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THE MOST GRACIOUS,
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The journal was initially name as Aljouf University Medical Journal (AUMJ). As the university name changed into Jouf University, the journal's name was also modified into Jouf University Medical Journal (JUMJ) starting from the 3rd issue of the year 2018.

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JUMJ full text articles and their serial code Digital Object Identifier (DOI) address number (according to the International DOI Foundation) are accessible online through searching Journals for Aljouf University Medical Journal at Al-Manhal Platform (https://platform.almanhal.com/Search/Result?q=&sf_21_0_3=Aljouf+University+Medical+Journal&opsf_21_0=1&f_title_type_exact_loc_en=Article&opf_title_type_exact_loc_en=2). JUMJ is indexed by the three major players in the world of academic library discovery services: EBSCO Discovery Services (EDS), OCLC (Worldcat Discovery) and ProQuest (SUMMON). The DOI of AUMJ is 10.12816.

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3. Expanding, increasing the depth, and spreading of internationally competent and updated medical knowledge among the JUMJ target audiences for the benefit of advancing the medical service to the local and international communities.
4. While insuring integrity and declaration of any conflict of interest, JUMJ is adopting an unbiased, fast, and comprehensively constructive one-month peer review cycle from date of submission to notification of final acceptance.

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Email: ajms@ju.edu.sa

Tel: 00966146252271

Fax:00966146247183

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Deanship of Library Affairs, Jouf University, POB: 2014 Sakaka, 42421, Aljouf, Saudi Arabia.

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Table of Contents

Jouf University Medical Journal (JUMJ), 2019 September 1; 6(3)

Original Article

Prevalence of Intestinal Parasitic Infection among Children with Chronic Liver Diseases, Assiut Governorate, Egypt

Shaban M. Sror[1], Shereen M. Galal[1], Yasser M. Mohamed[2*], Ahmed K. Dyab[2*]

[1]Gastroenterology and Hepatology Unit, Pediatric Department, and, [2]Department of Medical Parasitology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[*]Corresponding author: yasser_mokh@yahoo.com. ahmedsaf2001@yahoo.com.

Abstract

Background: Children with chronic liver diseases (CLDs), like immunocompromised individuals, are susceptible to infection with opportunistic parasites.

Objectives: To identify the frequency of intestinal parasitic infection among children with CLDs in Assiut Governorate, Egypt, and to correlate this infection with blood eosinophil count and patients' anthropometric measurements.

Patients and Methods: The present hospital-based case-control study was conducted on 200 children with CLDs of different etiologies (Cases) and age- and gender-matching 200 children complaining of gastrointestinal symptoms without CLDs as control patients. All children were investigated for liver function, complete blood count (CBC) (including differential and absolute eosinophil count), and stool examination. Infection was correlated to eosinophil count, and participant's weight and height.

Results: Among children with CLDs, the intestinal protozoa identified in order of frequency were; *Giardia lamblia* (16.5%), *Entamoeba histolytica/dispar* (13.5%), *Blastocystis spp.* (12%), *Cryptosporidium spp.* (8%), and *microsporidial* spores (3%). Intestinal helminthes identified in order of frequency were; *Hymenolepis nana* (8%), *Enterobius vermicularis* (6%), *Ancylostoma doudenale* (5%), *Ascaris lumbricoides* (3%), *Taenia saginata* (1.5%), and *Schistosoma mansoni* (0.5%). As compared to control patients, incidence of intestinal protozoa in CLDs children was significantly higher ($P < 0.001$), but there was insignificant difference regarding the incidence of intestinal helminthes ($P = 0.107$). The level of blood eosinophils in control patients was significantly higher than that in CLDs children ($P = 0.001$). While weight and height of children were apparently affected by parasitic infections, there was no significant correlation between intestinal parasitic infection and liver function tests. The prevalence of parasitic infections in children from rural areas was significantly higher than in those from urban areas in both groups ($P < 0.001$).

Conclusion: We reported a significant increase in the incidence of intestinal parasites with a lower eosinophilic immune response in cases with CLDs compared to controls. However, infection did not correlate with level of liver enzymes. Infection seemed to negatively affect the weight and height of the studied children.

Keywords: Intestinal parasites, Children, Chronic liver diseases, Eosinophils.

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Introduction

Intestinal parasites usually produce benign diseases in the immune-competent host. But, hosts with abnormal or compromised immune responses are at high risk for severe disease[(1,2)]. Chronic liver diseases (CLDs) and cirrhosis are major health problems[(3)]. Hyperglobulinemia and low cell-mediated immunity are common in different types of CLDs, and proportionately affect the patients' immunity[(4)]. Intestinal protozoa are common opportunistic pathogens and

can cause serious illnesses in immunocompromised individuals[5]

Parasitic infections can lead to malnutrition and micronutrient deficiency specially among children[6]. This would be worse in cases with CLDs that further affect the nutritional status of the patients leading to significant decrease in weight and height percentiles[7].

Eosinophils usually account for less than 7% of the circulating leukocytes. Eosinophilia is a condition in which eosinophils count exceeds 500 cells/µL and it is subjectively classified as mild (500 - 1500 cells/µL), moderate (>1500 - 5000 cells/µL), or severe (>5000 cells/µL) eosinophilia[8]. Diagnosis of eosinophilia is done as an integral part of the complete blood count (CBC). However, in some cases, a more accurate absolute eosinophil count may be needed[8].

Tissue-dwelling helminthes are parasitic infections that often produce mild to moderate eosinophilia. While, most protozoal infections usually do not cause eosinophilia, few of them may be accompanied by mild eosinophilia[9]. The most common cause of eosinophilia, worldwide, is helminthic infections and in industrialized nations is atopic disease[8].

Eosinophilia induced by parasitic infection is reliant on interleukin-5 produced by Th2 lymphocytes. Eosinophils contribute in the immune response against helminthic parasites by releasing their cytotoxic granular contents onto the parasites, which kill them[10].

In patients with CLDs, prompt recognition and management of parasitic infections avoids complications such as electrolyte disturbance, dehydration and development of hepatic encephalopathy[11].

This study was designed to identify the common intestinal parasites infecting children with CLDs in Assiut Governorate, Egypt, and assess their correlation with eosinophil count, and, weight and height of participants.

Patients and Methods

Participants and Setting

This hospital-based case-control study voluntarily enrolled 400 children attending the Hepatology and Gastroenterology Unit, Pediatric University Hospital, Assiut University, Assiut, Egypt during the period from September 2017 to August 2019. Study population was classified into two groups:; Cases Group I of 200 children with CLDs of different etiology (n = 115 males and = 85 females, a mean age of 9 ± 3.9 years, included 104 children from rural and 96 children from urban areas), and, age- and gender-matching control patient Group II of 200 children without CLDs but complaining of other gastrointestinal symptoms (n = 108 males and = 92 Females, mean age of 8 ± 4.5 years and n = 115 from rural and = 85 from urban areas). Children with acute liver diseases such as acute viral hepatitis and individuals with age above 15 years were excluded. The study was ethically approved by the Research Ethics Committee, Faculty of Medicine, Assiut University, and informed consent was obtained from the children's parents. The children were thoroughly clinically examined and treatment to the infected children was prescribed.

Investigations

Peripheral blood samples were collected for liver function tests including, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, prothrombin time and concentration, serum bilirubin, HBV, HCV and complete blood count (CBC).

Weight and height of each child were measured. Three stool specimens were collected over three successive days. For microscopic detection and identification of parasites' ova, protozoal cysts, trophozoites and/or oocysts, stool samples were examined by direct saline wet mount preparation with Lugol's iodine stain followed by formalin diethyl acetate concentration method[12]. Samples of stool specimens were smeared and stained with acid-fast trichrome (AFT) stain[13] and modified Ziehl-Neelsen stain[14] for coccidial identification. Baermann technique was made for the detection of *Strongyloides stercoralis*[15].

Statistical analysis

All analyses were done using SPSS (Statistical Package for Social Sciences) software version 18, Chicago, IL, USA. Chi-Square test (X^2) was used. A p value of ≤0.05 was set as significant.

Results

Table 1 presents the distribution of the CLDs children according to the etiology of their cases. More than 50% of cases were due to autoimmune hepatitis, biliary atresia and metabolic causes.

The observed incidence of intestinal protozoa in CLDs children was significantly higher than that in the control patients (P <0.001). There was no significant difference between both groups as regards to intestinal helminthes incidence (P = 0.107) (Table 2).

Table 1: Distribution of the chronic liver diseases in children stratified according to the etiology. Data shown are frequencies; n (%).

Disease	n (%)
Autoimmune hepatitis	40 (20.0)
Biliary atresia	36 (18.0)
Metabolic causes	32 (16.0)
Chronic hepatitis C virus	24 (12.0)
Budd-Chiari syndrome	20 (10.0)
Sclerosing cholangitis	15 (7.5)
Congenital hepatic fibrosis	12 (6.0)
Drug induced hepatitis	10 (5.0)
Criggler-Najjar syndrome	8 (4.0)
Choledochal cyst	3 (1.5)

Table 2: Intestinal parasitic infections identified by stool examination in children with chronic liver diseases (n = 200) vs. those with other gastrointestinal affection (control patients) (n = 200). Data shown are frequencies; n (%), and X^2 (P value).

Parasite group	Cases	Controls	X^2 (P)
Protozoa			
G. lamblia	22 (11)	14 (7)	17.414 (<0.001)
E. histolytica/dispar	18 (9)	10 (5)	
Cryptosporidium spp.	16 (8)	8 (4)	
Blastocystis spp.	16 (8)	8 (4)	
Microsporidium spp.	6 (3)	2 (1)	
Mixed *G. lamblia* and *E. histolytica/dispar*	6 (3)	4 (2)	
G. lamblia and *Blastocystis* spp.	5 (2.5)	3 (1.5)	
E. histolytica/dispar and *Blastocystis* spp.	3 (1.5)	2 (1)	
Total	92 (46)	51 (25.5)	
Helminthes			
Hymenolepis nana	16 (8)	10 (5)	2.592 (= 0.107)
Enterobius vermicularis	12 (6)	12 (6)	
Ancylostoma doudenale	10 (5)	4 (2)	
Ascaris lumbricoides	6 (3)	6 (3)	
Taenia saginata	3 (1.5)	1 (0.5)	
Schistosoma mansoni	1 (0.5)	1 (0.5)	
Total	48 (24)	34 (17)	

The prevalence of parasitic infection in children from rural areas was significantly higher than in those from urban areas in both groups (P <0.001) (Table 3).

In comparison to the controls, the weight and height of the studied CLDs children were significantly affected by parasitic infection (P <0.001) (Table 4).

There was no significant correlation between intestinal parasitic infection and liver function measured as serum levels of both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the CLDs group stratified for the presence or absence of infection.

Level of blood eosinophils in the controls was significantly higher than that in CLDs children (P <0.001). Level of blood eosinophils in children with intestinal helminthes was significantly higher than

that in children with intestinal protozoa especially in control group (Table 5).

Table 3: Intestinal parasitic infections identified by stool examination in children with chronic liver diseases (n = 200) vs. those with other gastrointestinal affection (n = 200) as controls stratified for their residence; rural vs. urban areas. Data shown are frequencies; n (%), and X^2 (P value).

Patient Group	Total infected	Rural	Urban	X^2 (P)
Cases	140 (70%)	90 (64.3)	50 (35.7)	21.773 (<0.001)
Controls	85 (42.5%)	60 (70.6)	25 (29.4)	27.232 (<0.001)

Table 4: Changes in the weight and height of children with intestinal parasitic infections identified by stool examination in those with chronic liver diseases (n = 200) vs. those with other gastrointestinal affection (n = 200) as controls. Data shown are frequencies; n (%), and X^2 (P value).

Parameter		Cases	Controls	Total	X^2 (P)
Weight percentile	<25	84 (42)	36 (18)	120 (30)	49.588 (<0.001)
	25 – 50	40 (20)	32 (16)	72 (18)	
	50 – 75	64 (32)	76 (38)	140 (35)	
	>75	12 (6)	56 (28)	68 (17)	
Height percentile	<25	100 (50)	44 (22)	144 (36)	63.429 (<0.001)
	25 – 50	60 (30)	40 (20)	100 (25)	
	50 – 75	16 (8)	56 (28)	72 (18)	
	>75	24 (12)	60 (30)	84 (21)	

Table 5: Correlation between type of intestinal parasitic infection identified by stool examination and eosinophil count in children with chronic liver diseases (n = 200) vs. those with other gastrointestinal affection (n = 200) as controls. Data shown are frequencies; n (%), and X^2 (P value). Eosinophilia; Mild = 500 - 1500 cells/µL, Moderate = >1500 - 5000 cells/µL, and, Severe >5000 cells/µL.

Group	Parasite	Total	Eosinophilia			X^2 (P)
			Mild	Moderate	Severe	
Cases	Protozoa	92 (46)	7 (7.6)	-	-	-
	Helminthes	48 (24)	14 (29.16)	3 (6.25)	-	5.882 (0.015)
Controls	Protozoa	51 (25.5)	8 (15.6)	2 (3.9)	-	2.500 (0.114)
	Helminthes	34 (17)	19 (55.9)	13 (38.2)	2 (5.9)	13.118 (0.001)

Discussion

CLDs incorporate a large number of disorders with different etiologies and present on a variety between hepatitis and cirrhosis[16]. CLDs in children are relatively common illnesses with longstanding risk of substantial morbidity and mortality predominantly in developing countries[17]. In the present study, common CLDs, in order of frequency, were autoimmune hepatitis, biliary atresia, metabolic disorders, chronic virus C hepatitis and Budd-Chiiari syndrome. Less common CLDs included sclerosing cholangitis, congenital hepatic fibrosis, drug-induced hepatitis, Criggler-Najjar syndrome. Similar distribution of etiology of CLDs among Egyptian children was previously reported by Abdel Fattah et al[18]. Several local and international studies reported that children with CLDs can be considered as immunocompromised and are predisposed to infection with opportunistic parasites[11,19,20]. A degree of immunocompromisation was evident in our patients with CLDs, as their eosinophilic counts were lower compared to controls - that correlated with their higher rate of parasitic infection.

In this study, the encountered parasites were diagnosed by stool examination using modest methods. However, some protozoa required special staining procedures to be identified under the

microscope. To reduce laboratory costs, acid-fast trichrome stain was used in this study to identify acid-fast oocysts of *Cryptosporidium* as well as *Microsporidial* spores. In agreement with EL-Shazly et al[20], this stain produced results equivalent to those obtained by modified Ziehl-Neelsen stain. This approach waives the need for invasive techniques for diagnosis of intestinal protozoa. Simplicity and low cost of such tool help prevalence studies, such as ours, to elucidate the pathogenic significance of intestinal parasites as a cause of chronic diarrhea in both immunocompromised and immunocompetent patients[21].

Since the identified protozoa associated with CLDs in our cases were all opportunistic protozoan parasites, this verifies the impaired immune state of these patients compared to other participants with gastrointestinal problems other than CLDs[22,23]. *G. lamblia* was the most common parasite detected singly or concomitantly with *E. histolytica/dispar* or *Blastocystis* spp; a result which was in agreement with other reports[24,25,11]. *G. lamblia* is often seen as an opportunistic pathogen and is considered as one of the major food- and water-borne parasites[26-28]. Bednarska et al[29] reported that the occurrence of giardiasis with blastocystosis is not surprising as *Blastocystis* spp. is also regarded as a human opportunistic parasite. In our study, *E. histolytica/dispar* was the second in order of prevalence which may be due to a simlar oral route of infection[30-32].

Similar to our results, *Cryptosporidium* spp. was frequently encountered in CLDs and immunocompromised children as one of the most common protozoan parasites; either singly or in mixed infections with other parasites[33-35]. We also noticed that detection of *Blastocystis* spp. was not an uncommon occurrence in CLDs children whether singly or as a mixed infection with other protozoan parasites. Symptomatic and asymptomatic genotype isolates of *Blastocystis* were commonly reported in immunocompromised children in Egypt[36-38].

Microsporidia are common opportunistic parasitic inhabitants of the small intestine of immunocompromised individuals. Our observed low prevalence is inconsistence with El-Shazly et al[20] investigating Egyptian CLDs children but was far lower than the figure reported in a Poland study on immune deficient patients[39] and a Thailand study conducted on HIV-infected children[40]. The low prevalence of this parasite in Egypt may be explained by its zoonotic transmission[41] and that association with animals is mandatory for its spreading. On the other hand, giardiasis, amoebiasis and cryptosporidiosis are transmissible mainly through contaminated food or drink with cysts or oocysts[42].

In the present study, the prevalence of parasitic infections (protozoan and helminthic) in CLDs children was found to be highly significant in favor of rural than urban children. This can be explained by the traditional customs, behavior, personal hygiene and health services[43,44].

The present study reported a significant difference in favor of stunted growth in CLDs cases, which is in agreement with several previous studies. Kidane et al[45] reported significant association between intestinal parasite infections and underweighted children in the age group 6-9 years. Moreover, Korpe et al[46] demonstrated the significant relation between *Cryptosporidium* spp. infection and the predisposition of linear growth faltering. Donowitz et al[47] reported that early life giardiasis was a risk factor for stunting growth at age of 2 years, while the data of Quihui-Cota et al[48] suggested that giardiasis and cryptosporidiosis may contribute to higher growth deficits in infected children. Furthermore, Rogawski et al[49] indicated that early persistent infection with giardiasis might contribute to stunted growth.

In our study, there was no significant correlation between intestinal parasitic infection and liver enzymes. Although levels of these enzymes may not directly reflect liver function, our results are in agreement with El-Shazly et al[20] who reported that no effect for intestinal parasitic infections on liver functions, measured as changes in serum enzymes.

This study demonstrated that the level of blood eosinophils in children with intestinal protozoal infections was not statistically significant in both groups. This observation is in agreement with Kovalszki and Peter[9]. On the other hand, with intestinal helminthes, eosinophilia was significantly higher in our controls than CLDs cases. In different patterns of CLDs, hyperglobulinaemia and low cell-mediated immunity are common, where the immunological disorders path consistent to the degree of the liver injury[4]. Moreover, helminth parasitic infection is known to be followed by mild to severe eosinophilia[50]. Also, Beers et al[8] reported that the most common cause of eosinophilia worldwide is helminthic infections.

Conclusion

Children with CLDs are immunocompromised individuals, as shown by significant increase in the incidence of intestinal parasites with a lower eosinophilic immune response in cases compared to controls. The weight and height of the studied children were affected by parasitic infections. Intestinal protozoa are essential opportunistic parasites and can cause serious infections in immunocompromised individuals including those with CLDs. Therefore, prompt recognition and management of parasitic infections in these patients are of a great value for avoiding complications as electrolyte disturbance, dehydration and development of hepatic encephalopathy.

Limitations of the Study

Molecular characterization studies for the encountered parasites were made difficult.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Sero-Molecular Epidemiology of Hepatitis E Virus among Hemodialysis Patients, Wad Medani, Gezira, Sudan: Effect of Demographics and Risk Factors

Nassir A. Babiker[1], Adam D. Abakar[2*], Mohamed A. Taha[3], Nawal T. Mohamed[4]

[1]Wad Medani Teaching Hospital for Obstetrics & Gynecology, and, [2]Department of Medical Parasitology, Faculty of Medical Laboratory Science, Gezira University, Gezira, Sudan. [3]Department of Medical Microbiology, Karari University, Khartoum. [4]Department of Parasitology, NPHL Research Unit, Khartoum, Sudan.

[*]Corresponding Author: nassirabakar31@gmail.com

Abstract

Backgrounds: Hepatitis E virus (HEV) causes hepatitis with significant morbidity and mortality both in human and animals.

Objectives: We aimed to determine the serological and molecular prevalence of HEV and its risk factors among Sudanese hemodialysis (HD) patients attending Wad Medani and El Managil Hemodialysis Centers that serve seven localities in Gezira State, Sudan.

Patients and Methods: The study adopted a cross-sectional descriptive design and was conducted among 300 HD patients in the period from 2014 to 2017 Participant's demographic and relevant clinical data and associating risk factors collected. Patients screened for anti-HEV IgG by specific quantitative commercially available ELISA kit. The strongly sero-positive samples were further screened for anti-HEV IgM using specific quantitative commercially available ELISA kit, also, the strongly IgG sero-positive samples assayed by RT-PCR.

Results: The rate of anti-HEV IgG sero-positivity among our HD patients was 57% (171/300), whereas, anti-HEV IgM prevalence was 2.1% among the stronger IgG sero-positive samples (2/96). The anti-HEV IgG & IgM positive samples tested for HEV-RNA by RT-PCR showed negative results. Considering the risk factors associated with the HEV infection, the study revealed higher prevalence rates among those who had no contact with animals 53% (159/300). The prevalence among those who had taken their food at home was 52.7% (158/300). The rate among those who consumed water from well sources was 52% (156/300) and among those who had received blood transfusions >4 times was 31.3% (94/300).

Conclusion: The rate of detection of anti-HEV-IgG was 57% among HD patients. Positive anti-HEV-IgM rate was 2.1%. HEV-RNA was undetected by using RT-PCR in all participants. The viral sero-prevalence exhibited a higher infection rates among males (36.3%), >40 years old age group (35.3%), and among the jobless patients (59.9%). In addition, a higher prevalence rates was recorded for those who had no contact to the animals (92.9%), those who depend on home-made food (92.4%), those who depend on wells as the water source (91%), those who had received blood transfusion >4 times (54.9%), and among those who had started dialysis from months to 3 years of the time interval (49%).

Key words: Hepatitis E virus, IgG, IgM, Hemodialysis patients, Sudan.

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Introduction

Hepatitis E viruses (HEV), formerly related to a group of unclassified viruses, called, non-A/non-B hepatitis viruses, was initially classified as a member in the Caliciviridae family of viruses.

Considering its replication cycle and genome structure, the virus was reclassified into Hepeviridae a family[1]. The HD patients gained the HEV infection either directly due to hygienic behavior of HD patient and chances to the virus exposure or by nosocomial infection during their setting in haemodialysis centers. i.e., by other routes rather than the common fecal-oral route of transmission[2]. Anti-HEV IgG antibodies were found to be positive in 11% of HD patients, 6 - 15.6% of renal transplant recipients and up to 30% of the general population in the USA, respectively[3,4]. Similar reports from Japan, Saudi Arabia and Greece have demonstrated a noticeable HEV sero-prevalence in HD patients [5-7]. A study from Sweden showed that the sero-prevalence of anti-HEV was significantly higher in patients older than 40 years[8]. In Africa, many studies investigated the epidemiology of HEV seroprevalence among various strata of the population. They revealed that the prevalence of the virus among pig handlers in Ghana was 34.8%[9], among Slaughterhouse workers in Madagascar was 14.1%[10], among poly-transfused patients in Tunisia was 28.9%[11], among urban community in Zambia aws 42%[12], and among pregnant women in central Tunisia was 12.1%[13]. HEV prevalence among Dutch blood donors showed higher prevalence rate among youg donors (<30 years) and lower prevalence among those aging >40 years[14].

Molecular detection of HEV RNA in some studies was negative[15,16], while other studies could detect HEV RNA[17]. Fischer et al verified a prevalence of >13.55% for anti-HEV IgG in 1,203 of HEV RNA-negative blood donors, while 6 of 7 HEV RNA-positive donors had positive anti-HEV IgM[18]. Rate of HEV infection increases with age[19].

While a few studies conducted in Sudan for screening for HEV infection among the public and refugees[20-27], no studies were reported for high-risk groups such as HD patients. We planned this serological and molecular diagnostic study to cross-sectionally assess the extent of HEV infection among HD patients from Gezira State, Sudan.

Patients and Methods

Patients and Setting

This a cross-sectional descriptive study that was conducted at Wad Medani and El Managil Hemodialysis Centers, Gezira State, Sudan in the period from May 2014 to December 2017. The study included the following localities: Medani, El Managil, South Gezira, Gurashy, Um-Algura, East Cezira, and Hasahesa. Ministry of health ethically approved the study and informed consent was collected from each participant. The study recruited patients attending Wad Medani and El Managil Renal Hemodialysis Centers who represent inhabitants of the seven localities of Gezira States. 187 patients were males and 113 were females who are on dialysis for months to about 20 years. The study included patients on HD even for one time and of all ages and excluded those patients with acute or chronic renal disease but were not treated by HD and patients from HD centers other than Wad Medani and El Managil centers. The impact of known risk factors for HEV infection; food and water sources, animal contact, frequency of blood transfusion, and the times on HD, was assessed.

Investigations

The screening was performed using microplate reader (Human®, Germany) and specific quantitative ELISA kits for anti-HEV IgG and anti-HEV IgM (Fortress Diagnostic®, UK; Cat# REF BXE0901A and BXE0902A). The results were calculated according to instructions of the manufacturer; dividing the sample absorbance by the cut-off value of the assay, where, cut-off value = Nc + 0.50. Nc = the mean absorbance value for three negative controls. The result of anti-IgM assay was considered negative when absorbance ratio/cut-off is <1, positive when absorbance ratio/cut-off is >1), and, equivocal when absorbance ratio/cut-off is 0.9 - 1.1. The anti-IgG assay the results was considered negative results when absorbance ratio/cut-off is <1, positive when absorbance ratio/cut-off is >1 and equivocal when absorbance ratio/cut-off is 0.9 - 1.1. The equivocal results were retested to confirm the reactivity and the persistent results of an absorbance

ratio/cut-off of 0.9 - 1.1 was regarded as positive.

For real time RT-PCR, the RT-PCR thermocycler (Rotor Gene 6000, Germany) was used. DNA/RNA extraction solutions (batch numbers, 66606, G25493, 66601, 66605, P15079S, 66628, 66622, SHB68127V, and 66596) and RT-PCR kit for HEV RNA (A HEV specific primer, probe mix, and TaqMan®; Serial number JN 160305-49345) from Primerdesign Ltd, UK, were used.

Statistical analysis

The collected data with the laboratory results were encoded and electronically entered in Microsot® Excel for Windows® 2007 database. The data was statistically analyzed using the Statistical Package for Social Science (SPSS), version 16.0. Descriptive statistics of the variables were conducted. For the variables of age, sex, localities, and anti-HEV IgG and IgM results and the risk factors frequencies were cross-tabulated and analyzed using Chi-square test and T-test. Statistical significance was set at p value ≤0.05.

Results

Demographics and general HEV seropositivity

Distributing the study population according to the gender revealed 187 males and 113 females. Out of the 300 renal hemodialysis plasma samples investigated, 57% (171/300) were reactive for anti-HEV IgG while the remaining 43% (129/300) were negative (Figure 1).

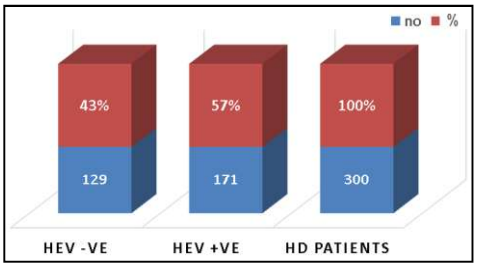

Figure 1: The distribution of hemodialysis patients according to their sero-reactivity for anti-hepatitis E virus IgG. Data shown are frequencies; number (no) and %.

Gender-wise, out of 171 patients with positive anti-HEV IgG 58.3% (109/187) were males and 54.9% (62/113) were females that constituted 36.3% (109/300) among males and 20.7% (62/300) among females of the total patients' number, (Figure 2). This reflects slightly insignificant higher infection rate among males (Pearson's Chi-square = 0.336).

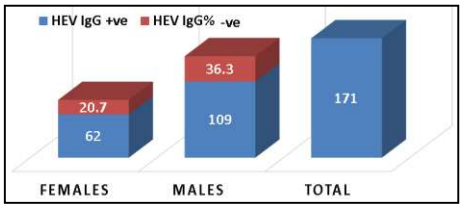

Figure 2: The distribution of hemodialysis patients seropositive for anti-hepatitis E virus IgG according to their gender. Data shown are frequencies; n (blue) and % (red). Total n of pateints investigated was 300.

Distributing the participants according to the age revealed 62 participants of 1 - 30 yrs group, 53 participants of 31 - 40 yrs group and 185 participants of >40 yrs group. Age-wise, out of 171 patients seropositive for anti-HEV IgG, 36 (21.052%) were from the 1-30 yrs group, 29 (16.959%) were from 31- 40 yrs group and 106 (61.988%) were from the >40 yrs group. The distribution of seropositive anti-HEV IgG within each age group showed that out of 62 patients of 1- 30 yrs group 36 (58.1%) were positive, out of 53 patients of 31 - 40 yrs age group 29 patients (54.7%) were positive, and, and out of 185 patients in >40 yrs age group 106 (57.3%) were seropositive (Figure 3). This reflects insignificantly higher liability for HEV infection among the older age group - although within age group, the infection prevalence was equivocal (Pearson's Chi-square = 0.148 and p >0.05).

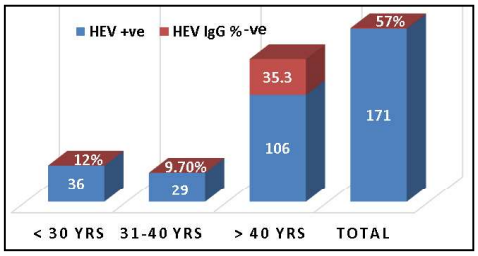

Figure 3: The distribution of hemodialysis patients seropositive for anti-hepatitis E virus IgG according to their age. Data shown are frequencies; n and %. Total n of pateints investigated was 300.

HEV IgG seroprevalence vs. the associated risk factors

Effect of the geographical locality:

Wad Medani, capital of Gezira state and Hasahisa are urban localities, and, the others are rural localities in Gezira state. Distributing hemodialysis participants' among various localities showed that 135 participants resided to Medani, 67 were from Managil, 43 were from South Gezira, 24 were from Gurashy, 16 were from Um-Algura, 14 were from East Cezira, and one was from Hishesa. Anti-HEV IgG reactive plasma was observed in 52.6% (71/135) of Medani, 62.6% (10/16) of Um-Algura, 65.1% (28/43) of South Gezira, 64.3% (9/14) of East Gezira, 59.7% (40/67) of Managil, 54.2% (13/24) of Gurashy and 0.0% of Hasahesa cases. The association of HEV infection among the localities was insignificant (Pearson's Chi-square = 4.330 and p >0.05).

Table 1: The anti-hepatitis E virus IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified for their localities. Data shown are frequencies; n and (%).

Locality	Anti-HEV IgG	
	Negative 129 (43.0)	Positive 171 (57.0)
Medani, n = 135	64 (47.4)	71 (52.6)
Managil, n = 67	27 (40.3)	40 (59.7)
South Gezira, n = 43	15 (34.9)	28 (65.1)
Gurshy, n = 24	11 (45.8)	13 (54.2)
Om Algura, n = 16	6 (37.5)	10 (62.5)
East Gezira, n = 14	5 (35.7)	9 (64.3)
Hasahesa, n = 1	1 (100.0)	0 (0.0)

Effect of the occupations

Distributing the dialysis patients according to their occupation (177 jobless, 37 casual workers, 4 drivers, 1 police officer, 1 engineer, 4 teachers, 2 healthcare workers, 9 students, 8 farmers and 57 housewives) had insignificant associations with HEV infection (Pearson's Chi-square = 7.400 & p >0.05). The Anti-HEV IgG seroprevalence among the occupation showed that 59.9% (106/177) of jobless, 45.9% (17/37) of casual workers, 50% (2/4) of drivers, 100% (1/1) of engineers, 25% (1/4) of teachers, 50% (1/2) of the healthcare workers, 55.6% (5/9) of students, 75% (6/8) of farmers, and 56.1% (32/57) of housewives were sero-positive (Table 2).

Table 2: The anti-hepatitis E virus IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified for their occupation. Data shown are frequencies; n and (%).

Occupation	Anti-HEV IgG	
	Negative 129(43.0)	Positive 171(57.0)
Jobless, n = 177	71 (40.1)	106(59.9)
Workers, n = 37	20 (54.1)	17 (45.9)
Drivers, n = 4	2 (50.0)	2 (50.0)
Policeman, n = 1	1 (100.0)	0 (0.0)
Engineer, n = 1	0 (0.0)	1 (100.0)
Teachers, n = 4	3 (75.0)	1 (25.0)
Healthcare workers, n = 2	1 (50.0)	1 (50.0)
Students, n = 9	4 (44.4)	5 (55.6)
Farmers, n = 8	2 (25.0)	6 (75.0)
Housewives, n = 57	25 (43.9)	32 (56.1)

Effect of the animal contact

Among HD patients whom had contact with animals (26/300), 12 (46.2%) patients were anti-HEV IgG sero-positive and 14 (53.8%) patients were negative. Out of the 274 patients with no animal contacts, 159 (58.0%) patients were seropositive and 115 (42.0%) were negative. HEV IgG seroprevalence insignificantly associated with the animal contact as a risk factor (Pearson's Chi square = 1.366 and p >0.05) (Table 3).

Table 3: The anti-hepatitis E virus IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified for their animal contact. Data shown are frequencies; n and (%).

Animal contact	HEV IgG	
	Negative 129 (43.0)	Positive 171 (57.0)
Yes, n = 26	14 (10.85)	12 (7.02)
No, n = 274	115 (89.15)	159 (92.98)

Effect of the food source

HD patients stratified on bases of their food source showed that 276 patients used

to take their food at home, 4 take ready-made and 20 patients take their food at home and outside home. Among the 171 anti-HEV IgG seropositive patients, 158 (57.2%) take homemade foods, 4 (100.0%) take ready-made foods while 9 (45.0%) take both. This reflected a nonsignificant association with HEV IgG seropositivity (Pearson's Chi square = 4.199 and p >0.05) (Table 4).

Table 4: The anti-hepatitis E virus IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified for their food source. Data shown are frequencies; n and (%).

Food source	HEV IgG	
	Negative 129 (43.0)	Positive 171(57.0)
Home-made, n = 276	118(91.47)	158(92.4)
Ready-made, n = 4	0 (0.0)	4 (2.34)
Both home- & Ready-made, n = 20	11 (8.53)	9 (5.26)

Effect of the water source

According to the drinking water sources, our results revealed that 275 of our patients depend on wells, four depend on Hafeer (a hole make in the ground where water is collected during the rainy seasons for later use), 2 depend on running river water and 19 depend on both wells and Hafeer water. Among the anti-HEV IgG sero-positive 171 patients, 156 (91.23%) patients consumed well water, 3 (1.75%) consumed Hafeer water, 2 (1.17%) consumed river water and 10 (5.85%) patients consumed both well and Hafeer waters (Table 5). The relationship between HEV IgG seropositivity and water source turned to be insignificant (Pearson's Chi-square = 2.194 and p > 0.05).

Effect of blood transfusion

According to times they received blood transfusion, records showed that 56 of the dialysis patients did not receive blood transfusion, 50 patients received blood once or twice, 25 received blood 3-4 times and 169 received blood >4 times. Among the anti-HEV IgG seropositive patients, 34 (19.88%) patients receive no blood transfusion, 27 (15.79%) received 1-2 transfusions, 16 (9.36%) received 3-4 transfusions, 94 (54.97%) received more than 4 transfusions (Table 6).

Table 5: The anti-hepatitis E virus IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified for their water source. Data shown are frequencies; n and (%).

Water source	Anti-HEV IgG	
	Negative 129 (43.0)	Positive 171 (57.0)
Well, n = 275	119 (92.25)	156 (91.23)
Hafeer, n = 4	1 (0.8)	3 (1.75)
River, n = 2	0 (0.0)	2 (1.17)
Well & Hafeer, n = 19	9 (6.98)	10 (5.85)

Table 6: The distribution of anti-HEV IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified according to the frequency blood transfusion. Data shown are frequencies; n and (%).

Times of blood transfusions	Anti-HEV IgG	
	Negative 129 (43.0)	Positive 171 (57.0)
None, n = 56	22 (17.05)	34 (19.88)
1 – 2, n = 50	23 (17.83)	27 (15.79)
3 – 4, n = 25	9 (6.98)	16 (9.36)
>4, n = 169	75 (58.14)	94 (54.97)

Out of 171 patients with positive anti-HEV IgG, 137 (80.12%) received blood transfusions (1, 2, 3, 4 times or more than 4 times), whereas only 34 (19.88%) did not receive blood transfusion (Pearson's Chi-square = 2.690).

Effect of the duration time on-dialysis

According to time since the start of HD, data revealed that 144 of patients had duration time of months - 3 years, 105 had dialysis duration of 3 - 7 years, 51 had dialysis duration of >7 years. Out of the 171 anti-HEV IgG seropositive patients, 84 (49.12%) had hemodialysis duration of months - 3 years, 56 (32.75%) were on dialysis for 4 - 7 years and, 31 (18.13%) were on dialysis for >7 years (Table 7). The relationship between HEV IgG seropositivity and the duration time on-dialysis was insignificant (Pearson's Chi-square = 0.978 and p >0.05).

Table 7: The distribution of anti-HEV IgG sero-reactivity among Sudanese hemodialysis patients (n = 300) stratified according to duration of their time-on-hemodialysis. Data shown are frequencies; n and (%).

Time since the start of hemodialysis	Anti-HEV IgG	
	Negative 129(43.0)	Positive 171(57.0)
Month - 3 years, n = 144	60(46.51)	84(49.12)
4 - 7 years, n = 105	49(37.99)	56(32.75)
>7 years, n = 51	20(15.50)	31(18.13)

Anti-HEV IgM seroprevalence

The HD participants also screened for anti-HEV IgM seroprevalence. Total of 96 strong positive (cut off >2) out of 171 HEV positive IgG were subjected to further screening to anti-HEV IgM. Only two cases 2.1% (2/96) had positive reaction. They were males; both were jobless, one belonged to Managil and one to Om Algura, both had >4 times blood transfusions, one was on HD for 4-7 years while the other was on HD for >7 years, both had no animal contact, both were depending on well water source, and were consuming home-made foods. All relationships with these criteria turned nonsignificant.

HEV-RNA:

The molecular diagnosis of HEV RNA conducted by RT-PCR for only 50 HEV patients with strong positive anti-HEV IgG (included the two positive cases with anti-HEV IgM) because of the limitation of the RT PCR reagent, that has a high cost. All results for HEV RNA were negative.

Discussion

HD patients are directly exposed to HEV infection or acquire it nosocomially[2]. The current study indicated that anti-HEV IgG sero-prevalence among the investigated 300 Sudanese HD patients (n male/female was 187/113) from Gezira State was 57%. Such prevalence is higher than that reported elsewhere[3-6]. This high IgG level observed among HD patients in the current study was probably due to the nosocomial infection, poor personal hygiene and/or through receiving of HEV-contaminated blood or other unrecognized factors. Nevertheless positive anti-HEV IgG indicates previous exposure to HEV.

Anti-HEV IgG prevalence was insignificantly higher among males (58.3%) than females (54.9%). This could be attributed to the fact that males are more likely to be exposed to viral sources of contamination such as animals or contaminated food consumption or other unrecognized factors. Our findings were similar to that reported from Japan where seroprevalence among males was higher than females (21.6 vs. 14.3%)[8].

The study revealed that anti-HEV IgG sero-prevalence among HD patients had insignificant association with age. The rate of HEV exposure among HD patients was insignificantly increased and decreased among <30 years and those of 31-40 years of age (58.1 and 54.7%). The reason for that it might be due to a degree of exposure to the virus by undefined root among <30 year agers. Our findings disagree with that of Netherland study on young blood donors (<30 years) showing lower seroprevalence among that age groups[14]. On the other hand, our results are similar to that reported by Swedish study revealing increased HEV infection among those aging >40 years[16].

Regarding the distribution of HEV seroprevalence within urban, suburban and rural localities, the study showed that Managil had the highest infection rate in comparison to Hasahesa and Wad Medani, which had the lowest infection rate. This could be due to Madeni being more urbanized with lower risk factors than other localities. However, the current results are higher than that observed among Urban community from Zambia[12].

Anti-HEV IgG seroprevalence has insignificant association to the occupation. However, the highest prevalence rate was observed among the jobless patients (59.9%). Furthermore, anti-HEV IgG seroprevalence had insignificant association with animal contact (7%). The food and water sources revealed insignificantly higher anti-HEV IgG seroprevalences with homemade food taking (92.40%) and well source water (91.23%). In addition, HD duration

showed insignificantly higher prevalence for those with the shorter duration-on-dialysis (months - 3 years; 49.12%). Frequency of blood transfusion showed insignificantly higher rates vs. those >4 times of blood transfusion (54.97%). Overall, seroreactivity of the virus among the participants was insignificantly associated with known risk factors. In studies elsewhere, pig handlers showed a prevalence of 34.8%[9], Slaughterhouse workers had a prevalence of 14.1%[10] and in poly-blood transfused patients had a rate of 28.9%[13]. These results seem to be lower in comparison to the current study results.

Molecular detection for HEV RNA conducted among our strong IgG positive samples revealed negative results for all of them. These results are similar to that conducted on 239 Ghanaian blood donors[15] and on American plasma donations[16]. In contrast, the HEV RNA detection was 0.012% among Swedish and 0.022% among German donations[16]. These European countries may have more sensitive detection methods that are not available for us. The negative results observed in our study could be due to the late immune response with a successful neutralization of the infection during viremic and/or early viral infection phases[18]. It could also be due to a transient or short viremic phase and the self-limiting characteristic known for HEV infection. With the early disappearance of the virus from the blood stream, it will not be detectable by RT-PCR. Also, it might be due to different sensitivity of kits and detection methods for HEV RNA.

Conclusion

The current study showed a high sero-prevalence rate of anti-HEV IgG among Sudanese renal HD patients (57%). Although, there were some HEV studies among other populations in the Sudan, this study is the first investigating HEV sero-prevalence among HD patients - adopting both serologic and molecular detectors. The latter did not detect the viral RNA that may reflect the self-limiting nature of the disease. The viral seroprevalence exhibited a higher infection rates among males (36.3%), those of >40 years age (35.3%), jobless (59.9%), those with no animal contact (92.9%), those taking home-made food (92.4%), those depending on wells as the water source (91%), those received blood transfusion >4 times (54.9%), and among those the shorter duration-on-dialysis (months - 3 years; 49%).

Limitations of the Study

For the study being cross-sectional, limits the chances of detecting rapid resolving infections like that of HEV. Although we included all willing participants, the subdivision stratifications for gender, localities, and the several risk factors investigated lowered number of patients per subgroup and weakened the statistical association analysis.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Quality and Capacity of Clinical Laboratories for Microscopy-Based Malaria Diagnosis at N'djamena City, Republic of Chad

Mohamat Ali Youssouf[1], Alfatih Saifudinn Aljafari[2,3]

[1]Higher National Institute of Science and Technology of Abeche (INSTA), Abeche, Republic of Chad. [2]Pathology Department, College of Medicine, Jouf University, Sakaka, Saudi Arabia. [3]Medical Parasitology Department, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.

Corresponding author: aljafari@ju.edu.sa

Abstract

Background: Microscopy-based diagnosis of malaria (parasite-based diagnosis) remains the gold standard method for diagnosis in endemic areas, where 600 million individuals are infected. Malaria is the most common cause of hospital visits in Chad.

Objectives: This study aimed to assess and evaluate the capacity and quality of the Clinical Laboratories of the governmental and private sectors at N'djamena city, Chad, for the parasite-based malaria diagnosis.

Materials and Methods: Eighty nine clinical laboratories from the governmental (n = 35) and private (n = 54) sectors were enrolled in this cross-sectional analytical survey study. The quality and capacity for the study subjects were assessed and evaluated using scoring system. Six quality identifiers were selected as key performance indicators. The quality identifiers and their weight (score) were; personnel (20%), microscope (25%), staining (20%), blood film and slide (15%), result and report (10%), facility (10%). For the evaluation of the quality and capacity of study subjects, an overall score of 60% was identified as the lower limit of accepted quality. Governmental vs. private lab data were compared using X^2-test.

Results: For the personnel quality, sixty-three (70.8%) individuals in this study have a diploma degree in medical laboratory sciences; ten (11.2%) individuals have a bachelor degree in medical laboratory sciences, and, only one person (1.1%) had a major in parasitology), and the other 15 individuals (16.9%) had other degrees. Seventy four (83.1%) of the study personnel have more than three years of experience in malaria diagnosis. Seventy-two individuals (80.9%) scored at least 10 out of 20 for the total personnel quality score. For the quality of microscope, 87.3% the study labs scored a least of 15 points out of 25. 85.4% of the study subjects use Giemsa's stain. For the staining quality, 83 subjects (71.9%) scored more than 10 points out of 20. Only 9% of study subjects report the species of Plasmodium. 10.1% of study personnel report the stage of the parasite.

Conclusion

68.5% of the subjects are of a good quality and acceptable capacity for microscopic parasite-based malaria diagnosis without significant differences between governmental and private labs. However, the status needs to be improved by implementing an obligate in-service training program of minimum quality and duration and applying a mandatory official program for quality control surveillance that would be able to evaluate several criteria particularly the positive and negative detection rate.

Key words: Clinical laboratory, Quantity, Malaria, Microscopic Diagnosis, N'djamena, Chad.

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Introduction

The number of global malaria cases (90% of them in Sub-Sahara Africa) was decreased from 237 million cases in 2010 to 211 million in 2015, but it starts to increase again to reach 216 million in 2016. However, the death toll remains the same; 44600 in 2015 and 44500 in 2016. Meanwhile, some countries have been certified by WHO as malaria free[1]. In the Republic of Chad, malaria is 100% caused by *Plasmodium falciparum*, and is transmitted mostly (i.e., 94.9 %) by *Anopheles gambiae*[2]. Eighty percent of Chad's population live in high malaria transmission areas, with 2.0 million estimated cases and 9000 maximum estimated death cases reported in 2016. High level of malaria transmission (67%) during the short rainy season was reported in Central and Western areas of Chad. Malaria accounts for 10% of all health facility visits and 12% of all hospital admissions. Malaria diagnosis and treatment is very expensive to the already exhausted healthcare system and cost millions of Chadian Central African Francs[1,3].

The Republic of Chad faces many difficulties about malaria. Malaria drug resistance emerged in 2006[4], also insecticide resistance[5,6], together with local and international funding scarcity, all are on the surface[7]. The fund allocated for malaria control dropped to the minimum "less than 1 million US$" in 2016 which may affect the efforts of improving case detection and treatment[8]. An accurate and prompt diagnosis of malaria is important for early case management. This is achieved by detection and identification of malaria parasite, through microscopic examination of Giemsa's stained thick and thin blood films, by competent personnel followed by qualitative and quantitative adequate reporting. Microscopy remains the option of choice for routine diagnosis of malaria[9]. Diagnosis of malaria is fundamental in epidemiological and control surveys[10].

It is mandatory to confirm the infection by parasite-based microscopy diagnosis for clinically suspected patients of malaria before administration of anti-malarial treatment. The reliability of rapid diagnostic tests (RDTs) is questionable. The positivity rate of RDTs falls from 90% in 2013 to 70% in 2015 due to histidine-rich protein-2 gene deletions[11,12]. Many factors affect the sensitivity and specificity of microscopic parasite-based malaria diagnosis. Poor infrastructure, heavy workload, and absence of proper training are the leading factors that have negative impact on both quality of the lab and the competency of personnel[13-15].

Many action plans were designed to perform gap analysis for malaria diagnosis. They adopt quality programs (internal or external) that target the efficiency of microscopy atmosphere[16] with some other indicators including the quality of personnel and the facility[17]. Health authorities in The Republic of Chad struggle their best to tackle many synchronized health issues.

This study was planned to provide a scientific-based situation analysis for the decision makers to bridge the gap with minimum but effective measures. Specifically, the objective of this study was to assess and evaluate the capacity and quality of the Clinical Laboratories of the governmental and the private sector at N'djamena city, Chad for the microscopic parasite-based malaria diagnosis.

Materials and Methods

Study design and area

This is a analytical cross-sectional and facility-based survey study of a qualitative approach. In this study, a total number of 89 clinical laboratories from the governmental and private sector were enrolled (n = 35 and 54, respectively). The subjects were selected randomly from the governmental and private clinical laboratories at N'Djamena city, Chad.

Study area

N'Djamena, the capital of the Republic of Chad, population (1,092,066), is located southwest of the country at 12°6′47″N/15°2′57″E coordinates, on the confluence of the Chari and Logone rivers. It is facing and connected to

the Cameroonian town of Kousséri. N'Djamena has a semi-arid climate with a short wet season and a lengthy dry season. The city receives 510 mm (20 in) of rain annually during the rainy season that lasts from June to September. It's a regional center of trade and industrial economic activity in Chad.

Data collection and analysis

For data collection, the study utilized structured data performa validated by Hamdy et al[18]. A scoring system was applied to report the quality of the study subjects. Each variable has given a percentage that represents its weight in relation to the overall quality evaluation. The percentages (which are also equivalent to quality score) were as follows: Personnel (20%), microscope (25%), staining (20%), blood film and slide (15%), result and report (10%), and building and safety (10%).

For the evaluation of the quality of study subjects, the following are the predefined quality level scores: Excellent (≥80%), very good (70 - <80%), good (60 - <70%), and poor quality (<60%). For the capacity of selected subjected, we define 60% as a lower limit for acceptance.

Data were tabulated, processed, and analyzed using IPM Statistical Package for Social Sciences software (SPSS19.0). Statistical inference was achieved based on the descriptive statistics and Pearson Chi-Square test to compare with significance of P value <0.05.

Ethical consideration

The proposal of this study has been ethically approved by Higher National institute of Science and Technology (Abeche, Chad).

Results

This study involved 89 clinical laboratory; 35 governmental and 54 private (39.3% and 60.7%, respectively).

Personnel quality

Among the study population, 10 individuals (11.2%) have a bachelor degree in Medical Laboratory Sciences with only one of them with a major in parasitology. Table 1 shows detailed frequencies for the qualifications of lab personnel. Seventy four (83.1%) of the personnel have more than three years' experience in malaria diagnosis. Table 2 shows detailed frequencies for personnel experience. Minority of the personnel in this study (23.6%) attended a special in-service training on malaria diagnosis. Table 3 shows the frequency of in-service training periods among study personnel. Seventy-two individuals (80.9%) scored at least 10 points out of 20 for the total quality score. Details of the quality scores for lab personnel in this study are listed in Table 4.

Table 1: Frequency of the education qualifications of studied personnel for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.71.

Qualification	Gov. n (%)	Priv. n(%)	Total n(%)
Medical Laboratory B.Sc. (Parasitology)	0 (0)	1 (1.85)	1 (1.1)
Medical Laboratory B.Sc. (Non-Parasitology)	3 (8.6)	7 (13)	10 (11.2)
Medical laboratory diploma	25 (71.43)	38 (70.4)	63 (70.8)
Other	7 (20)	8 (14.8)	15 (16.9)

Table 2: Frequency of personnel's experiences in malaria diagnosis. Data shown are frequencies; n (%) and X^2 test p value = 0.27.

Experience	Gov. n (%)	Priv. n (%)	Total n (%)
≥3 years	31 (88.57)	43 (79.6)	74 (83.1)
1 - <3 years	4 (11.43)	11(20.4)	15(16.9)
<1 year	0 (0)	0 (0)	0 (0.0)

Table 3: Frequency of in-service training of study personnel on parasite-based malaria diagnosis for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.30.

Type of in-service training	Gov. n (%)	Priv. n (%)	Total n (%)
Basic	2 (5.7)	8 (14.8)	10 (11.2)
Refreshing	8 (22.9)	3 (5.6)	11 (12.4)
None	25 (71.43)	43 (79.6)	68 (76.4)

Table 4: Frequency of personnel's quality score for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.83.

Score	Gov. n (%)	Priv. n (%)	Total n (%)
<10	26(74.3)	39 (72.2)	65 (73)
10 - 14	9 (25.7)	14 (25.9)	23 (25.8)
15 - 20	0 (0)	1 (1.8)	1 (1.1)

Microscope utility

The quality of the microscope was determined by the availability and efficiency of its parts and accessories that are essential for proper blood film examination. All study subjects scored at least 15 points out of 25. Table 5 shows a detailed score for microscope quality.

Table 5: Frequency of microscope quality scores for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.38.

Score	Gov. n (%)	Priv. n (%)	Total n (%)
<15	3 (8.75)	8 (14.8)	11(12.7)
15 - <20	12(34.2)	8 (14.8)	20(22.3)
20 - 25	12(34.2)	38 (70.4)	58 (65)

Staining

The majority of the study subjects (85.4%) used Giemsa's stain, with only 14.6% use Field's stain. Many key indicators were investigated to determine the quality of staining; shown in Table 6. Concerning staining quality score, 28 subjects (31.4%) score more than 10 out of 20. Table 7 shows detailed evaluation for staining quality.

Only eight of study subjects (9%) report the species of Plasmodium, and 10.1% of study personnel report its stage. Table 8 summarizes the frequencies of the quality indicators in regard to reporting of blood film examination for malaria. Few of the studied facilities were adhering to standard safety measures. The overall quality ranking for the study subjects are illustrated in Table 9.

Table 6: Frequency of staining quality key indicators for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.24.

Staining quality key indicators	Gov. n(%)	Priv. n(%)	Total n (%)
Optimal concentration of working solution	27 (77.1)	31 (57.4)	58 (65.2)
pH adjustment	10 (28.6)	25 (46.3)	35 (39.3)
Working solution disposal after 30 min	3 (8.6)	2 (3.7)	5 (5.6)
Optimal staining time	11 (31.4)	18 (33.3)	29 (32.6)
Using stain control	5 (14.3)	9 (16.7)	14 (17.5)
Thin and thick film in the same slide	8 (22.9)	3 (5.6)	11 (12.4)
Proper air drying	33 (94.3)	51 (94.5)	84 (94.4)
Safety Measures	16 (45.7)	13 (24.1)	29 (32.6)

Table 7: Frequency of staining quality score for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.70.

Staining quality score	Gov. n (%)	Priv. n (%)	Total n (%)
<10	23 (65.7)	38(70.37)	61 (68.6)
10 - <15	9 (25.7)	13 (24.1)	22 (24.7)
15 - 20	3 (8.75)	3 (5.5)	6 (6.7)

Table 8: Frequencies of report quality indicators for 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are frequencies; n (%) and X^2 test p value = 0.62.

Indicator	Gov. n (%)	Priv. n (%)	Total n (%)
Report Species	2 (5.7)	6 (11.1)	8 (9)
Report Stage	2 (5.7)	7 (13.0)	9 (10.1)
Report parasite density	20 (57.1)	33 (61.1)	53 (59.6)
Maintain a copy of the result	35 (100)	47 (87)	82 (92.1)

Table 9: Distribution of clinical laboratories according to the capacity level in 89 governmental (Gov.) and private (Priv.) clinical laboratories surveyed. Data shown are total score and frequencies; n (%), and X^2 test p value = 0.52.

Capacity level (Grade)	Score	Gov., n (%)	Priv., n (%)	Total., n (%)
Excellent	80 - 100	2 (5.7)	8 (14.8)	0 (0.0)
Very good	70 - <80	12 (34.3)	10 (18.5)	10 (11.2)
Good	60 - <70	10 (28.6)	19 (35.2)	22 (24.7)
Poor	50 - <60	11 (31.4)	17 (31.48)	29 (32.6)
Fail	<50	2 (5.7)	8 (14.8)	28 (31.5)
Non acceptable	> 60	21 (60)	36 (66.7)	57 (64)
Acceptable	≥ 60	14 (40)	18 (33.3)	32 (36)

Discussion

In spite of the fact that mass screening for malaria is undertaken and case reporting from private sector is mandatory, but the Republic of Chad is among the countries with no updated status of malaria[2,10]. This is because of the poor infrastructure and scarcity of resources. The government spends less than 3% of its budget for non-wage expenditure of the health sector. The global fund is rolling back with less than 1.0 million US$ donation[19]. Nevertheless, the public healthcare sector provides free services especially in rural areas. The private sector is rigorous and shares more than 60% of the healthcare services in N'djamena city. The latter is characterized by non-affordable high price that may constitute major obstacle for proper delivery of the services[20]. For clinical laboratory services, the Republic of Chad, like most of Sub-Saharan African countries, does not have an accredited clinical laboratory[21].

This work has investigated the capacity and quality of clinical laboratory at in N'djamena city for their capability and readiness for conducting reliable malaria microscopy. The assessment was based on the fulfillment of the minimum requirements and benchmarking that are now widely used[18,22]. Sixty-three (70.8%) of the laboratory personnel in this study graduated with Diploma in Medical Laboratory Sciences; this is a trend in Central and West Africa. This means that they have attended lesser contact hours in training on malaria diagnosis than those with Bachelor Degree. About 16.9% of the study personnel were non-clinical laboratory specialists; they are paramedical workers. Although they are not licensed for laboratory practice, they took over the work because of the shortage. This study has exposed a great defect in the in-service training. The absence of this essential practice may be attributed to the lack of funding, to the unavailability of expertise trainers and to absence of mandatory official quality control surveillance. The majority of personnel seemed to have enough experience. However, years of experience might not be counted unless accompanied by structured training that develops competence[23].

Many countries focus on the strengthening of the personnel quality. They differ in the identification of the quality indicators. However, the baseline-identifiers are the same[24]. In this study 68.5% of lab personnel were of an acceptable quality, considering the score achieved in regard to quality identifiers. However, only 11.2% of personnel were of a very good quality – representing those who graduated with bachelor degree major in parasitology and underwent basic & refreshing in-service training.

Many quality key performance indicators have been assigned to identify the quality of staining. Almost all laboratories in the study use Giemsa stain with a variable degree of adherence to its standard operation procedures. None of the study subjects pay attention to the pH adjustment; they are using commercially ready-made buffer solutions. Also, they are working with Giemsa stain without considering safety precautions. For them, Giemsa stain is safe and the method contains no risk, which is untrue. The rest of key performance indicators were good enough to ensure that, at least 17.5% of the subjects of this study were using stain & staining procedure in an acceptable quality.

Proper reporting of microscopic examination is one of the quality indicators. Only 9% of the personnel in this study report the species of Plasmodium, although it seems that they all know how to do it. They claim that the treating doctors don't care about the species and density. Interestingly, 10.1% of the personnel in this study report stage of the parasite. In addition, a majority of them maintain archive for results.

Results show no difference between governmental and private clinical laboratories. This conclusion may be attributed to the lack of market competition and also to the absence of quality and accreditation policy.

The identification for the capacity and quality of the clinical laboratories gives an indication for their preparedness for achieve reliable parasite-based malaria diagnosis. Some malaria endemic countries reach a level of capacity and quality that fits with the basic requirements of parasite-based malaria diagnosis[24]. Some countries try to adjust, whereas others still struggle to provide basic needs[25]. There are many benchmarks and quality standards that ensure the readiness for a facility to reach a quality parasite-based diagnosis of malaria. These key performance indicators define the capacity (and quality) of clinical laboratory for proper and accurate parasite-based diagnosis of malaria. They ensure good laboratory practice in the same regard[18,22].

The healthcare system of the Republic of Chad is fragile and embarked by emerging priorities which drain the available resources. However, with well-trained leadership, the malaria microscopy could be improved[26].

Conclusion

This study revealed that 68.5% of the subjects were of a good quality and they are of acceptable capacity for microscopic parasite-based malaria diagnosis with no difference between governmental and private clinical laboratories. However, this status could be improved by implementing an obligate in-service training program of minimum quality and duration and applying a mandatory program for quality control inspection. Further control sample and slide-based quality assurance study should be conducted to confirm the quality of positive and negative detection rate.

Limitations of the Study

The findings of this study are limited to study area and population. A nationwide quality assurance program should be done.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Accuracy of Visual Estimation of Blood Loss among Healthcare Providers: A Cross-Sectional Comparative Study

Nasser A. N. Alzerwi

Department of Surgery, College of Medicine, Majmaah University, Al-Majmaah, Kingdom of Saudi Arabia.

For Correspondence: n.alzerwi@mu.edu.sa

Abstract

Background: Actual blood loss (ABL) measurement is laborious and time consuming. Conversely, visual estimation of blood loss (VEBL) is convenient for healthcare providers (HCPs). However, its lack of accuracy and reliability is concerning.

Objectives: To compare VEBL accuracy among HCPs in Riyadh, Saudi Arabia.

Participants and Methods: A quantitative cross-sectional study targeting different HCP categories with relevant characteristics such as training levels, years of practice, and center type was conducted between March 1st and 15th, 2019. VEBL was estimated using supersaturated 10 × 10- and 45 × 45-cm gauze pads. Significant intergroup differences regarding accuracy, which was defined as the mean VEBL within 20% of ABL for each grouping variable and was the "correct estimation" by >60% participants) were determined using the Mann–Whitney U, Eta squared, Chi-square, and Cramer's V tests.

Results: Of the 200 participants recruited (mean age: 35.8, [SD = 9.02] years), majority were males (56%) and surgeons (40%). No significant differences were observed in mean VEBL or frequency of events in estimation categories (underestimation vs. correct estimation vs. overestimation) across professions, training levels, center designation, and nationality for both pad sizes. However, anesthetists accurately estimated VEBL for the 45 × 45-cm pad and more likely correctly estimated VEBL for both pad sizes than surgeons and operating department practitioners. Females more likely correctly estimated/underestimated and less likely overestimated than males for the 10 × 10-cm pads (p = 0.02, Cramer's V = 0.197). Experienced participants more correctly estimated, less likely underestimated, and more likely overestimated for the 45 × 45-cm pads (p = 0.03, Cramer's V=0.190).

Conclusions: VEBL is an inaccurate method for intraoperative blood loss quantification across all professions, training levels, and center designations. Significant differences occurred in accuracy between years of experience groups and gender groups. Educational intervention is strongly recommended to improve accuracy.

Keywords: Visual estimation of blood loss, Actual blood loss, Healthcare providers, Accuracy.

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Introduction

Intraoperative blood loss is one of the most important predictors of operative mortality and morbidity[1-3]. Early recognition and appropriate management of significant intraoperative bleeding to prevent under- or over-resuscitation is dependent on accurate assessment of blood loss by surgeons, anesthetists, and operating department practitioners (ODPs)[4]. Accurate determination of the actual blood loss (ABL) is crucial; however, it requires more sophisticated

methods such as the gravimetric and photometric measurements[4-6]. This makes ABL less widely adopted and prone to being substituted by less expensive and more rapid and convenient methods of visual estimation of blood loss (VEBL) by surgeons, anesthetists, and ODPs. This substitution is especially so in developing countries with limited access to expensive high-tech methods of objective measurements of ABL[5,7-9]. Nevertheless, VEBL is the most commonly used method to assess blood loss in the United States of America (USA) by healthcare providers[5]. Many studies have shown that VEBL, although more pragmatic and convenient, is fraught with concerns of accuracy, reliability, and inter-observer & intra-observer variability of under- & overestimation[5,7,8,10-15]. Studies have also shown that most healthcare providers had no formal training in VEBL and that most use self-notion instead[16-18]. Such variability and lack of reliability raise concerns about the risk of underestimation of ABL using VEBL. This might lead to delayed resuscitation or under-resuscitation with the consequent risk of increased mortality and morbidity[19]. Desalu et al failed to appropriately transfuse 38% of the patients in their study when using VEBL combined with physiological end points[9]. The reported relevant factors that might affect the accuracy of VEBL include gender, age, members of different professions (e.g., obstetricians, midwives, ODPs, anesthetists, paramedics), years of experience, level of training, pad size and volume lost, type of surgical procedure or access and length, and amount of irrigation fluid[7,13,15,16,20-23]. However, none have studied factors such as center designation, and very few have included general surgeons[7,13,22]. Other studies have shown VEBL to be an accurate method for estimating blood loss at childbirth and postpartum hemorrhage among well-trained midwives when the volume is less than 200 mL. This suggests the effect of blood loss volume on VEBL accuracy[23]. Most previous studies have focused on either midwife and/or obstetricians, while few studies have evaluated the accuracy of VEBL of intraoperative bleeding by general surgeons. Specifically, no study has been conducted in Saudi Arabia on the accuracy of VEBL among general surgeons. Therefore, the goal of the current study was to explore the accuracy of VEBL regarding intraoperative bleeding by general surgeons and compare to that estimated by other healthcare providers in the operating room (anesthetists and ODPs) at varying levels of training and experience as well as at different center designations in the Riyadh region of Saudi Arabia.

Participants and Methods

Study Design

This study is multicentric cross-sectional comparative study.

Materials

We performed literature review (Google scholar and PubMed) and conducted dry lab experiments to determine the absorptive capacity of dry 10 × 10-cm gauze (12 mL) and 45 × 45-cm lap (160 mL) pads. Then we supersaturated the 10 × 10-cm gauze pad with 15 mL of blood and the 45 × 45-cm lap pad with 160 mL of blood (blood was collected from the author voluntarily). This absorptive capacity was reported as ABL volume. Then photographs of the supersaturated gauze pads in the fully spread out configurations were taken (Figure 1).

Figure 1: The 10 X 10-cm (left) and 45 X 45-cm (right) gauze pads supersaturated with whole blood in a fully spread out configuration.

Online Survey

An online survey was created and distributed to the 200 (100% response rate) recruited participants (operating department nurses, anesthetists, and surgeons [consultants and trainees]) in the Riyadh region on March 1, 2019. The first section of the survey was used to collect personal data (age, gender, specialty, level

of training, years of practice, nationality, center designation [secondary vs. tertiary]). The second section presented the photographs of the supersaturated 10 x 10-cm gauze (spread out) and the 45 x 45-cm lap pad (spread out), and the participants were asked to estimate the amount of blood loss in milliliters (mL).

Inclusion Criteria and Exclusion Criteria

Secondary and tertiary hospitals in Riyadh as well as general surgeons, anesthetists, and ODPs at different levels of training from the same region were included. Medical and nursing students, surgeons other than general surgeons, hospitals outside Riyadh, non-operating general surgeons for the last 6 months, and participants who had received formal training (workshops or lectures) on VEBL were excluded.

Blinding

The survey administrator and the participants were unaware of the ABL for each pad size.

Sampling Technique and Sample Size Calculations

Sampling was a simple random sampling. The survey administrators (residents in training) randomly selected participants from operating departments in 26 public and private hospitals in the Riyadh region. Power analysis for a Chi-square test was conducted in G-POWER to determine a sufficient sample size using an alpha of 0.05 (two-tailed p values < 0.05 were considered to indicate statistical significance). A sample size of 197 participants was calculated to achieve 80% power, to detect a 20% difference in effect size (small effect size w = 0.2) of outcome variation (VEBL – ABL/ABL X 100), and 1 degree of freedom [24].

Participants

Operating department nurses and ODPs, surgeons (consultants and trainees), and anesthetists (consultants and trainees) in Riyadh were approached and recruited by a survey administrator (surgical resident). They were informed about the survey and consented for taking the survey on-site, from March 1st to 15th, 2019.

Ethical Considerations

Written informed consent was obtained from all participants, and participants were all informed about their right to withdraw their consent at any moment without any consequences, and the study protocol was approved by King Fahd Medical City (KFMC) institutional review board (IRB Log Number 20-225E).

Statistical Analysis

Analyses were conducted using IBM SPSS Statistics for Macintosh, Version 26.0 (IBM Corp., Armonk, NY, USA). Differences in characteristics between the comparison groups (surgeons vs. anesthetists; residents vs. consultants; and physicians vs. nurses, male/female comparison, Levels of training comparisons, Saudi vs. Non-Saudi and tertiary vs. secondary center designation comparisons) were determined using descriptive statistics. Normal distribution and homogeneity of variance assumptions were assessed using Kolmogorov-Smirnov and Shapiro-Wilk normality tests and were violated for all variables. Therefore, nonparametric tests were used to compare different categories of variables. For continuous variables, the Mann-Whitney U test was used to detect significant differences in characteristics between groups, followed by Eta-square estimation of effect size for the clinical significance of such differences if any. Kruskal-Wallis tests were used for continuous variables with more than two categories. Games-Howell post-hoc test was conducted when there was a significant difference between categories. For categorical variable comparisons, we performed Pearson's χ^2-test to detect any significant differences, followed by Cramer's V estimation of effect size for the clinical significance of such differences if any. Adjusted standardized residuals were used for the post hoc correction of multiple comparisons by χ^2-test.

Operational Definitions of Accuracy and Estimation Categories

Any difference in mean VEBL for any grouping variable within 20% of ABL (ABL – 20% to ABL + 20%, which was within 12-18 cm for the 10 X 10-cm pads,

and within 128-192 mL for the 45 X 45-cm pads) was considered negligible, i.e., "Accurate" (correct estimation). Any difference of more than 20% was deemed to be significant, i.e., "Inaccurate" (over-estimation: more than ABL + 20%, which is >18 mL for the 10 X 10- cm pads and >192 mL for the 45 X 45-cm pads; or under-estimation: less than ABL-20% which is <12 mL for the 10 X 10-cm pads and <128 mL for the 45 X 45-cm pads). Furthermore, the accuracy for each grouping variable was defined as >60% of participants that "correctly estimated" VEBL to within 20% of ABL. If less than 60% of participants in any grouping variable "correctly estimated" VEBL to within 20% of ABL, then VEBL was considered "Inaccurate" for this grouping variable.

Results

The definitions of "Accurate" vs. "Inaccurate" mean VEBL and "Accurate" vs. "Inaccurate" VEBL categories of estimation are given in the participants and methods section. Owing to the on-site survey administrator ensuring completion of each survey instrument, a 100% response rate was achieved.

General participants' characteristics

The characteristics (age, gender, nationality, profession, level of training, years of practice, and center designation) of all 200 participants recruited (100% response rate) are detailed in Table 1.

Table 1: Comparison of participants' characteristics and visually estimated blood loss (VEBL) for 10 X 10- and 45 X 45-cm gauze pads. Data shown are frequencies; n (%) or mean ± SD, confidence interval (CI) and p values, and effect size (Eta squared for Mann-Whitney U Test and for Kruskal-Wallis test). Total n = 200. ODPs = Operating Department Practitioner.

Variable		Value	VEBL for 10X10 cm	CI	p	Effect Size	VEBL for 45X45 cm	CI	p	Effect Size
Age, years		35.8 ± 9.02	-	-	-	-	-	-	-	-
Gender	Female	88 (44)	38.4 ± 37.2	-29, 19	0.14	0.05	237 ± 327.2	-141.2, 61.8	0.16	0.003
	Male	112 (56)	43.4 ± 80.4				197.3± 176.4			
Profession	Surgeon	80 (40)	44.6 ± 80.8	18.8, 70.4	0.46	0.04	218.8± 226.4	146, 291	0.87	0.038
	Anesthetist	42 (21)	49.8 ± 44.7	29.5, 70.1			189.3± 135.6	127, 251		
	ODPs	78 (39)	31.5 ± 37.9	19.2, 43.8			224.5± 323.3	119, 329		
Level of Training	Consultant	44 (22)	38.1 ± 31.6	25.7, 65.7	0.58	0.01	203.4± 157.9	106.8, 300.2	0.90	0.003
	Resident	84 (42)	45.7 ± 45.1	25.0, 51.2			203.5± 228.9	133.4, 273.4		
	Registered Nurse	72 (36)	31.9 ± 39.2	18.7, 45.2			208.5± 330.8	96.6, 320.4		
Years of Practice	<5 years	74 (37)	32.8 ± 27.7	-37, 12.2	0.05	0.015	188.2± 198.7	-146.5, 62.2	0.52	0.04
	>5 years	126 (63)	45.2 ± 72.3				230.4± 280.9			
Center Designation	Secondary	50 (25)	37.4 ± 36.4	-23.3, 31.8	0.80	0.03	215.4 ± 160	-117.5, 115.9	0.98	0.037
	Tertiary	150 (75)	41.7 ± 66.0				214.6± 278.5			
Nationality	Saudi	96 (48)	43.1 ± 74.6	-19, 28.7	0.68	0.01	214.8± 215.9	-101.2, 101.1	0.86	0.002
	Non-Saudi	104 (52)	38.3 ± 42.6				214.7± 285.7			

Accuracy of VEBL between genders

Regarding the mean VEBL, it was "Inaccurate" for both genders for both pad sizes (Table 1). For the categories of estimation accuracy, it was "Inaccurate" for both genders were for both pad sizes (Tables 2 and 3). There was no significant difference in accuracy between genders regarding the mean VEBL for either the 10 X 10-cm gauze pads or the 45 X 45-cm pads (Table 1). A significant difference was observed in genders for the categories of estimation accuracy (underestimation vs. correct estimation vs. overestimation) of VEBL for the 10 X 10-cm pads (Table 2). However, no such significant difference was found for the 45 X 45-cm pads (Table 3). Furthermore, the practical significance (effect size) of the gender variable was small (Table 2).

Accuracy of VEBL between professions

Mean VEBL was "Inaccurate" across all professions for the 10 X 10-cm pad size. However, it was "Accurate" for anesthetists for the 45 X 45-cm pad size (mean VEBL was within + 20% of ABL). It was "Inaccurate" for general surgeons and ODPs for the same pad size (Table 1). However, this difference (in accuracy between anesthetists and general surgeons) did not reach statistical significance in both pad sizes (Table 1). Regarding categories of estimation accuracy, VEBL was also inaccurate across all three professions for both pad sizes (Tables 2 and 3). Significant differences were not found between professions for the categories of estimation accuracy of VEBL for either the 10 X 10-cm pads (Table 2) or the 45 x 45-cm pads (Table 3).

Table 2: Comparison of participants' visual estimation of blood loss (VEBL) reliability (VEBL - actual blood loss (ABL)/actual blood loss x 100) by personal characteristics for 10 X 10-cm supersaturated gauze pad. Data shown are observed frequency of participants; n who estimated correctly, or over or under estimated vs. the (Expected number of participants), %; percentage of observed number of participants out of total number of participants for each grouping variable, X^2: chi-square test of independence, df: degree of freedom, Effect size (Cramer's V, correct estimation: VEBL within 20% of ABL, Under-estimation: VEBL 20% less than ABL, Overestimation: VEBL 20% more than ABL). ODPs = Operating Department Practitioner.

Variable	Category	Under-estimation	Correct Estimation	Over-Estimation	X^2 (df)	p	Effect Size
		Observed Frequency (Expected),%	Observed Frequency (Expected),%	Observed Frequency (Expected),%			
Gender	Female	34 (26.4), 39%	8 (6.2), 9%	46 (55.4),52%	7.8 (2)	0.02	0.197
	Male	26 (33.6), 23%	6 (7.8), 5%	80 (70.6),72%			
Profession	Surgeon	18 (23.2), 23%	16 (13.2), 20%	46 (43.6),57%	4.8 (4)	0.31	0.109
	Anesthetist	11 (12.2), 26%	7 (6.9), 17%	24 (22.9),57%			
	ODPs	29 (22.6), 37%	10 (12.9), 13%	39 (42.5),50%			
Level of Training	Consultant	12 (12.3), 27%	8 (6.2), 18%	24 (25.5),55%	6.8 (4)	0.15	0.131
	Resident	17 (23.5), 20%	11 (11.8), 13%	56 (48.7),67%			
	Registered Nurse	27 (20.2), 37%	9 (10.1), 13%	36 (41.8),50%			
Years of Practice	<5 years	16 (21.1), 22%	6 (6.7), 8%	52 (46.3),70%	3.2 (2)	0.20	0.126
	>5 years	41 (35.9), 32%	12 (11.3), 10%	73 (78.8), 58			
Center Designation	Secondary	9 (15.5), 18%	7 (4.8), 14%	34 (29.8),68%	5.9 (2)	0.053	0.171
	Tertiary	53 (46.5), 35%	12 (14.3), 8%	85 (89.3),57%			
Nationality	Saudi	23 (26.4), 24%	14 (12), 15%	59 (57.6),61%	1.5 (2)	0.50	0.088
	Non-Saudi	32 (28.6), 31%	11 (13), 11%	61 (62.4),58%			

Accuracy of VEBL between consultants, residents, and registered nurses

Mean VEBL was "Inaccurate" for both pad sizes across all levels of training (Table 1); regarding categories of estimation accuracy, VEBL was also "Inaccurate" (Tables 2 and 3). There were no significant differences between the three categories of levels of training regarding mean VEBL for the 10 X 10-cm pads or for the 45 X 45-cm pads (Table 1). No significant differences were found between the level of training categories for the categories of estimation accuracy of VEBL for the 10 X 10-cm

pads (Table 2) or for the 45 X 45-cm pads (Table 3).

Accuracy of VEBL between categories of years of practice (<5 years vs. >5 years)

Although the mean VEBL was not significantly different between categories of years of practice for the 10 X 10-cm or the 45 X 45-cm pads, it had trend toward statistical significance for the 10 X 10-cm pads (p = 0.05) (Table 1). However, both categories of experience were "Inaccurate" regarding mean VEBL.

Both categories of experience were "Inaccurate" regarding the categories of estimation of VEBL for both pad sizes (Tables 2 & 3). Although there was no significant difference in the categories of estimation accuracy for VEBL for the 10 X 10-cm pads (Table 2), significant difference was observed between categories of years of practice regarding categories of estimation accuracy of VEBL for 45 X 45-cm pads (Table 3). The more experienced categories of participants were more likely to correctly estimate & less prone to under- or overestimate than the less experienced participants; with a small practical significance (Cramer's V = 0.190) (Table 3).

Table 3: Comparison of participants' visual estimation of blood loss (VEBL) reliability (VEBL - actual blood loss (ABL)/actual blood loss x 100) by personal characteristics for the 45 X 45-cm supersaturated gauze pad. Data shown are observed frequency; n of participants who estimated correctly, or over or under estimated vs. the (Expected number of participants), %: percentage of observed number of participants out of total number of participants for each grouping variable, Chi-square test of independence, df: degree of freedom, Effect size (Cramer's V, correct estimation: VEBL within 20% of ABL, Under-estimation: VEBL 20% less than ABL, Overestimation: VEBL 20% more than ABL). ODPs = Operating Department Practitioner.

Variable	Category	Under-estimation	Correct Estimation	Over-Estimation	X^2	p	Effect Size
		Observed Frequency (Expected), %	Observed Frequency (Expected), %	Observed Frequency (Expected), %			
Gender	Female	37 (40.9), 42%	15 (12.3), 17%	36 (34.8), 41%	1.8 (2)	.41	0.095
	Male	56 (52.1), 50%	13 (15.7), 12%	43 (44.2), 38%			
Profession	Surgeon	38 (37.2), 48%	9 (10.8), 11%	33 (32.0), 41%	1.04 (4)	.90	0.072
	Anesthetist	21 (19.5), 50%	6 (5.7), 14%	15 (16.8), 36%			
	ODPs	34 (36.3), 44%	12 (10.5), 15%	32 (31.2), 41%			
Level of Training	Consultant	16 (20.5), 36%	7 (5.7), 16%	21 (17.8), 48%	3.5 (4)	.47	0.094
	Resident	42 (39.1), 50%	8 (10.9), 9%	34 (34),41%			
	Registered Nurse	35 (33.5), 49%	11 (9.4), 15%	26 (29.2), 36%			
Years of Practice	<5 years	43 (34), 58%	6 (9.3), 8%	25 (30.7), 34%	7.3 (2)	.03	0.190
	>5 years	49 (58), 39%	19 (15.8), 15%	58 (52.3), 46%			
Center Designation	Secondary	20 (23), 40%	7 (6.3), 14%	23 (20.8), 46%	0.97 (2)	.62	0.070
	Tertiary	72 (69), 48%	18 (18.8), 12%	60 (62.3), 40%			
Nationality	Saudi	49 (44.2), 51%	8 (13), 8%	39 (38.9), 41%	4.7 (2)	.09	0.153
	Non-Saudi	43 (47.8), 42%	19 (14), 18%	42 (42.1), 40%			

Accuracy of VEBL between secondary and tertiary centers

Mean VEBL was "Inaccurate" for both secondary and tertiary centers, for both pad sizes (Table 1). Regarding the categories of estimation, VEBL was "Inaccurate" for both types of center designations for both pad sizes (Tables 2 and 3). There was no significant difference in mean VEBL between secondary and tertiary centers for the 10 X 10-cm pads or the 45 X 45-cm pads (Table 1). No significant differences occurred between center designation categories for the categories of estimation accuracy for the 10 X 10-cm pads (although it tended toward significance at p = 0.053) (Table 2) or for the 45 X 45-cm pads (Table 3).

Accuracy of VEBL between Saudis and non-Saudis

Mean VEBL was "Inaccurate" for both Saudis and non-Saudis for both pad sizes (Table 1). Regarding categories of estimation, VEBL was "Inaccurate" for both Saudis and non-Saudis for both types

of pad sizes (Tables 2 and 3). There was no significant difference between Saudis & non-Saudis regarding mean VEBL for the 10 X 10-cm or 45 X 45-cm pads (Table 1) or the categories of estimation accuracy for both types of pads (Tables 2 and 3).

Discussion

The results of this study showed that the mean VEBL estimated by the general surgeons was "Inaccurate" (>20% overestimation of the ABL) for both the 10 X 10- and 45 X 45-cm pads (Table 1). Only 20% and 11% of general surgeons correctly estimated VEBL (within -20% underestimation to +20% overestimation of the ABL) for the 10 X 10- and 45 X 45-cm pads, respectively (Tables 2 and 3). However, the general surgeons were more likely, though non-significantly, to correctly estimate and overestimate VEBL for the categories of estimation than anesthetists or ODPs, for both pad sizes (Tables 2 and 3). These results are in agreement with those of another study showing that anesthetists tend to underestimate blood loss [25]. However, the findings are in contrast to the popular belief that general surgeons tend to underestimate blood loss compared to anesthetists who tend to overestimate blood loss[21]. The findings were in contrast with those of other studies that reported the accuracy of VEBL by both junior and senior surgeons compared to anesthetists, where only senior anesthetists were accurate in their estimation[26].

The other two professions (anesthetists and ODPs) were also "Inaccurate," although non-significantly, in terms of categories of VEBL estimation for both pad sizes (Tables 2 and 3). However, ODPs were more likely to correctly estimate than anesthetists for the 45 X 45-cm pads (Table 3) while anesthetists were more likely to correctly estimate than ODPs for the 10 X 10-cm pads (Table 2). These results are in agreement with those of Ashraf et al., showing that anesthetists provided the closest estimation to the ABL[25]. In this study, anesthetists were the only professionals met the definition of "Accurate," although non-significantly, in terms of the mean VEBL for the 45 X 45-cm pads (Table 1). The anesthetists were also "Inaccurate" in terms of the mean VEBL for the 10 X 10-cm pads (Table 1). This result reveals the important effect of pad size and volume of blood loss on the accuracy of VEBL[13,16,23,27-29]. Several previous studies have shown inaccurate VEBL, associated with high intra- and inter-observer variability[11,13,19,30].

This study also showed inaccurate VEBL not only across all the three professional categories (surgeons, anesthetists, and ODPs) but also across all levels of training (residents, consultants, and registered nurses). These findings are supported by those of other studies where medical students and experienced faculty demonstrated similar error rates[15,16], and in contrast to those of another study showing that although both junior and senior surgeons were accurate (i.e., no difference across levels of training of surgeons), only senior anesthetists were accurate[26].

Further, many studies have shown inaccurate VEBL across both genders and nationalities[11,13,15,19,30-34]. This study showed a significant difference between genders regarding the frequency of events in the estimation categories of VEBL for the 10 X 10-cm pads (Table 2). This result is in contrast with that of other studies that have shown no gender impact on estimation accuracy[15,34]. However, this difference had a small practical significance (effect size = 0.197, Table 2) but no significant difference between genders, neither for the mean VEBL for both types of pad sizes (Table 1) nor for the frequency of events for the estimation categories of VEBL for the 45 x 45-cm pads (Table 3).

The findings of this study are in contrast with those of other studies showing that accuracy is affected by the type of professional membership[7,35]. However, they are consistent with those of many other studies that have shown no impact of professional membership on estimation accuracy[13,36]. Our study results were also consistent with those of other studies that have shown the impact of pad size and volume lost on the accuracy of estimation[13,16,27,28]. To the best of our

knowledge, this is the first study to compare the impact of center designation on the accuracy of VEBL, and there were no significant differences between secondary and tertiary centers. This might suggest that institutional practices and patient volume load has no impact on the accuracy of estimation. This result is also consistent with other findings, showing no differences across levels of training and showing that informal and unintentional education by exposure to blood loss is not effective in improving accuracy and that more formal and intentional educational interventions are necessary[16,19,27].

The type of surgical access, even for the same procedure, has been shown as an independent factor of the accuracy of estimation[20]. This study showed that the only significant difference for years of practice occurred for the estimation categories of VEBL for the 45 X 45-cm pads (Table 3), with a small practical significance of 0.190 (Table 3). The more experienced category was more likely to correctly estimate, less likely to underestimate, and more likely to overestimate VEBL than the less experienced category. These results are in contrast with those of other studies showing that experience has no effect on estimation accuracy[11,13,15,16,27,36]. However, this effect had a small practical significance (Cramer's V = 0.190, Table 3).

This study showed that, in general, healthcare providers tended to underestimate or overestimate VEBL and were less likely to correctly estimate VEBL within 20% of ABL. This finding is in line with the results of other studies that VEBL is inaccurate and unreliable, with high inter-observer variability, which makes VEBL highly subjective and non-valuable in decision making regarding blood loss management. Thus, the inaccuracy necessitates either abandoning VEBL and reverting to the more objective gravimetric and photometric estimations of ABL[5,13] or searching for interventional educational measures to improve its accuracy and reliability [8,11,16]. Multiple studies have shown the accuracy and reliability of ABL measurement methods, e.g., gravimetric, photometric, colorimetric, calculated blood loss using laboratory hematocrit, or red blood cell radioisotope tagging[5,6,13,22,25,37]. However, these objective ABL measurement methods are too expensive and logistically demanding to be widely adopted and implemented as the standard.

Many studies have shown the positive impact of educational interventions on VEBL accuracy and reliability, using multiple educational measures, e.g., gauze visual analog scale, web-based training, simulation-based training, conventional training, graded bag collector measurement, clinical reconstructions, Objective Structured Clinical Examinations training sessions, and written and photographic instructions[16,19,27,38-43]. However, most studies in the literature have focused on VEBL accuracy and reliability and the impact of a randomized educational intervention on such accuracy and reliability targeted midwives and obstetrics and gynecology practitioners, or anesthetists, operating department practitioners, and paramedics; very few have targeted general surgeons. Therefore, this study is the first in Saudi Arabia to target general surgeons of all levels of training and compared their level of accuracy and reliability to those of anesthetists and ODPs. Further research in Saudi Arabia should explore the impact of different educational intervention programs using a randomized controlled trial design, repeated measure methodology, systematic review, and evidence synthesis to improve the objectivity and reliability of VEBL and its impact on decision making.

Conclusion

This study results showed that VEBL was an inaccurate method of intraoperative blood loss quantification and was associated with gross under/overestimation that made it an unsafe strategy to inform decision-making intraoperatively, for the detection or management of serious intraoperative blood loss. This inaccuracy was not related to professional membership, level of training, center designation, or nationality. However, this study is the first to show the effect of years of

experience and gender on VEBL and is the first study on general surgeons' accuracy in visually estimating blood loss in Saudi Arabia. There were significant differences with small clinical significance in the accuracy of VEBL between categories of years of experience; better with the more experienced group for the large pad size, and, between genders; better with female participants for the small pad size. Proper educational interventions might help in improving VEBL accuracy and reliability. However, competence in VEBL training should be a standard and a target for quality assurance in operating departments, which should be regularly measured and maintained.

Limitations of the Study

Limitations of this study include the fact that participants were limited to the Riyadh region, which might have led to selection bias in terms of qualifications of participants compared to those in peripheral hospitals, and the inherent limitations of a cross-sectional study design. The strengths of this study included its sample size, making it well powered. Furthermore, the use of whole fresh blood to ensure authenticity instead of either diluted or stored blood, might be a confounding factor, as the color intensity, which is determined by the hematocrit percentage, might have led to an underestimation. Further research should study the impact of educational interventions on the accuracy of VEBL, the durability of such impact, and its effect on operative morbidity and mortality as well as the timeliness and appropriateness of blood transfusion.

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Conflict of Interests

The author declared no conflict of interests.

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A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided. Non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords

Immediately after the abstract, provide a maximum of 10 keywords for full papers, or 5 keywords for Short Communications, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Please use terms from the most current issue of medical subject headings of Index Medicus. The key words should cover precisely the contents of the submitted paper and should give readers sufficient information as to the relevance of the paper to his/her particular field. Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Introduction

Provide adequate background that highlights the importance and gap information of your research point in relation to previous studies but avoiding a detailed literature survey. State the hypothesis or rationale and objectives of the work and a brief description of how you planned to approach them.

Materials or Patients and Methods

Provide sufficient detail to allow the work to be reproduced, with details of supplier and catalogue number when appropriate. Methods already published should be indicated by a reference: only relevant modifications should be described.

Patients and Normal Subjects

If human participants were used in the experiment please make a statement to the effect that this study has been approved by your Institution Ethics Review Board for human studies (the number of the approval should be stated in the methods section and JUMJ may ask for submission of the original ethical approval with the manuscript), and, that patients or their custodians have signed an informed consent that also states right of withdrawal without any consequences. Sample sized should be appropriately calculated. The manuscript should describe how the size of the experiment was planned. If a sample size calculation was performed this should be reported in detail, including the expected

difference between groups, the expected variance, the planned analysis method, the desired statistical power and the sample size thus calculated. For parametric data, variance should be reported as 95% confidence limits or standard deviations rather than as the standard error of the mean. Normal participants and patients criteria, inclusion and exclusion criteria should be stated. Name and address where the work was done and when it was done (time period, from …. to …..) should be clearly stated, too.

Experimental animals

When animals were used in the experiments, a local Institutional Ethics Review Board for animal studies should review and approve the experiment and that all animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and according to National Institute of Health (NIH) protocol. The precise species, strain, sub-strain and source of animals used should be stated. Where applicable (for instance in studies with genetically modified animals) the generation should also be given, as well as the details of the wild-type control group (for instance littermate, back cross etc.). The manuscript should describe the method by which animals were allocated (randomized) to experimental groups, particularly for comparisons between groups of genetically modified animals (transgenic, knockout etc.), the method of allocation to for instance sham operation or focal ischemia should be described.

Experimental

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Where and when the study was conducted should be stated.

Results

Results should be clear and concise. Data should be presented in an appropriately organized tables, figures and/or artworks. The statistical analysis used should be suitable for the objectives of the study and type of data analyzed. Prospective authors are highly advised to consult a biostatician.

Footnotes

Footnotes should be used sparingly. For table footnotes, indicate each footnote in a table with a superscript lowercase letter or add them into the title.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. It is preferable to be inserted at its normal place to the relevant text or otherwise be submitted as a separate TIFF, EPS, PDF or MS Office files.

Discussion

This should explore the significance, interpretation and reasoning of the results of the work vs. other studies. Do not repeat describing the results in this section. A combined Results and Discussion section is acceptable. Avoid extensive citations and discussion of published literature. In the same time, avoid speculations without a supporting literature. Avoid discussion based on "Data not Shown" or "Personal Communications".

Limitations and Future Prospective

The authors may wish to pinpoint the limitations of the study and their reason and foresee the next step to go from their study. This may be presented in a short Limitations and Future Prospective section standing alone or as a separate paragraph in the Discussion or Results/Discussion section.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section standing alone or as a separate paragraph at the end of the Discussion or Results/Discussion section. Conclusions should not be biased and should be based on the data, presented and discussed inside the manuscript only.

Gain of Knowledge

Following the conclusion section, it is mandatory for manuscripts submitted for final publication in JUMJ to have a Gain of Knowledge section that is consisted of 2 - 5 bullet points (maximum 90 characters, including spaces, per bullet point) that convey the core findings of the article.

Acknowledgements and Funding

Collate acknowledgements in a separate section at the end of the article before the references. List individuals or organizations that provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Whoever would

be acknowledged should be informed and a verification for that could be requested by JUMJ Editor. If funded, the source of funding should be mentioned.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly, for tables and figures: Table A.1; Fig. A.1, etc.

CASE REPORT WRITING TEMPLATE

Title. Include the words "case report" in the title. Describe the phenomenon of greatest interest (e.g., symptom, diagnosis, diagnostic test, intervention, and outcome).

Abstract. Summarize the following information if relevant: 1) Rationale for this case report, 2) Presenting concerns (e.g., chief complaints or symptoms, diagnoses), 3) Interventions (e.g., diagnostic, preventive, prognostic, therapeutic exchange), 4) Outcomes, and 5) Main lesson(s) from this case report.

Key Words. Provide 3 - 8 key words that will help potential readers search for and find this case report.

Introduction. Briefly summarize the background and context of this case report.

Presenting Concerns. Describe the patient characteristics (e.g., relevant demographics - age, gender, ethnicity, occupation) and their presenting concern(s) with relevant details of related past interventions.

Clinical Findings. Describe: 1) the medical, family, and psychosocial history including lifestyle and genetic information; 2) pertinent co-morbidities and relevant interventions (e.g., self-care, other therapies); and 3) the physical examination (PE) focused on the pertinent findings including results from testing.

Timeline. Create a timeline that includes specific dates and times (table, figure, or graphic).

Diagnostic Focus and Assessment. Provide an assessment of the; 1) diagnostic methods (e.g., PE, laboratory testing, imaging, questionnaires, referral), 2) diagnostic challenges (e.g., financial, patient availability, cultural), 3) diagnostic reasoning including other diagnoses considered, and, 4) prognostic characteristics (e.g., staging) where applicable.

Therapeutic Focus and Assessment. Describe: 1) the type(s) of intervention (e.g., preventive, pharmacologic, surgical, lifestyle, self-care) and 2) the administration and intensity of the intervention (e.g., dosage, strength, duration, frequency.

Follow-up and Outcomes. Describe the clinical course of this case including all follow-up visits as well as 1) intervention modification, interruption, or discontinuation, and the reasons; 2) adherence to the intervention and how this was assessed; and 3) adverse effects or unanticipated events. In addition, describe: 1) patient-reported outcomes, 2) clinician-assessed and -reported outcomes, and 3) important positive and negative test results.

Discussion. Please describe: 1) the strengths and limitations of this case report including case management, 2) the literature relevant to this case report (the scientific and clinical context), 3) the rationale for your conclusions (e.g., potential causal links and generalizability), and 4) the main findings of this case report: What are the take-away messages?

Patient Perspective. The patient should share his or her experience or perspective of the care in a narrative that accompanies the case report whenever appropriate.

Informed Consent. Did the patient or their custodian give the author of this case report informed consent? Provide if requested .

Case Report Submission Requirements: 1) Competing interests, are there any competing interests? 2) Ethics Approval, Did an ethics committee or institutional review board review give approval? If yes, please provide if requested, 3) De-Identification, has all patient's related data been de-identified?

RANDOMIZED CLINICAL TRIALS WRITING TEMPLATE

In this particular type of original study, individuals are randomly allocated to receive or not receive a preventive, therapeutic, or diagnostic intervention and then followed up to determine the effect of the intervention. All randomized clinical trials should include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; http://www.consort-statement.org) and a trial protocol.

Authors of randomized controlled trials are encouraged to submit trial protocols along with their manuscripts.

All clinical trials must be registered (before recruitment of the first participant) at an appropriate online public that must be independent of for-profit interest, e.g.:

- http://www.clinicaltrials.gov;
- http://www.anzctr.org.au;

- http://www.umin.ac.jp/ctr;
- http://isrctn.org;
- http://www.trialregister.nl/trialreg/index.asp).

Each manuscript should clearly state an objective or hypothesis; the design and methods (including the study setting and dates, patients or participants with inclusion and exclusion criteria, or data sources, and how these were selected for the study); the essential features of any interventions; the main outcome measures; the main results of the study; a comment section placing the results in context with the published literature and addressing study limitations; and the conclusions.

Data included in research reports must be original. A structured abstract not exceeding 300 words is required. Clinical trials are limited to 2700 words (not including abstract, tables, figures, and references), 40 references, and no more than 5 tables and figures.

REVIEW, MINIREVIEW AND META-ANALYSIS PAPERS

These papers will not have empirical data acquired by the authors but will include historical perspectives, analysis and discussion of papers published and data acquired in a specific area.

Systematic reviews and meta-analyses are a particular type of original articles that perform systematic, critical assessment of literature and data sources pertaining to clinical topics, emphasizing factors such as cause, diagnosis, prognosis, therapy, or prevention. All articles or data sources should be searched for and selected systematically for inclusion and critically evaluated, and the search and selection process should be described in detail in the manuscript. The specific type of study or analysis, population, intervention, exposure, and tests or outcomes should be described for each article or data source. A structured abstract of less than 300 words is required. The text is limited to 3500 words (not including abstract, tables, figures, and references); about 4 tables (a flow diagram that depicts search and selection processes as well as evidence tables should be included) - and no reference limit.

Minireview is a brief historical perspective, or summaries of developments in fast-moving areas covered within the scope of the journal. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of the journal. The goal of the minireview is to provide a concise very up-to-date summary of a particular field in a manner understandable to all readers.

SHORT COMMUNICATION AND SHORT RESEARCH ARTICLE

Short Communications are urgent communications of important preliminary results that are very original, of high interest and likely to have a significant impact on the subject area of the journal. A Short Communication needs only to demonstrate a 'proof of principle'. Authors are encouraged to submit an Original Research Paper to the journal following their Short Communication. There is no strict page limit for a Short Communication; however, a length of 2500-3500 words, plus 2-3 figures and/or tables, and 15-20 key references is advisable. Short Research Article may be smaller single-result findings as a brief summary that include enough information, particularly in the methods and results sections, that a reader could understand what was done.

POLICY PAPER

The purpose of the policy paper is to provide a comprehensive and persuasive argument justifying the policy recommendations presented in the paper, and therefore to act as a decision-making tool and a call to action for the target audience.

COMMENTARIES/OPINION ARTICLES

An opinion-based article on a topical issue of broad interest, which is intended to engender discussion.

STUDY PROTOCOLS AND PRE-PROTOCOLS

JUMJ welcomes publishing protocols for any study design, including observational studies and systematic reviews. All protocols for randomized clinical trials must be registered and follow the CONSORT guidelines; ethical approval for the study must have been already granted. Study pre-protocols (i.e., discussing provisional study designs) may also be submitted and will be clearly labeled as such when published. Study protocols for pilot and feasibility studies may also be considered.

METHOD ARTICLES

These articles describe a new experimental or computational method, test or procedure, and should have been well tested. This includes new study methods, substantive modifications to existing methods or innovative applications of existing methods to new models or scientific questions. We also welcome new technical tools that facilitate the design or performance of experiments or operations and

data analysis such as software and laboratory and surgical devices, or of new technologies to assist medical diagnosis and treatment such as drug delivery devices.

Maximum length of submissions

Full length original research articles should not exceed 10000 words (maximum 60 references), and up to 6 tables and/or figures.

Short communications comprising up to 1800 words of text, maximum 15 references, and two illustrative items (Tables and/or Figures).

Letters and Case Reports (provide novel insight into disease mechanisms, diagnostic and management applications). *Clinical Laboratory Notes* (technical evaluation or important insight into analytical methodology), or *Letters to the Editor* (focused on a specific article that has appeared in JUMJ within 4 weeks of print issue date of article). For all 3 types of letters listed above, the text should not exceed 600 words, with no abstract, a maximum of 1 table or figure and up to 5 references.

Review Articles, Surveys, Essays, and Special Reports may exceed the word and reference limit for Full-length articles as per the comprehensive nature of these articles. However, both of these articles (Reviews and Special Reports) will still require an abstract (unstructured, 350 word maximum).

Editorials, Meeting summary, Commentaries, Book review and Opinion pieces will not require an abstract and will be limited to 2000 words and up to 20 references. A book review is a brief critical and unbiased evaluation of a current book determined to be of interest to the journal audience. Publication of a submitted book review is at the discretion of the editor.

Artwork

General points

Make sure you use uniform lettering and sizing of your original artwork. Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier. Number the illustrations according to their sequence in the text. Use a logical naming convention for your artwork files. Indicate per figure if it is a single, 1.5 or 2-column fitting image. For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below). Please do not supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low, supply files that are too low in resolution, and, submit graphics that are disproportionately large for the content.

- EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
- TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
- TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
- TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures the Journal will ensure that these figures will appear in color on the Web regardless of whether or not these illustrations are reproduced in color in the printed version. Because of technical complications which can arise by converting color figures to 'gray scale' please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption (Legend). A caption should comprise a brief title below the figure that describes its content and not to be general. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used in the legend. Figure caption should stand for itself (self-explanatory) without the need for consulting the text.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters within the table. If necessary, such footnotes could be placed at the end of the table title. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article (Figures or text). The table caption (Title) should be brief but describes its content and not to be general. Explain all symbols and abbreviations used in the table in the footnote. Table title should stand for itself (self-explanatory) without the need for consulting the text. The table structure should be scientifically organized

(columns and rows) and its message should be easily comprehendible.

The Editor-in-Chief, on accepting a manuscript, may recommend that additional tables and/or graphs containing important backup data, too extensive to be published in the article, may be published as supplementary material. In that event, an appropriate statement will be added to the text. However, the author should submit such material for consideration with the manuscript.

References

References cited should be relevant, up-to-date and adequately cover the field without ignoring any supportive or conflicting publications. Please ensure that every reference cited in the text is also present in the reference list (and vice versa). If present, unpublished results and personal communications may be mentioned in the text and not in the reference list. Citation of a reference as 'in press' implies that the item has been accepted for publication and shows up on PubMed literature search or a copy of the title page of the relevant article must be submitted. DOI of the references - whenever applicable should be presented. Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.

Reference management software

This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to word processing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style, which is described below.

Reference formatting

There are no strict requirements on reference formatting at submission but should be consistent, complete and up-to-date. Where applicable, author(s) name(s), chapter title/article title, journal title/book title, year of publication, volume number-issue number/book chapter and the pagination must be present. For the book reference, the edition number, editors (if they are not the authors), publisher and its main address (City and Country) should be added as described below in the example. The reference style used by the journal should be applied to the accepted article at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. Use peer-reviewed references only except for national and international organizational reporting and registers. If you do wish to format the references yourself, they should be arranged according to the following examples:

Reference style

Indicate references by number(s) in curved brackets as a bolded superscript at the end of the cited text(s) before the full stop, e.g., …… shorter hospital stay and lower cost[20]. The actual authors can be referred to, but the reference number(s) must always be given. Number the references in the list in the order in which they appear in the text. The authors list should not be shortened, all authors' names should be mentioned up to 10 authors and end longer list by et al. For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997; 277: 927-34) (see also http://www.nlm.nih.gov/bsd/uniform_requirements.html).

Examples:

Reference to a journal publication: Format your journal publications according to the following examples depending on whether; 1) It is already published with specific page numbers, 2 and 3) It is already published with article ID number and pages from 1 to …, 4) It is published put ahead of print, or, it is accepted for publication.

1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J. Sci. Commun., 2010;163(1):51-9.
2. Leta S, Dao TH, Mesele F, Alemayehu G. Visceral Leishmaniasis in Ethiopia: An Evolving Disease. PLoS Negl Trop Dis., 2014; 8(9):e3131;1-7.
3. Arjmand MH, Ahmad Shah F, Saleh Moghadam M, Tara F, Jalili A, Mosavi Bazaz M, Hamidi Alamdari D. Prooxidant-antioxidant balance in umbilical cord blood of infants with meconium stained of amniotic fluid. Biochem Res Int., 2013;2013:ID270545;1-4.
4. Teferra RA, Grant BJ, Mindel JW, Siddiqi TA, Iftikhar IH, Ajaz F, Aliling JP, Khan MS, Hoffmann SP, Magalang UJ. Cost minimization using an artificial neural network sleep apnea prediction tool for sleep studies. Ann Am Thorac Soc., 2018 Jul 28 (ahead of print).
5. Alduraywish AA, Almani AZ, Alanazi AD-A, Alruwaili FS, Alolaywi AN, Almaeen AH, El-Metwally TH. Vitamin D insufficiency among healthy participants

and type 2 diabetic patients from the northern Al-Jouf region of Saudi Arabia: Correlation with the prognostic indices of the disease. International Medical J, 2019 (Accepted for publication; http://www.seronjihou.co.jp/imj/).

Reference to a book: Strunk Jr W, White EB (Editors). The elements of style, 4th Edition, Longman, New York; 2000, pp. 210-9.

Reference to a chapter in an edited book: Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ (Editors), Introduction to the electronic age, 1st Edition, E-Publishing Inc., New York, 2009, Chapter 2: pp. 281-304.

Reference to a homepage: It is acceptable to refer to an Organizational Guidelines, Reports, Forms, Data sheets, Questionnaires, etc. It should follow the following format. World Health Organization. Non-communicable Diseases (NCD) Country Profile, 2014 (http://www.who.int/globalcoordinationmechanism/publications/ncds-country-profiles-eng.pdf; last accessed March 1, 2017).

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Abbreviations and units

Standard abbreviations as listed in the Council of Biology Editors Style Manual may be used without definition. Use non-standard abbreviations sparingly, preceding their first use in the text with the corresponding full designation. Use units in conformity with the standard International System (SI) of units.

Video data

The journal accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. To ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article. Since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Audio Slides

JUMJ encourages authors to create an Audio Slides presentation with their published article as supplementary material. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. Authors of this journal will automatically receive an invitation e-mail to create an Audio Slides presentation after acceptance of their paper.

Supplementary data

JUMJ accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file.

Supplementary material captions

Each supplementary material file should have a short caption which will be placed at the bottom of the article, where it can assist the reader and also be used by search engines.

THE COPYRIGHTS

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

To avoid unnecessary errors, you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details for all authors:

- E-mail address.
- Full postal address.
- Telephone.

All necessary files have been uploaded, and contain:

- Keywords.
- All figures and their captions.
- All tables (including title, description, footnotes).

Further considerations:

- Manuscript has been 'spell-checked' and 'grammar-checked'.
- All references mentioned in the Reference list are cited in the text, and vice versa.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- Color figures are clearly marked as being intended for color reproduction on and in print, or to be reproduced in color electronically and in black-and-white in print.

PEER REVIEW PROCESS

High quality manuscripts are peer-reviewed by minimum of two peers of the same field along with a biostatistician in the case the study requires. Pre-reviewing advice and help will be provided by the Editor-In-Chief on first submission for initial improvements to meeting the minimum criteria of peer-reviewing. The journal follows strict double-blind fold constructive review policy to ensure neutral evaluation. During this review process identity of both the authors and reviewers are kept hidden to ensure unbiased evaluation. A cycle of one-month reviewing process is the target of the journal from submission to final acceptance. For meeting this goal, the Editor-In-Chief is expecting strict compliance from author hastening corrections and replying editorial requests. Continuous post-publication open peer reviewing is highly encouraged through submitting comments to the Editor on any of the published article that will show up with author reply in the subsequent issue to the journal.

The reviewers' comments are sent to authors once received. With the help of the reviewers' comments, FINAL decision (accepted or accepted with minor revision or accepted with major revision or rejected) will be sent to the corresponding author. Reviewers are asked if they would like to review a revised version of the manuscript. The editorial office may request a re-review regardless of a reviewer's response in order to ensure a thorough and fair evaluation.

In order to maintain this journal's mission of one-month publication cycle, authors are encouraged to submit the revised manuscript within one week of receipt of reviewer's comment (in case of minor corrections). However, revised manuscript submission should not go beyond 2 weeks (only for the cases of major revision which involves additional experiment, analysis etc.). Along with corrected manuscript authors will be requested to submit filled a point-by-point answers to the reviewers' comments and any rebuttal to any point raised. The Editor-In-Chief of the journal will have exclusive power to take final decision for acceptance or rejection during any dispute.

Under special circumstance, if the review process takes more time, author(s) will be informed accordingly. The editorial board or referees may re-review manuscripts that are accepted pending revision. Manuscripts with latest and significant findings will be handled with the highest priority so that it could be published within a very short time. The journal is determined to promote integrity in research publication. In case of any suspected misconduct, the journal management reserves the right to re-review any manuscript at any stages before final publication.

Our massage to JUMJ potential reviewers says "Although the Manuscript General Evaluation Form is attached, we like to instigate a policy of constructive reviewing and to do our best to make the submitted manuscripts publishable - provided that it is genuine and contain no major frauds of republication, duplicate use of self-data or plagiarism of intellectual properties of the others. Please, make your changes and insert your corrections, comments and suggestions directly into the manuscript text but in a different color. Please also make sure that the author(s) presented an inclusive and updated list of genuine references, applied proper statistics and extracted justified conclusions".

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The Manuscript Assigned Number and Title: ……..

Although the following manuscript general evaluation form is sent to reviewers, JUMJ asks the reviewer for further meticulous one-

word-at-a-time revision, please. Please insert corrections, changes, suggestions, questions, comments and points of deficiency directly into the manuscript text but in a different color. Also, please do not worry much for the formatting.

The Manuscript Evaluation Score: Please, score the manuscript from 0 to 4 (highest) for each of the following items, and sum the total score. Please, also check if the statistics of the results require revision.

Items \ Scores	0	1	2	3	4	Item Score
The Study is a Priority Problem						
Originality						
Significance of the Work						
Research Design						
Quality and Clarity of the English Writing						
Standard and Reproducible Methodology						
Results Presentation and Appropriate Statistics						
Relevant Discussion and Justified Conclusions						
Tables/ Illustrations/ Figures						
References: Inclusive and Updated						
Total score						
Requires Statistical Revision (Mark Please)				Yes		No

Justification for Decision & Feedback for the Author (REQUIRED): JUMJ recommends the reviewer to introduce such justifications and feedback into the text at the appropriate places within the manuscript (Specific and Comprehensive Revision).

Note to the Editor (WELCOMED): JUMJ welcomes reviewer writing a note to the Editor including conflict of interest that will not be disclosed to the Author(s).

The Decision: Should JUMJ publish the manuscript? Please, check the appropriate box.

Yes, without any revision:	Accepted	
Yes, with minor revision and alterations:	Accepted with revision	
Yes, with major revision and alterations:	Re-Evaluation after substantial revision	
No, this manuscript should be denied publication:	Denied	

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with annotation for correction of MS format proofs and resend the corrected version. All instructions for proofing will be given in the e-mail we send to authors. We will do everything possible to get your article published quickly and accurately - please upload all of your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via email (the PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use). For an extra charge, paper offprints can be ordered at any time via the offprint order form, which is sent once the article is accepted for publication.

Policy Regarding Corrections & Retractions

JUMJ differentiates the post-publication amendments into: Comments, Addenda, Corrections, and Retractions (or Partial Retractions).

Comments and Replies: An interested reader may approach the Editorial Office in cases of questionable results or experimental design and methods of a published article. Upon request, the Editorial Office will ask the reader to formulate his/her argument in a one-page evidence-supported comment on the article. In

case the comment is valid after revision, the Editorial Office will approach the authors of the article in question and invite them to prepare a Reply. Reply will be reviewed, and if accepted, will be compiled with the comment for publication in the current issue of JUMJ, with page numbers added - with a hyperlink to the Comments and Replies appearing on the original publication. If the reader's complaints are substantiated without a valid refute from the authors, the authors in collaboration with the Editorial Office may consequently publish a Correction or decide to retract the paper entirely.

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