by Da'dara *et al* 2016 to accelerate the formation of blood clots in murine blood in vivo, however possibly compensated by a rapid, abnormal, fibrinolytic clot breakdown due to reduced blood platelet count and fibrinolysis [**28**], Kabuyaya M, Chimbari *et at* [**29**], Lagler *et al*.[**30**]**,** Da'dara *et al*. hypothesized that this may be a result of reduced levels of coagulation control proteins which promotes rapid blood clotting, and as counter balance, rapid fibrinolytic clot break down may occur due to reduced blood platelet count and fibrinolysis[**21,31,32**], also this finding is harmonized with the previous study done by adult S. *mansoni* and eggs induced alteration in endothelial function[**33-35**], and schistosomes have several electronegative charges on their tegument that could potentially activate platelets and coagulation cascade[**36**], leading to hypercoagulation granulomas, increased consumption of coagulation factors and decreased hepatic synthesis of these factors due to liver abnormality were the possible reason for the occurrence of prolonged PT, INR and APTT in S. mansoni-infected children [**21, 37-40**].

When compared hematological parameters Hb g/dl, PCV%, platelet count in Schistosoma infected students was fount statistically lower than non- infected with *p- value* = 0.001. This study revealed that the platelet count had significantly decreased in S.mansoni-infected students more serious than Schistosoma haematobium compared to non-infected controls p value < 0.001. This is in agreement with studies done in *North America*, Korean, Brazil, China, and Sudan [**41-48**]. These results explain by splenic retention due to poor portal blood drainage or platelet draped sinusoidal space of liver fibrotic [**48**].

In this study Schistosoma was evaluated only by basic coagulation profile [APTT, PT, INR], and platelets count as well as CBC], further studies contacted iron profile, D-dimer, factors assay particularly contact factors.

Conclusion

Prothrombin time, and Activated partial thromboplastin time were significantly higher**,** while the platelet count was lower in schistosomiasis infected than non-infected students.

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