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Research Article

Reliability of TNF-α as a Screening Test for Cerebral Stroke

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Abstract: In spite of the pivotal role of TNF-α in the pathogenesis and clinical outcomes of cerebral ischemia, this cytokine was never investigated before as an indicator of stroke. The aim of this study was to investigate the reliability of TNF-α as screening test for ischemic stroke. The study enrolled 35 patients with stroke and 81 apparently healthy volunteers. Following clinical examination, blood samples were collected from each volunteer and used for estimation of concentration of TNF-α, random blood glucose (RBG), and lipid profile. TNF-α level ≥ 0.135 pg/ml was consider as significantly high concentration. Chi-square test and conditional ratios were used to assess association between higher levels of TNF-α and stroke. Higher TNF-α levels were associated with higher risk of stroke (Odds Ratio (OR) = 6.37, 95% confidence interval (CI) = 2.671 - 15.2), sensitivity = 62.86%, specificity = 79.01%, positive predictive value (PPV) = 56.41%, negative predictive value (NPP) = 83.12 %, false positive rate (FPR) = 20.99%, false negative rate (FNR) = 37.14% and accuracy = 74.14%.TNF-α achieved significantly higher levels in patients suffering from recent stroke compared to apparently healthy subject. However, our results fail to demonstrate TNF-α as a reliable screening test for recent stroke.
Keywords: Sensitivity, Specificity, Stroke, TNF- α

INTRODUCTION

There are accumulating evidences that inflammation plays a key role in the pathophysiology and clinical outcomes of stroke [1]. Following stroke, microglial cells are activated together with some other inflammatory cells like neutrophils, T lymphocyte and macrophages [2, 3]. Reactive oxygen species (ROS) and certain cytokines are released from the damaged brain tissue [4]. Inflammatory mediators, in turn, enhance the expression of the adhesion molecules which stimulate margination and diapedesis of circulating leukocytes [5]. Later, infiltrating leukocytes release more ROS and cytokines, which augment the brain-inflammatory responses and ultimately disruption of the blood brain barrier (BBB) and cerebral hemorrhage [2].

Tumor necrosis factor alpha (TNF-α) is among the important cytokines released during inflammatory response to stroke [6-13]. Previous studies reported neurotoxic as well neuroprotective effects of TNF-α [6, 7]. Inactivation of TNF-α was proved to minimize infarct volume [8] and improve neurological deficits in stroke animal models [9] and human being [10, 11]. Alternatively, mice with knockout TNFα receptors showed worsened neuronal damage following stroke [12] suggesting a neuroprotective role of TNFα [13].

In spite of the pivotal role of TNF-α in the pathogenesis and clinical outcomes of cerebral ischemia, this cytokine was never investigated before as an indicator of stroke. This study aims to investigate the reliability of TNF-α as screening test for ischemic stroke.

MATERIALS AND METHODS

Ethical clearance was approved by the ethics review committee at Alneelain research center – Alneelain University – Sudan. All volunteers signed informed written consents before being enrolled in the study.

The study involved a test group of 35 patients (21 males and 14 females) with stroke and a control group of 81 apparently healthy volunteers (55 males and 26 females). The stroke patients were recruited from neurology wards and refer clinics at Al-Shaab teaching hospital - Khartoum – Sudan. The control subjects were mostly staff members of central national laboratory - Khartoum – Sudan. Following clinical examination, 5 ml blood sample was collected from each volunteer and coded. Half of the blood sample was centrifuged at 6000 rpm for 10 minutes and the stored at

−80°C until being processed for measurement of TNF-α. The other half was dispensed into tube containing lithium heparin and stored as plasma at -20°C until used for measurement of fasting blood glucose and lipids profile. Serum TNF-α level was measured by enzyme-linked immunesorbent assay (ELISA) using Technique kits (KOMA BIOTECH, ELISA Complete Kit; cat. No. K0331131; Lot No. 06151). Random blood glucose and lipid profile were measured according to the standard enzymatic methods. TNF-α level ≥ 0.135 pg/ml was consider as significantly high concentration.

All data were analyzed using the statistical package for social science (SPSS, version 19) for Windows. The Body mass index (BMI) and Mean arterial blood pressure (MABP) were calculated by SPSS using the formulae BMI = Weight (Kg) / (Height (m))2 and MABP = diastolic blood pressure (DBP) + [(systolic blood pressure (SBP) – DBP)/3] respectively. The data were described by mean (M) ± standard deviation (SD). Significant differences between measured variables in the patients with stroke and the control group were assessed using Student T-test. Chi-square test and conditional ratios were used to assess association between higher levels of TNF-α and stroke. *P* value < 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes characteristics of the patients with stroke and the control group. The patients with stroke are proved to have significantly higher age (58.25±10.88 years), MABP (98.45±15.99 mmHg), RBG (156.11±65.70 mg/dl) and TNF-α (65.83±81.00 pg/ml) compared to the control group (50.37±12.56 years, *P* < 0.001; 92.65±5.21 mmHg, *P* = 0.017; 86.58±3.92 mg/dl, p < 0.001 and 25.90±27.75 pg/ml, *P* = 0.001 respectively). In contrast, BMI and lipids profile were comparable in patients with stroke and the control group (table 1).

Based on Pearson Chi-square test, higher TNF-α levels were associated with higher risk of stroke (Odds Ratio (OR) = 6.37, 95% confidence interval (CI) = 2.671 - 15.2), sensitivity = 62.86%, specificity = 79.01%, positive predictive value (PPV) = 56.41%, negative predictive value (NPP) = 83.12 %, false positive rate (FPR) = 20.99%, false negative rate (FNR) = 37.14% and accuracy = 74.14% .

Table-1: Characteristics of the control group and patients with stroke.

	Stroke Patients N = 35 M±SD	**Control Group N = 81 M±SD**	**p Value**
Age (years)	58.25±10.88	50.37±12.56	0.000*
BMI (kg/m^2)	27.64±4.82	26.63±20.51	0.247
MABP (mmHg)	98.45±15.99	92.65±5.21	0.017*
RBG (mg/dl)	156.11±65.70	86.58±3.92	0.000*
Triglyceride (mg/dl)	88.57±21.57	93.09±30.69	0.629
Cholesterol (mg/dl)	141.88±24.05	140.39±24.54	0.779
HDL (mg/dl)	34.19±6.29	33.86±6.52	0.780
LDL (mg/dl)	89.97±24.49	87.91±25.12	0.652
TNF-α (pg/ml)	65.83±81.00	25.90±27.75	0.001*

Table-2: Association between TNF-α and stroke

	Stroke Patients	**Control Group**	**Total**
TNF-α ≥ 0.135 pg/ml	22 (19%)	17(14.7%)	39 (33.6%)
TNF-α < 0.135 pg/ml	13 (11.2%)	64 (55.1%)	77 (66.4%)
Total	35(30.2%)	81 (69.8%)	116 (100%)
Pearson Chi^2 = 19.20, P < 0.001, OR (95% CI) = 6.37 (2.671 - 15.2), Sensitivity = 62.86%, Specificity = 79.01%, , PPV = 56.41%, NPP = 83.12 %, FPR = 20.99%, FNR = 37.14%, Accuracy = 74.14%			

DISCUSSION

It is obvious from the present results that serum levels of TNF-α tend to be higher in patients suffering from recent stroke compared to apparently healthy subject. Comparable findings were reported by several previous reports [14, 15] but failed to reach statistical significance in others [16]. TNF-α was among the cytokines evaluated by Licata *et al*. to address the relationship between blood inflammatory biomarkers and neurological deficit caused by cardio-embolic stroke (CES) [14]. Licata *et al*. demonstrated significantly higher median plasma levels of TNF-α in patients with CES compared to subjects with other subtypes of stroke. In addition, Licata *et al*. suggested enhanced immuno-inflammatory activation as a probable cause of the worse clinical presentation in patients with stroke. In a comparable study, Beridze and Shakarishvili assessed the correlation between the initial levels of pro-inflammatory cytokines in cerebrospinal fluid (CSF) and neurological outcome following acute ischemic stroke. Their results confirmed significantly higher CSF levels of TNF-α in

patient with stroke compared to the controls after 48 hours of stroke onset, but not earlier [15]. Beridze and Shakarishvili findings were further supported by Vila and his research group who evaluated the potential influences of TNF-α on the degree of neurological deficits in stroke patients. About one third of the patients studied by Vila *et al* showed signs of neurological deteriorations concomitant with increased CSF and plasma concentrations of TNF-α, although the higher TNF-α levels remained insignificant on multivariate analysis [16].

In the present study age, RBG and MABP were significantly higher in patients with stroke compared to the healthy control, which may explain the difference of TNF-α levels in the studied groups. There are increasing evidences that elderly subjects with high TNF-α concentrations are at higher risk of atherosclerosis regardless of their serum total cholesterol, LDL or BMI [17]. Age-related increase in TNF-α may explain higher mortality rate in elderly following cerebral injury [18]. Moderate increases in TNF-α may also induce hypertension by enhancing salt retention and changing renal hemodynamics [19]. Alternatively, there is strong association between TNF-α levels and insulin resistance which explain pro-inflammatory nature of diabetes mellitus [20]. The influence of age, RBG and MABP on TNF-α level also explains why high concentration of this cytokine is not an appropriate indicator of stroke. According to our results, high TNF-α level is more specific (79.01%) but less sensitive (62.86%) to stroke. Consequently, high TNF-α level is better to be used in excluding (NPP = 83.12%) rather than confirming (PPV = 56.41%) the diagnosis of stoke. In a comparable study, Shubair and his group investigated the reliability of TNF-α as screening test for detection of patients with atherosclerotic coronary heart disease (CHD) [21]. Based on Shubair *et al* results, the conditional ratios of TNF-α as a tool for CHD detection was as follows: sensitivity = 33.00%, specificity = 79.01%, PPV = 66.00%, NPV = 48.85%. The accuracy of TNF-α as screening test for CHD detection was 53.59% according to Shubair *et al*., which was far less compared with the accuracy achieved by the same cytokine for detection of stroke (74.14%). According to Shubair *et al*. data and our results, it seems logical that TNF-α is neither suitable for screening of CHD nor ischemic stroke.

CONCLUSION

In conclusion, TNF-α achieved significantly higher level in patients suffering from recent stroke compared to apparently healthy subject. However, our results fail to demonstrate TNF-α as a reliable screening test for recent stroke, probably because of the diversity of the diseases associated with high TNF-α level.

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