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Activated Protein C Resistance in Adult Sudanese Patients diagnosed with Deep Vein Thrombosis

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ABSTRACT

Resistance to activated protein C in both its acquired and hereditary forms is a known risk factor for the development of venous thromboembolic disorders. It is reported to be found in 3% to 5% of the general population and it is responsible for 20% to 50% of thrombosis in patients. The prevalence of Activated Protein C Resistance (APCR) is estimated to be high in Caucasians, while Asians and Africans are thought to have a low incidence . Among Arabs, there is a difference in the prevalence of APCR in different sub-nationalities. The prevalence in Sudanese subjects has not so far been determined in spite of the high incidence of DVT, so this cross- sectional study was carried out to determine the prevalence of activated protein C resistance among adult Sudanese patients with Deep vein thrombosis (DVT). One hundred and thirty eight adult patients admitted to Khartoum State hospitals who were diagnosed as having DVT were included in this study. APCR ratios were determined for them using a semi automated coagulometer. (Thrombotimer 4 channel coagulometer). Prevalence of APCR in the studied group was found to be (33.3%). Inherited APCR seemed to be more prevalent among those patients than the the acquired one. Family history, age, and some associated clinical conditions like postnatal period, pregnancy and cancer were significantly associated with APCR.

KEYWORDS: Activated Protein C Resistance (APCR), Deep vein thrombosis (DVT), Thrombophilia

INTRODUCTION

Protein C (PC) is a Vitamin K dependent glycoprotein and is a potent natural inhibitor of coagulation which provides a mechanism to limit clotting at the vicinity of tissue injury (Cheryl A, John A et al ,1998). After its activation on the endothelial cells by a complex of thrombin with thrombomodulin, activated protein C (APC) inhibits coagulation by selectively degrading coagulation factors Va and VIIIa. Protein S functions as a cofactor in this reaction (Cheryl A et al. ,1998).

Activated Protein C Resistance (APCR) is a recently described haemostatic disorder discovered by Dahlback et al in Holland (Dahlback B et al,1993), and it remains an important heritable and the most common cause of hypercoagulability since its discovery. Cheryl A et al, (1998) ,Hoffbrand A et al, (2003). It characterized by a poor response to the anticoagulant effect of APC due to inability of PC to cleave factor Va and VIIIa, which allows for longer duration of thrombin generation leading to a hypercoagulable state, thus resulting in an increased risk of venous thrombosis. Dahcback B, (2003), Adam R et al ,2007). APCR may be hereditary or acquired, FV Leiden mutation (FVLM) which was originally described by Bertina et al in Leiden (Bertina RM et al, 1994), is the most common hereditary form of APCR (Cheryl A et al, 1998) , and it's responsible for 90% of hereditary APCR cases Cheryl A, John A et al, (1998) ,Dacie J and, Lewis S , 2006) .There are other genetic mutations in factor V leading to APCR and venous thromboembloism such as FV Cambridge, Hong Kong and Liverpool mutations but these are very rare (Ruiz G et al ,2004). Certain clinical conditions, such as cancer, pregnancy, and oral contraceptives use are associated with acquired resistance to APC (Sarig G et al, (2005) , Robert C et al , (2004) , Younis JS et al ,2000). Several studies have been conducted worldwide to determine the prevalence and risk of APCR in different countries and ethnic groups.,. It is reported to be found in 3% to 5% of the general population and is responsible for 20% to 50% of thrombosis in patients. (Cheryl A et al ,1998) .The prevalence of APCR is estimated to be high in Caucasians (Hala Tamim et al, (2000), Hoffbrand A et al ,2003), while Asians and Africans are thought to have a low incidence (Hoffbrand A, petit et al ,2003). Among Arabs,, there is a difference in the prevalence of APCR in different sub-nationalities (Dashti A et al, (2010), Almawi W et al ,2005). There is a high incidence of Deep vein thrombosis (DVT) in Sudan but studies concerning the role of APCR in patients with DVT are lacking.

Study Objectives

The aim of this study was to evaluate the role of APCR in the etiology of DVT among Sudanese patients for purposes of screening those at risk, thus - achieving good prevention of DVT and improving management of patients with DVT.

To find out the prevalence of hereditary APCR due to factor FV Leiden mutation (FVLM) and the prevalence of acquired APCR among studied population.

MATERIALS AND METHODS

Study population

One hundred and thirty eight adult patients from both sexes admitted to Khartoum State hospitals in the period from 23rd of September 2009 to 25th of October 2011 who were diagnosed as having DVT were included in this study, regardless of whether they were taking anticoagulant treatment or not. Children and patients with arterial thrombosis were excluded. The samples were collected from patients in the departments of medicine, surgery, obstetrics, and orthopedic surgery in Khartoum Teaching Hospital, Academic Charity Teaching Hospital, Ibn Sina Hospital,

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Omdurman Teaching Hospital, Military Hospital, Khartoum North Teaching Hospital, and Ribat University Hospital (formerly Police Hospital). The practical work was carried out in the dermatological diseases laboratory in Khartoum Teaching Hospital.

Forty three patients were males and 95 were females, with an age range of 18-84 years. Thirty one of these patients (22.5%) had family history of DVT. Ninety patients presented with a first attack while the rest of the patients had history of previous attacks. After obtaining permission from the Ethical research committee in Islamic University (Appendix 1) and verbal consent from all patients who participated in this study, 2.5 ml of venous blood were obtained from each patient in Tri-Sodiumc eCitrate containers.

APCR assay

APCR coagulation test was done by using thrombotimer 4 channels using APCR kit from TC.

Principle of APCR assay

APCR test is a plasma based functional clotting assay that differs from other functional APC resistance tests by acting specifically on the prothrombinase complex level. It is based on a FV-dependent prothrombin activator isolated from snake venom. Robustness and specificity of the assay is enhanced by elimination of possible disturbing influences by factors upstream the coagulation cascade and independence from calcium.

Interference from unfractionated heparin (UFH), low molecular weight heparin (LMWH), and pentasaccharide in the blood sample is precluded by addition of a heparin inhibitor. Test plasma is pre-diluted and incubated at (37^{0C}) with FV activator from snake venom (RVV-V from Doboia russelli) and coagulation is triggered by the addition of a FV dependent prothrombin activator from snake venom (Notechis Scutatus scutatus) in the absence of calcium. The clotting times in presence and absence of APC are recorded and the ratios (Clotting time in the presence of APC/Clotting time in the absence of APC) are calculated.

Interpretation of APCR test

Differentiation of homozygous, heterozygous, acquired and inherited APCR resistance is based on the typical ratio ranges calculated from results obtained from patients' plasma. The ratio range differs according to the instruments used. The ratio ranges with thrombotimer 4 channel coagulometer, which are similar to those obtained with KC 4 micro coagulometer, are as follows:

– Negative APCR ≥3.

– Heterozygous hereditary APCR 1.3-1.9.

–Homozygous hereditary APCR 0.9-1.1.

According to the kit used , ratios more than 1.9 and less than 3 are were considered as indicative of acquired APCR.

Statistical Analysis

Statistical package for social science (SPSS) computer program version (14) was used for data analysis. Descriptive data was used to show the frequency and prevalence of APCR among studied population and other parameters like gender, age, geographical distribution, and clinical findings. Chi-square test was used to show the significant differences between the, results of APCR obtained and other studied parameters. All p-values less than 0.05 were considered as statistically significant.

RESULTS

Forty six patients (33.3%) were positive for APCR (ratio 0.9-2.9). Out of these,, 26 (18.8 %) had low ratios (0.9 -1.9) indicating a hereditary etiology, while 20 (14.5 %) had ratios of 2.1-2.9 which were consistent with acquired APCR, Figure (1).

According to the ratios obtained,, 4 patients (2.9%) with hereditary APCR were homozygous while 22 (15.9%) were heterozygous. Figure (2).

The frequency of APCR was higher in females, but the difference was not statistically significant (P = 0.337). Age significantly affected the frequency of APCR (P = 0.001) with more cases seen in younger adults; Figure (3).

Heterogeneity in prevalence of APCR was noted among the different geographical areas in Sudan exemplified by a high prevalence in central Sudan and low prevalence in south of Sudan although the difference was not statistically significant (P = 0.411). The frequency of APCR was slightly higher in patients with first episodes of DVT, but the difference was not statistically significant.

Thirty one patients had positive family history, of whom 23 patients (74.2%) had positive APCR. Seventeen of these (54.8%) had hereditary APCR while 6 (19.4%) had acquired APCR. One hundred and seven patients had no family history of DVT, of whom 23 patients (21.5%) were positive for APCR, 9 (8.4%) had hereditary APCR, while 14(13.1%) had acquired APCR, Table (1) . Therefore, the prevalence of APCR was significantly higher in patients with positive family history (P = 0.000).
Most of the acquired positive APCR's's showed a high significant correlation with clinical conditions known to be associated with high risk of developing venous thrombosis,, while most of the hereditary ones were not associated with such conditions.,, Table (2).

DISCUSSION

APCR is the most common cause of venous thrombosis (Cheryl A et al (1998), Hoffbrand A et a,l (2003) ,Y Chen et al, (2003) , Dacie J and Lewis S, 2006). In the Western countries,, 40-60% of hereditary causes of venous thrombosis are due to APCR, this is mainly due to FVLM (Cheryl A et al (1998) ,Dacie J and Lewis S, 2006). APCR is reported to be very rare in Africans and blacks (Hoffbrand A et al, (2003) , Vizcanio G et al, 2000) . In contrast to that current study's results revealed a high incidence of APCR in

Sudanese patients where 33% of the studied patients showed resistance to activated protein C;: thus indicating a significant role of APCR in the etiology of DVT in Sudanese patients. Similar results were reported by Aboghoush and Alrashed in 2003 who found a prevalence of 33.6% in a study involving 104 Jordanian patients with DVT (Aboghoush M and Alrashed M ,2003). The prevalence of hereditary causes in the study's patients was 18.8% while that of acquired causes was 14.5%. The prevalence of acquired APCR in the current study is similar to results obtained by Rodeghiero F and Tosetto A, who reported a similar prevalence of acquired APCR in Italy (Rodeghiero F and Tosetto A,1999).

However, the high incidence of hereditary APCR in this study is in contradistinction to the previously recorded results in the literature,, which indicated that it is rather rare or does not exist in Africans and blacks (Hoffbrand (2003), Vizcanio G, et al 2000) .This difference in results can be explained by the mixed African - Arab origin of the Sudanese population, since those studies may have been carried on pure Africans . Ethnic variation in the incidence of APCR is demonstrated in many literature reports which have clearly shown different prevalence of APCR in different ethnic groups (Hala Tamim et al,(2000), Palomo I et al 2009).

Most of the studied patients with hereditary APCR (84.6%) were heterozygous for FVLM with a prevalence of (15.9%) among the whole studied population,, while only 4 patients (15.4%) were homozygous for FVLM with prevalence of 2.9% among the whole studied population. The lower incidence of homozygous FVLM may indicate that there were aa few cases of consanguineous marriages in the studied group. Geographical origin did not affect the prevalence of APCR in this study significantly (P = 0. 411). This does not agree with the result of other studies which reported a significant difference in prevalence of APCR in different ethnic groups among the same nationality (Dashti A et al, (2010) , Almawi W et al ,(2005) , Moerloose P et al ,2000) , and may be explained by the unequal participation of different geographical areas in the present study. Age significantly affected the prevalence of APCR (P = 0.001) where a higher incidence of APCR was found among younger adults and this is in agreement with results reported by Sevensson and Dahlback . A significantly higher incidence of APCR was shown in those with a positive family history (P value 0.000), this has been confirmed in other studies worldwide (Hala Tamim et a l ,(2000) , Kalakanli S et al, 2006).

The present results showed a prevalence of 14.5 % for acquired APCR. Most of these patients were suffering from diseases known to be strongly associated with APCR. Eleven out of 20 females (55 %) who developed DVT during the post partum period were positive for APCR, 8 of these patients were shown to have acquired APCR. Three out of 4 pregnant women with DVT were positive for APCR, all of them having acquired APCR.

CONCLUSION

APCR has a high prevalence among Sudanese patients with DVT. Inherited APCR seems to be more prevalent among those patients than acquired. This result highlights the importance of screening for APCR, therefore it is recommended that testing for APCR is done in all patients with DVT and for people who are at risk for developing thrombosis. For patients with positive results. It is recommended that screening is carried on their first degree relatives, since appropriate prophylactic measures can help prevent a lot of morbidity and mortality.

Recommendations for further studies

1. A case control study should be carried out since the results have highlighted the significance of this problem

2. All patients diagnosed with Deep vein thrombosis (DVT) must be screened for thrombophilia and dealt with accordingly, to reduce the possibility of a new attack.

3. Activated Protein C Resistance should be looked for in all susceptible patients and family members of the affected . Special attention regarding testing for APCR should be given to females who are pregnant, on pills or in the postnatal period

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TABLES AND FIGURES

Table 1: Frequency of APCR according to family history of DVT

Results	Family history							P. value
	Negative	Father	Mother	Sister- brother	Sons- daughter	Others	Total	
Negative	84	3	1	2	0	2	92	0·000
Homozygous	3	0	0	0	0	1	4	
Heterozygous	6	3	3	5	1	4	22	
Acquired	14	1	1	2	0	2	20	
Total	107	7	5	9	1	9	138	

Table 2: Frequency of APCR according to associated Clinical conditions

Results	Clinical diagnosis									Total	P. value
	postpartum	pregnancy	multiple miscarriages	SLE	cancer	contraceptives	others	two	Nil		
Negative	9	1	1	1	1	2	22	1	54	92	0·000
homozygous	1	0	0	0	0	0	0	0	3	4	
heterozygous	2	0	0	2	1	0	0	2	15	22	
Acquired	8	3	0	0	2	0	4	1	2	20	
Total	20	4	1	3	4	2	26	4	74	138	

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Figure (1) The prevalence of positive and negative APCR in the study sample

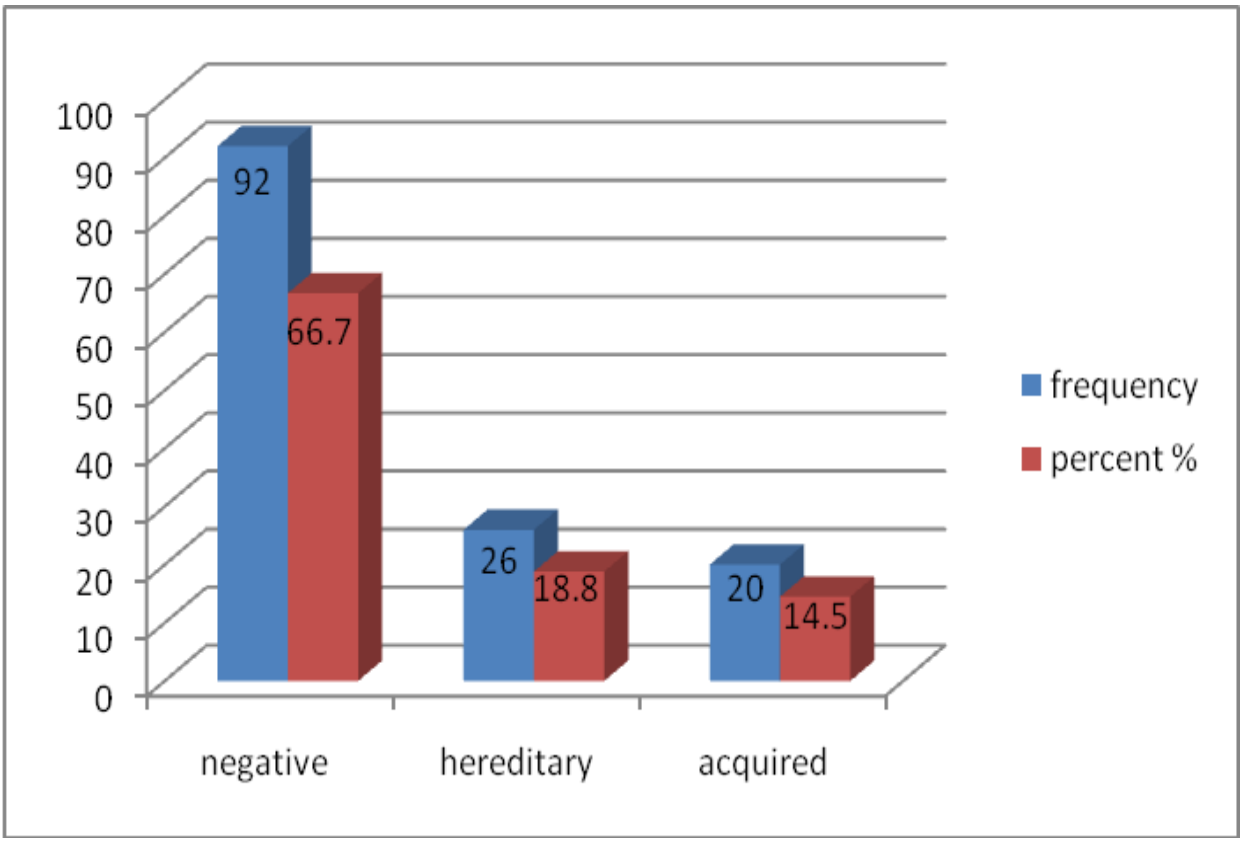


Figure (2) The prevalence of homogeneity in APCR in the study sample

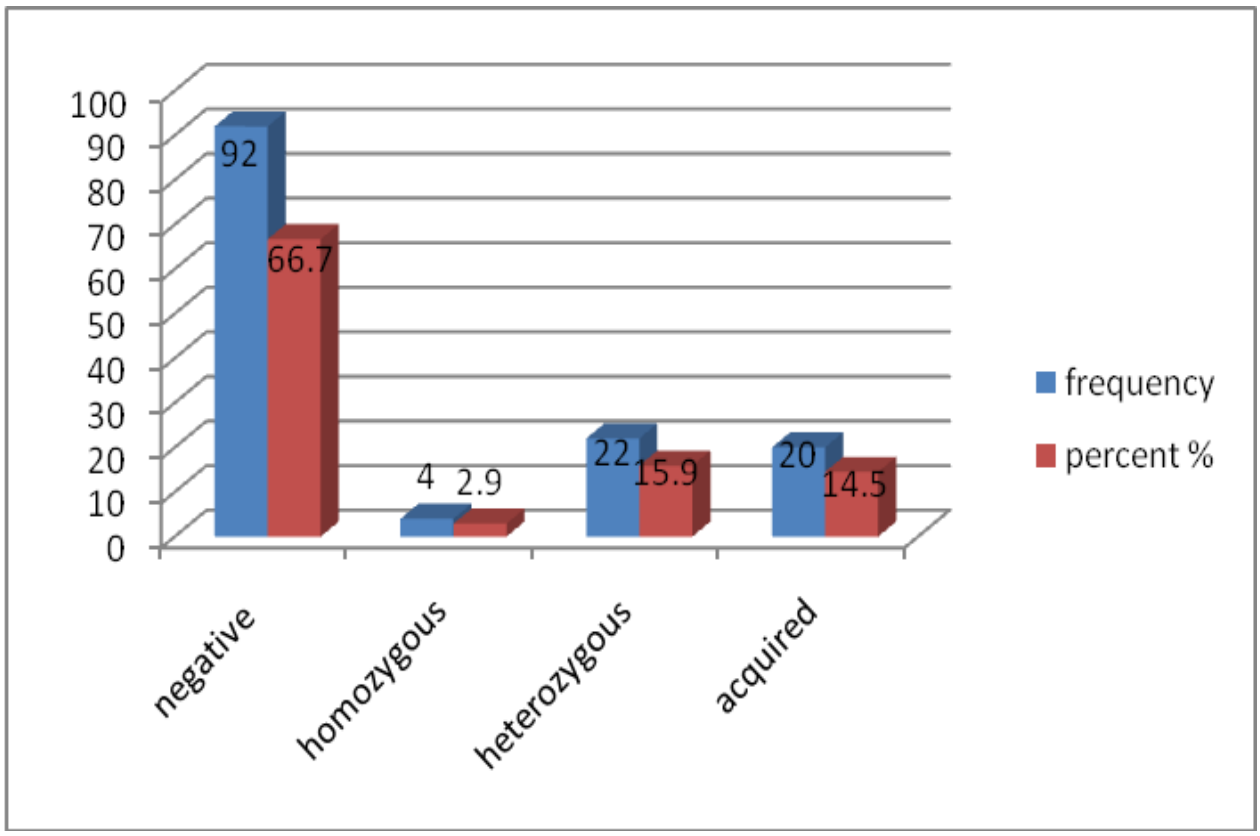
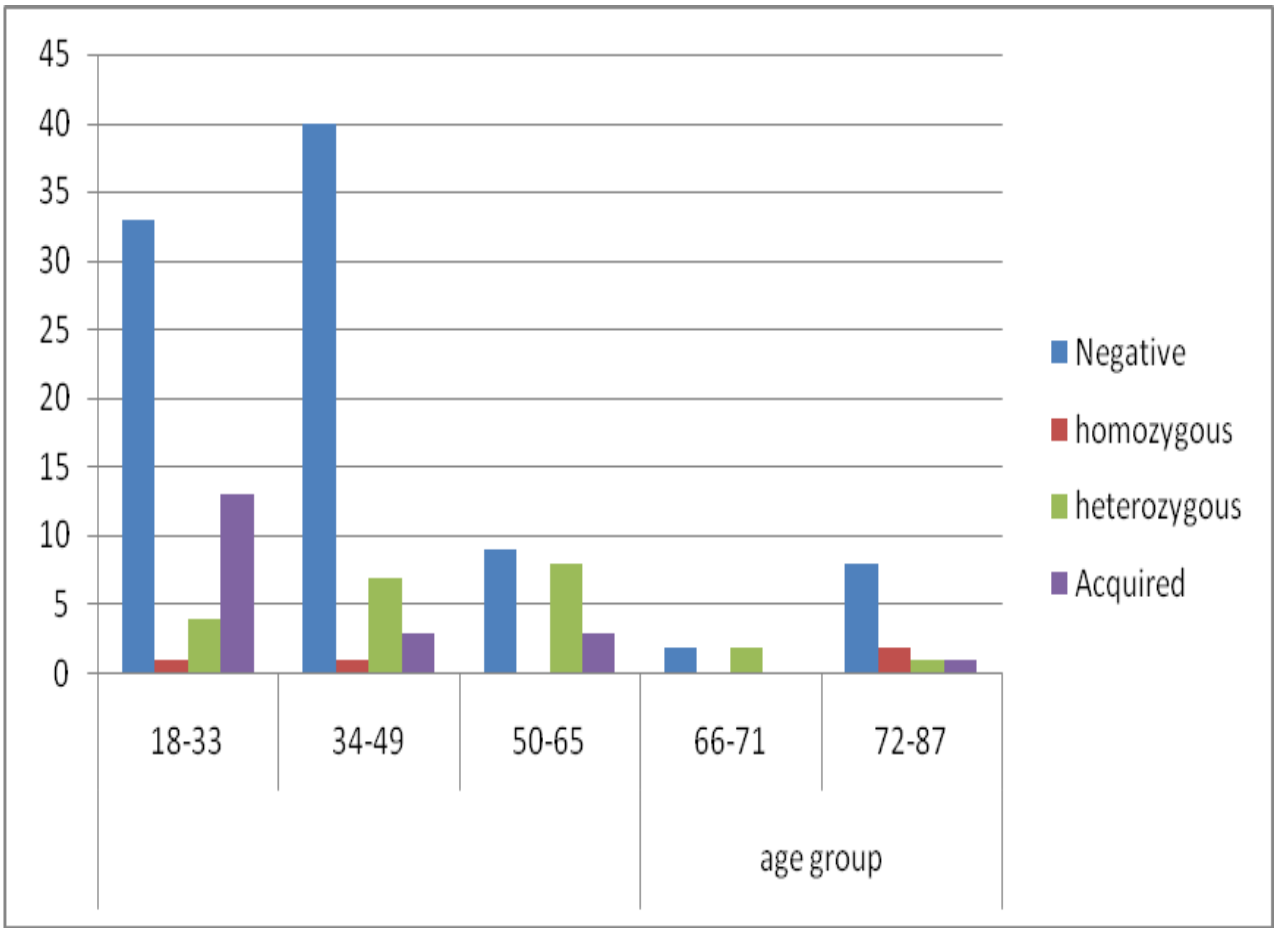


Figure (3) The frequency of APCR according to age distribution



Appendix (1)

جامعة أم درمان الإسلامية
إدارة الخدمات الطبية والصحية

التاريخ :2011/6/22 م مكتب المدير العام الثمرة: ج أ س/ إ خ ط ص/ ع

السيد/ أ.د. رئيس مجلس الابحاث-كلية علوم المختبرات الطبية

المحترم

السلام عليكم ورحمة الله وبركاته

الموضوع/ أخلاقيات البحوث

للباحثة: صفية خليل علي طه

بالإشارة للموضوع أعلاه نفيد سيادتكم بأن الرسالة بعنوان :

prevalence of Activaed Protein Resistance among Adult SudanesePatient,sWith Deep Venous Thrombosis (Khartoum State)

أن الموضوع يستحق إجازة البراءة الأخلاقية وذلك لإستيفائة كل شروط أخلاقيات البحث لذلك أوصي بأن تستمر الدراسة .

ولكم خالص شكرنا وتقديرنا

أ.د. يوسف عبد المعبود السيسي

أستشاري طب المجتمع وطب الاسرة و والصحة المهنية

رئيس لجنة أخلاقيات البحوث

بجامعة ام درمان الاسلامية

ص.ب ٣٨٢ - أم درمان - السودان

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