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A comparison of C-reactive protein and Serum Ferritin as Acute Phase Reactants in Venous Thromboembolism

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ABSTRACT

Background:

Serum ferritin and C – reactive protein are non-specific markers of inflammation. They have been useful in predicting the outcome of stroke.

Aims and Objectives:

High sensitive C-reactive protein (hs-CRP) and serum ferritin were analysed in patients with venous thromboembolism (VTE) as a preliminary study to determine their usefulness in predicting VTE.

Materials and Methods:

Serum ferritin and hs-CRP were analyzed in 37 consenting patients with VTE by Enzyme Immunoassay. The type of VTE, risk factors for VTE, and haematological parameters were obtained from

the case files. Twenty healthy individuals, mostly blood donors also had their sera analyzed for hs-CRP and serum ferritin. The study was conducted between June and November 2012.

Results:

The mean age of the patients was 56±18.3yrs, 16 had identifiable risk factors while no risk factor was identified in 21 patients (idiopathic group). The mean haematocrit was 35±6.5%, four individuals had values less than 30% of which 3(75%) were in the idiopathic group. The mean MCH and MCV were 27.1±3.1pg and 79.4±10.5fl respectively, low MCH was observed in 70% of patients while more than half (59%) had elevated hs-CRP (>10µg/L). The mean serum ferritin was 373.9±331ng/ml, the mean serum ferritin was lower for those with elevated Hs-CRP (333.5µg/L vs 484.6µg/L; p=0.57). The mean serum ferritin was similar in those with or without identifiable risk factors (373.8 vs 373.9ng/ml), those with risk associated VTE had non-statistically significant higher values of CRP than those with idiopathic VTE (68% vs 61% respectively, p>0.05). The mean serum ferritin and hs-CRP of the control was 74.7ng/ml and 3.5µg/L respectively.

Conclusion:

Neither CRP nor serum ferritin appear discriminatory enough for predicting type of VTE.

Keywords: inflammatory markers, idiopathic VTE, anaemia, microcytosis.

INTRODUCTION

Serum ferritin and C-reactive protein (CRP) are non-specific markers of inflammation so are useful in correlating the association between inflammation and disease. The role of CRP and other markers of inflammation have been studied extensively in acute stroke such that CRP may eventually become useful in predicting future cardiovascular events or even death in stroke patients [1]. More so, elevated levels of CRP is associated with recurrent and worse outcome of stroke [2,3], it is also found

useful in the classification of acute ischaemic stroke [4]. The usefulness of CRP as a marker of inflammation and a predictor of the outcome of illness is not limited to stroke but has also been observed in venous thromboembolism (VTE) where it was observed that levels of high sensitive CRP (hs-CRP), fibrinogen and factor VIII are higher in patients with idiopathic VTE compared to risk associated VTE. [5] Very few studies have included serum ferritin in the panel of inflammatory markers studied, but a study which included serum ferritin noted that high levels of the markers are associated with poor

outcome in stroke and that the markers are useful in appreciating the role of inflammatory markers in the pathogenesis of ischaemic stroke. [6]

The increasing awareness and diagnosis of VTE in the Nigerian population requires that markers that can prognosticate outcome of VTE be developed, serum ferritin and hs-CRP are markers which are readily available and expected to be within the budget limit of this patient population. [7] This is a preliminary study to measure levels of these two markers in patients with VTE and determine its role as markers of inflammation in VTE.

MATERIALS AND METHODS

Patients' description

Thirty seven consenting patients with Doppler confirmed VTE had their blood samples taken and analyzed for hs-CRP and serum ferritin by enzyme immunoassay (EIA). Demographic data of the patients were obtained from the case files, predisposing factor for the thromboembolism was sought for clinically from medical history, clinical examination and in the case file and where none was identified it was assumed to be idiopathic. The most common co-morbidity identified was cerebrovascular disease (CVD), risk factors identified include surgery, trauma, diabetes mellitus and malignancy, immobility is a common pathway for most of the risk factors. None of the patients was investigated for autoimmune disorder by the attending physician. A clotted blood sample from which serum was extracted was also obtained from twenty apparently healthy volunteers who consisted of 13 blood donors and seven apparently healthy volunteers.

Diagnosis of Venous Thromboembolism

The extent/type of the thromboembolism (whether proximal, distal, bilateral, extensive or chronic DVT or pulmonary embolism) was obtained from the report of Doppler's ultrasound or pulmonary angiography but this was not taken into consideration because 77% of the patients had what could be considered as extensive VTE which included proximal,

bilateral and extensive deep venous thromboembolism (DVT) or pulmonary embolism (PE).

Haematological Parameters

Baseline laboratory parameters such as haematocrit, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) (by automation) were extracted from the case files.

Analysis of serum for hs-CRP and Serum Ferritin by Enzyme Immunoassay

High sensitive C reactive protein and ferritin were measured using an enzyme linked immunosorbent assay (ELISA) according to the manufacturers' instruction provided by IBL International, Germany, Ref: EU59151; LOT:1111312 and Ref:DB59111; LOT:121560 respectively.

Principle: The assay employs the principle of the quantitative sandwich enzyme immunoassay technique in which antibodies specific for hs-CRP/ ferritin are precoated onto a microtitre plate. Standards and samples are pipette into the wells with any hs-CRP/ferritin present in serum bound by the immobilized antibody.

Management of Patients

All patients were enrolled and blood samples were taken while on admission for anticoagulation after diagnosis by Doppler ultrasound. Dose of warfarin therapy was adjusted based on the international randomized ratio (INR) with the aim of achieving INR between 1.5-2.5. Upon achieving the desired INR, patients were discharged to be followed up in the outpatients' clinic for 3-6 months.

The study was approved by the University of Ibadan/ University College Hospital, Ibadan ethical committee NHREC/05/01/2008a. The study was carried out between June and November 2012.

Statistical Analysis

Data were entered and analyzed using SPSS software, version 16.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were recorded as mean and standard deviation while nominal variables were reported in percentages. The level of significance was set at 5%.

RESULTS

The mean age of the patients was 56±18.3yrs with a male/female ratio of 1.2:1, sixteen patients had identifiable risk factors while no risk factor was identified in 21 patients. The mean haematocrit on admission for all patients was 35±6.5%, male patients had a mean haematocrit which was not significantly higher than female patients 36%vs 33% (p=0.28). Ten

per cent (4/37) of the patient population had a haematocrit that was less than 30% and 75% (3/4) of this was observed in the idiopathic group. The mean MCH and MCV were 27.1±3.1pg and 79.4±10.5fl respectively (Table 1), microcytosis (MCH<27pg and/or MCV <80fl) was observed in 70% of the patients, the mean MCH was not significantly different between the two gender (p=0.48). More than half of the patients (59%) had elevated levels of hs-CRP (>10µg/L). The mean serum ferritin was 373.9±331 ng/ml (9.4 -1472.2 ng/ml), the serum ferritin levels of male did not differ significantly from that of female (420.7 ng/ml vs 334. 4 ng/ml; p=0.45). Almost half (45.7%) of the patients had elevated serum ferritin (>300 ng/ml) while only one patient had subnormal serum ferritin (<15ng/ml).

Table 1: Haematological Variables of Patients with Venous Thromboembolism

Haematological Variables	Mean values SD)
Haematocrit (Hct)	35±6.5%
Mean Corpuscular Haemoglobin (MCH)	27.1±3.1pg
Mean Corpuscular Volume (MCV)	79.4±10.5fl
Serum ferritin	373.9±331ng/ml

The mean serum ferritin level of patients with elevated hs-CRP was lower than those with normal hs-CRP (333.5µg/L vs 484.6µg/L; p=0.57). The mean serum ferritin of the patients with identifiable risk factors was similar to those without an identifiable risk factor (idiopathic group) (373.8 vs 373.9ng/ml). A higher percentage of patients with risk associated VTE had elevated Hs-CRP (68% vs 61%) (Table 2). There was a non-significant positive correlation between serum ferritin and haematocrit and a negative correlation between serum ferritin and MCH (r=0.17; p=0.47; and r=-0.09; p=0.72 respectively). The mean serum ferritin and hs-CRP of the healthy individuals was 74.7ng/ml (5.5-209ng/ml) and 3.5µg/L (0.5-10.5µg/L) respectively, none of the healthy individuals had elevated serum ferritin and only two had suboptimal level, these two were not blood donor but a blood donor had hs-CRP above 10.5µg/L.

DISCUSSION

The elevated serum levels of ferritin and hs-CRP in the patients in comparison to the healthy subjects possibly reflect the inflammatory aspect of the thromboembolic disorder suggesting a possible role of these markers in predicting outcome in VTE. Surprisingly, these elevations were not observed in all the patients, more so high levels of ferritin was recorded in less than half of the patients. The lower serum ferritin level observed in patients with elevated hs-CRP compared to those in whom the hs-CRP was not elevated would suggest that hs-CRP may be a better index of inflammation in these patients and that the elevated serum ferritin level may not be solely due to an inflammatory process. Though risk factors were not rigorously sought for in these patients, an assumption of absence of a risk factor was made in those in whom it was not seen. This study is at variance with the findings of

Table 2: A comparison of the Parameters between Idiopathic and Risk Associated Venous Thromboembolism

Parameters	Idiopathic VTE (n=21) Mean±SD	Risk Associated VTE (n=16) Mean±SD	P-value
Hct (%)	33.9±6.7	37.1±6.1	0.88
MCH (pg)	27.0±3.6	27.3±2.2	0.32
MCV (fl)	78.7±9.7	80.8±12.6	0.43
Serum Ferritin (ng/ml)	373.9±367.2	373.8±295.8	0.6
CRP (µg/L)	10±2.9	8.9±3.4	0.31
CRP ≤ 10 µg/L	8	5	0.66
CRP >10 µg/L	13	11	0.66

Luxembourg *et al* (2009) that higher levels of CRP is seen in patients with idiopathic VTE. [5]

It should also be noted that the mean serum ferritin level is similar in those with identified risk factor and those with unidentified risk factor (idiopathic group).

The baseline haematological parameters of these patients are also very informative in that all the mean values of the haematological parameters i.e. haematocrit, MCH and MCV were suboptimal or borderline of normal value, unfortunately these parameters were not analysed in the healthy control. The question therefore is to decide if this abnormal finding is due to the thromboembolic disorder alone, an underlying unidentified factor or a combination of both which this study may not be able to provide a direct answer. Moderate anaemia was found in the majority of patients in the idiopathic group. Iron deficiency, folic acid deficiency, chronic renal failure and cobalamin deficiency were the five leading causes of anemia in 53% of hospitalized elderly patients with inflammation [8]. Though the mean age (79.7yrs) of patients in the study differ greatly from our cohort of patients, inflammation and chronic renal failure as a cause of anaemia cannot be ruled out in our patients but the values of the red cell indices (MCH & MCV) and the serum ferritin levels preclude the diagnosis of the other three conditions that is, iron deficiency, folic acid deficiency and cobalamin deficiency. It is however unlikely that the inflammation of thromboembolism alone will account for the associated anaemia, this would have been thought to be due to comorbidities but for the fact that the anemia was noted more in the idiopathic group in whom associated risk

factors were not identified.

The observation of microcytosis in 70% of the patients is not unusual since reviews of peripheral blood film of hospitalized patients in this environment mostly show a similar prevalence of microcytosis. Iron deficiency had always been thought to be responsible for this but this is not supported by the findings of this study more so, with only one patient and two of the healthy subjects having low serum ferritin levels, other studies have also observed normal serum ferritin levels in healthy Nigerians. [9,10] The positive correlation between the haematocrit and serum ferritin is also not surprising as this has also been previously reported, it was also observed that the correlation for haemoglobin was highest for MCH and lowest for serum ferritin, but the reason for the negative correlation between serum ferritin and MCH remains a puzzle. [11,12]

Idiopathic venous thromboembolism has recently become a separate entity with an incidence rate of 25-50%, peculiarities observed in this group of patients in this study include a higher prevalence of anaemia and lower levels of CRP than those with identified risk factors, but the mean serum ferritin is similar in both groups. [13] Genetic causes of VTE (thrombophilia) are now closely associated with idiopathic VTE, though thrombophilia is rarely screened for in Nigerian patients, but a few of these deficiencies have been identified in the setting of VTE. It is therefore necessary to be aware of this; it also calls for a large population based survey to determine the prevalence of these genetic causes of VTE in the population. It is also

important to always identify associated risk factors in patients with VTE in order to avoid unnecessary investigations.

This study observed that idiopathic VTE is commoner than risk associated VTE in the Nigerian population and that anaemia is commoner in this group of patients. Both hs-CRP and serum ferritin are not discriminatory enough as markers of prognosis in VTE. As observed in other studies, the microcytosis in the Nigerian patients is less likely due to iron deficiency. The lack of significance in most of the outcome measures can be attributed to the fact that the study is not sufficiently powered, so increasing the sample sizes and a comparison of baseline characteristics of both patients and controls may yield more significant differences between the groups. It may also be worthwhile to follow up the patients after resolution of the VTE to determine if there are alterations in these laboratory parameters.

CONCLUSION

Idiopathic VTE is commoner than risk

associated VTE and more associated with anaemia. Low red cell indices found in the majority of patients was not due to low levels of serum ferritin. Neither CRP nor serum ferritin appear discriminatory enough in predicting the type of VTE.

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Conflict of Interest:

Authors declare no conflict of interest.

Author's Contributions:

TRK, FAF conceived the idea, TRK, FAF, OEB, and PEO designed the study. OEB and PEO recruited participants and collected data for the study. FMA and OE carried out the biochemical analysis and interpreted the results. TRK performed the statistical analysis and prepared the first draft. All authors revised the manuscripts and approved the final draft.

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