Original Article

Assessment of Hematological and Electrolytes Levels and Oxidative Stress Predictive Factors by Logistic Regression Analysis of Acute Ischemic Stroke Disease

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ABSTRACT

Background: Ischemic stroke is a common disease that affects the blood vessels in the brain. This disease is considered one of the most dangerous ailments that affect the nervous system all over the world.

Objectives: The aim of this study was to evaluate some hematological and biochemical parameters in men with ischemic disease in Touggourt (Algeria) region.

Methods: The study was done on 40 voluntary individuals divided into healthy men reserved as control with Mean±SD age of 56.13±3.32 years and ischemic stroke man (patients) with Mean±SD age of 57.75±4.01 years; their origin covered the whole Touggourt (Algeria) region. Some hematological and biochemical parameters were analyzed, Sensitivity and specificity of oxidative stress biomarkers in serum, erythrocytes, and leucocytes were estimated using a receiver operating characteristics curve (ROC) design.

Results: The results suggest that when compared to the controls, blood glucose levels significantly increased ($P>0.05$) while serum sodium, potassium, and chloride concentrations and serum alkaline phosphatase activity significantly decreased ($P<0.05$) in stroke patients. Red blood cell, hemoglobin, glutathione (GSH), and total thiol levels significantly decreased ($P<0.05$) while white blood cell, neutrophile, platelets, malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC) and vitamine C levels significantly increased ($P<0.05$) in ischemic stroke patients compared to the controls with high sensitivity and specificity values of oxidative stress markers.

Conclusion: The results indicate that change in electrolytes, hematological, alkaline phosphatase, and oxidative stress markers contributes to the development or complications of ischemic stroke. Investigations on oxidative stress markers can early identify predictors of stroke disease.

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Introduction

Ischemic stroke is a common disease that affects the blood vessels in the brain [1]. This disease is considered one of the most dangerous ailments that affect the nervous system all over the world [2]. The number of stroke patients is gradually increasing worldwide, with an estimated 5.71 million people with stroke in 2004. According to the World Health Organization (WHO), this number is expected to reach 7.8 million in 2030 [3]. The Magrebia region has reported varied epidemiological transitions among all Arab and North Africa from 1990 to 2017, there are 13.008,01 cases of ischemic stroke and 5.184,36 cases of hemorrhagic stroke in Algeria [4]. According to the last WHO data published in 2017 stroke deaths in Algeria reached 22.917 or 13.19% of total deaths. Many risk factors cause stroke, including gender, age, and genetic factors, and the daily habits and behaviors of individuals play a large role in the occurrence of the disease [5].

In addition, many factors related to the lifestyle and way of life of individuals may play an important role in the etiology of this disease, including diet [6]. Some diseases may be a direct cause of stroke, including cardiovascular disease, obesity, diabetes, smoking, and dyslipidemia [7]. Several inflammatory markers identify and detect stroke, such as tumor necrosis factor (TNFα) and interleukin 6 (IL-6), as well as lipid markers, including low-density lipoprotein (LDL) and high-density lipoprotein (HDL), along with imaging features, especially in patients without external symptoms [8]. For stroke treatment, t-PA can be the only FDA-approved stroke drug but has a limited treatment time window of 4.5 hours [9].

On the other hand, oxidative stress may be considered a strong cause of stroke, as oxidative stress is an abnormal condition in the body that results from an increase in the production of oxidants compared to antioxidants [10]. Oxidative stress is generally considered an influential and direct cause of injury and several diseases [11], including acute stroke [12]. The main cause of exacerbation of brain injury due to cerebral ischemia is the abnormal increase in oxygen free radicals, and the enhancement of oxygen free radical reactions is an important cause of cerebral edema secondary to CH [13]. Faced with these data, this study aimed to evaluate some biological parameters and the sensitivity and specificity of oxidative stress markers in men with ischemic stroke in the Touggourt (Algeria) region.

Materials and Methods

Subjects

Ethical approval was obtained from the ethics committee (15 EC/DCMB/FNSL/EU2019) of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued. The study was done on 40 voluntary males divided into healthy reserved as controls with an average age of 56.13±3.32 years and ischemic stroke patients with an average age of 57.75±4.01 years. All of the volunteers (control and patients) in this study were living in the Touggourt area located in the South East of Algeria.

Inclusion and exclusion criteria

Ischemic stroke patients were selected based on clinical diagnosis by specialist doctors who confirmed stroke suffering for at least one month. In addition, patients had not received treatment for other types of chronic diseases for 30 days. Controls were healthy people not suffering from chronic or acute diseases and had not consumed drugs for 30 days. All other types of diseases were excluded.

Sample collection and analyses

Blood sampling was done for both groups in the morning and fasting. It was performed using the vein at the end of the elbow. Blood samples were collected in three tubes. Dry tubes were centrifuged at 3000 rpm for 10 minutes, then the serum was obtained to achieve the dosage of blood glucose, electrolytes, and oxidative stress (malondialdehyde (MDA), glutathione (GSH), vitamin C, superoxide dismutase (SOD), oxygen radical absorbance capacity (ORAC), and total thiol parameters. The anticoagulant tube (EDTA) was mixed well and then the hematological and oxidative stress (MDA and GSH) parameters were assayed. Hematological analysis (FNS) was performed by the hematology auto analyzer. Determination of electrolytes (potassium, sodium, and chlorine) was done by an automatic electrolyte analyzer (Easylute).

Preparation of erythrocyte and leukocyte homogenate

Blood EDTA tube contents were centrifuged at 2000 rpm for 10 minutes and the plasma was removed. The cap of the EDTA tube was lyzed with 50 mL of TBS buffer (EDTA 2.92M; tris 1.21M; pH=7) and incubated for 30 minutes in the freezer. After incubation, centrifugation was done at 2500 rpm for 10 minutes and the supernatant was obtained. Erythrocyte homogenate [14] was used for the determination of antioxidant activity [15]. After removing the plasma and separating of erythrocyte, the rest of the EDTA tube contents

were centrifuged at 2000 rpm for 10 minutes. The pellet was washed with lysis buffer and shaken followed by incubation in the freezer for 30 minutes. After incubation, centrifugation was done at 2500 rpm for 10 minutes followed by washing with lysis buffer until the leukocyte pairing, followed by recovering to make the dosage of stress tests [15].

Measurement of oxidative markers

The method of MD assay was based on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid according to the method of Yagi (1976) [16]. The level of reduced GSH was determined according to Weak and Cory (1988) [17] by measuring the optical density results from the formation of 2-nitro-5-mercapturic acid from the reduction of dithiol-bis-2-nitrobenzoic acid, which is called Ellman reagent with SH groups exist in GSH. SOD activity was assessed using the NBT by the superoxide anion (O_2^-) and was based on detecting the presence of SOD by measuring the absorbance at 560 nm [18]. The total antioxidant power (ORAC) and total thiol of the serum were estimated according to the method of Oyaizu (1986) [19] and Ellman [20], respectively. The plasma vitamin C was measured according to the method of Jacota and Dani (1982) [21] using the Folin reagent and a range of ascorbic acids.

Statistical analysis

Statistical analysis was performed by SPSS software, version 20.0 software and comparisons were carried out by the Student t-test. Correlation analysis was carried out using the Pearson Correlation coefficient and regression analysis was used for other analyses. Differences were considered statically significant at $P<0.05$.

Results

Description of the study population

The general data on the socioeconomic characteristics of the two groups of subjects included age, body weight, BMI, and blood pressure levels. These indicators did not

Table 1. Demographic, clinical, and laboratory features between the study groups

Parameters		Mean±SD/%		P
		Control (n=20)	Stroke (n=20)	
Age (y)		56.13±3.32	57.75±4.01	0.098
Body weight (kg)		75.37±1.83	73.68±1.73	0.163
Body mass index, (kg/m^2)		28.02±0.698	27.936±0.666	0.152
Systolic blood pressure (mmHg)		120±2.033	142±3.65	0.042
Diastolic blood pressure (mmHg)		70.02±1.24	79.95±2.03	0.053
Fasting blood glucose (mg/dl)		97.80±2.58	132.6±13.1	0.038
Triglycerides (mg/dl)		90.6±11.9	125.4±24.9	0.001
High-density lipoprotein cholesterol (mg/dl)		34.0±2.69	41.87±4.28	0.052
Low-density lipoprotein cholesterol (mg/dl)		83.58±7.02	88.17±8.11	0.086
Total cholesterol (mg/dl)		132.42±5.63	143.77±6.02	0.063
Blood type	A	8.62	9.48	0.125
	B	15.52	6.90	0.023
	AB	3.45	0.86	0.000
	O	28.45	26.7	0.632
Social case	Married	46.15	50	0.087
	Single	3.85	00	0.000

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Table 2. Changes in the hematological serum electrolytes and alkaline phosphatase levels between the control and ischemic stroke patients

Parameters	Mean±SD		P
	Control (n=20)	Stroke (n=20)	
White blood cells (×10³/µL)	4.87±0.55	9.48±0.78	<0.0001
Lymphocytes (×10³/µ)	1.64±0.17	2.04±0.20	0.081
Neutrophiles (×10³/µ)	2.96±0.46	7.08±0.97	0.001
Red blood Cells (×10⁶/µL)	4.90±0.11	3.55±0.23	<0.0001
Hemoglobin (g/dl)	15.14±0.28	10.69±0.69	<0.0001
Platelets (×10³/µL)	268±16.6	238.8±22.6	0.198
Blood glucose (g/L)	0.9780±0.0258	1.326±0.131	0.038
Serum sodium (mmol/L)	141.29±10.76	139.28±09.79	0.023
Serum potassium (mmol/L)	4.342±0.134	3.88±0.07	0.016
Serum chloride (mmol/L)	105.12±10.698	97.36±0.66	0.02
Serum alkaline phosphatase (U/L)	210±12.03	155±13.65	0.002

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have any statistically significant (P>0.05) differences but blood pressure increased in male stroke patients compared to controls as shown in Table 1.

Hematological and biochemical markers

Our results (Table 2) showed that the leukocyte lineage (WBC and NEUT) significantly increased (P<0.001 and P<0.01, respectively) in the stroke group than the control group. Erythrocytes (hemoglobin and RBC) significantly decreased (P<0.001) in stroke men compared to the controls. But lymphocytes and PLT showed no significant differences (P>0.05). The result of blood glucose and blood electrolyte analysis (Table 2) showed that blood glucose levels significantly increased (P>0.05) while sodium, potassium, and chloride concentrations significantly decreased (P<0.05) in the stroke group to the control group. On the other hand, a significant decrease (P<0.01) in alkaline phosphatase activity was found in the stroke group than in the control group.

Oxidative stress markers

The analysis of blood oxidative stress parameters in control and stroke patients is shown in Table 3. There was a significant increase in MDA levels and SOD activity in erythrocytes (P<0.001), leukocytes (P<0.01) and in serum (P<0.05) and total antioxidant capacity (TAC) (P<0.01) in stroke group compared to male control subjects, in contrast there was a significant decrease in leukocyte GSH (P<0.05) and serum GSH (P<0.05) and total thiol (P<0.01) levels of the male stroke patients compared to the male control subjects. However, our results showed no significant differences in erythrocyte, GSH, and serum vitamin C levels.

Predictive factors

We found that erythrocyte MDA and leukocyte SOD were a significant predictive factors for stroke in men (P<0.05) with a high percentage of specificity (100; 100%) (AUC value: 1; 0.83) and sensitivity (40; 60 %), respectively (Table 4 and Figure 1).

On the other hand, our results showed that serum MDA, leukocyte GSH, and serum SOD levels were not significant predictive factors for stroke (P>0.05).

Discussion

The obtained results showed a significant increase in blood glucose levels in the stroke group compared to the control group. This result is in agreement with that of Xue et al. [22]. Hyperglycemia in acute stroke is probably the result of multiple factors, including cytokine-induced resistance to insulin action [23]. In our study,

Table 3. Oxidative stress parameters in blood of control and stroke patients

Parameters		Mean±SD		P
		Control (n=20)	Patient (n=20)	
MDA (nmol/mgHb)	Erythrocytes	3.32±0.51	13.55±1.61	0.0001
	Leukocytes	4.017±0.79	12.25±2.35	0.002
	Serum	10.25±0.72	19.16±3.96	0.042
GSH (µmol/L)	Erythrocytes	0.30±0.04	0.26±0.02	0.065
	Leukocytes	0.13±0.03	0.09±0.01	0.049
	Serum	0.20±0.02	0.14±0.01*	0.038
SOD (U/mg Hb)	Leukocytes	11.84±0.34	18.40±1.57**	0.008
	Serum	11.72±1.49	17.07±1.68**	0.006
TAC (U/L)	Serum	1.10±0.02	1.15±0.01**	0.003
Vit C (mmol/L)	Serum	6.08±1.19	9.80±2.80	0.072
Total thiol (mol/L)	Serum	1.66±0.18	7·28±1.46**	0.007

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MDA: Malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; Vit C: vitamin C.

stroke patients had significantly low serum sodium, potassium, and chlorine levels compared to controls. Disorders of sodium and potassium concentration are the commonest electrolyte abnormalities found in the cerebral vascular accident (CVA) [24]. Low sodium after stroke results from either the syndrome of inappropriate antidiuretic hormone secretion, inappropriate fluid intake and fluid loss, or a high level of brain natriuretic peptides (BNPs) [25].

In our experimental study, the result showed a significant decrease in alkaline phosphatase (ALP) activity in

Table 4. Sensitivity, specificity, and AUC values of some oxidative stress markers

Variables	%		AUC	$CI_{95\%}$	P
	Sensitivity	Specificity			
Erythrocytes MDA	40	100	1	1.000-1.000	0.014
Leukocytes MDA	00	100	0.75	0.326-1.000	0.221
Serum MDA	40	75	0.48	0.068-0.882	0.903
Leukocytes GSH	60	50	0.40	0.000-0.839	0.624
Serum GSH	80	25	0.30	0.000-0.681	0.327
Leukocytes SOD	60	100	1	1.000-1.000	0.014
Serum SOD	60	100	0.83	0.538-1.000	0.111
Serum TAC	20	50	0.40	0.000-0.824	0.624
Serum THIOL	00	100	0.30	0.000-0.680	0.327

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MDA: malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; AUC: area under curve.
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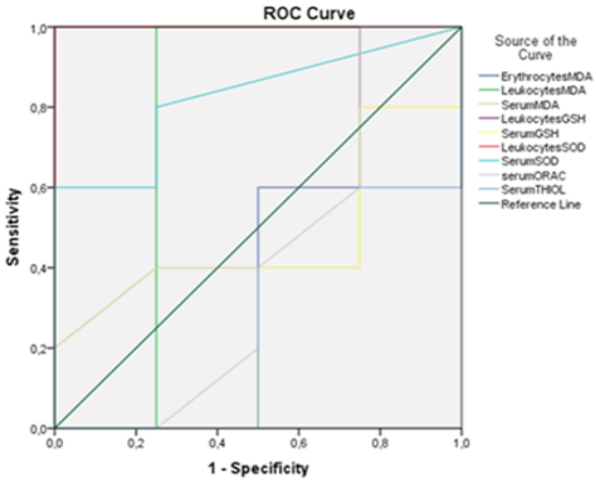

Figure 1. ROC curve for oxidative stress markers in ischemic stroke patients.

both men and women patients as controls. This can be related to inflammation and oxidative stress after stroke [26]. Blood analyzes of stroke patients showed a clear increase in WBC and NEUT rates, which is consistent with a study by Laridan et al. [27] where their results found that levels of WBC in stroke are significantly elevated, as they are the 1st cell to be recruited after a stroke in the affected area leading to the increased expression of adhesion molecules, cytokines/chemokines, proteases, and reactive oxygen species [28]. Moreover, our results indicated that there was a significant decrease in RBC and HGB counts in men with stroke compared to healthy individuals, which is in agreement with the study by Santos-Silva et al. [29]. During ischemic stroke, inflammation can reduce the survival rate of RBC [30] by WBC activation post-stroke, which also leads to induced hemoglobin oxidation and lipid peroxidation, resulting in hemolysis and a decrease in erythrocyte count and hemoglobin levels [31].

In our experimental study, our results show that the MDA level is significantly increased in men with stroke compared to controls. This result is in agreement with the study by Antonio et al. [32] who explained that the MDA showed higher levels in stroke patients than in controls at hospital admission and its levels did not change in the following seven days. Stroke is accompanied by increased formation of free radicals [33]. The brain is particularly sensitive to oxidative injury because of the high content of polyunsaturated fatty acids [34]. Oxidative stress is an abnormal condition caused by an excess production of oxidants compared to the antioxidant [35], it has been considered the main cause of several pathologies [36].

In our experimental study, the results showed a significant decrease in GSH and total thiol levels both in female and male patients compared to controls. This result is in harmony with that of Zimmermann et al. [37] who stated that nearly two-thirds of patients with stroke showed decreased GSH levels. GSH is a sulfhydryl-containing tripeptide (Glu-Cys-Gly). It acts as an antioxidant and detoxifier [38]. As a consequence of post-stroke inflammation, tissue GSH is rapidly consumed and depleted. GSH is required for the synthesis of LTC4 from leukotriene A4 (LTA4; a pro-inflammatory lipid mediator) [39]. Concerning total thiol levels, our results are in agreement with the study by Işık et al. [40], who showed a significant decrease in serum total thiol levels in ischemic patients compared to the control groups. The subsequent decrease in plasma GSH levels was previously demonstrated both in an experimental model of cerebral ischemia and in clinical studies on acute stroke [41, 42]. Thus, the reduction of GSH levels contributes to the reduction of total thiol levels. SOD is known as one of the major enzymatic (oxidoreductases) defenses against ROS production [43].

Our experimental study demonstrated a significant increase in SOD activity in patients with stroke compared to controls, which is in agreement with the study by Abdullah et al. [44] who observed that during the acute phase, the MDA and SOD levels were increased in the serum of the patients with ischemic stroke. ORAC method is used to measure antioxidant capacity versus oxygen free radicals [45]. In our study, the serum TAC increased in ischemic stroke patients than in controls Lorente et al. [46] reported similar results to ours regarding higher levels of TAC in their ischemic stroke patients. The TAC up-regulation in patients with ischemic stroke might be a compensatory mechanism for higher ROS production in response to ischemia [46].

Conclusion

This study indicated that hematological parameters changed and oxidative stress was associated with the ischemic stroke disease as a cause or as a development factor for it and through the ROC analysis results where MDA, SOD, and TAC are considered to be the most important markers, which contributes to their early detection in the stroke diseases.

Ethical Considerations

Compliance with ethical guidelines

All human studies were approved by the appropriate ethics committee. All subjects gave their informed consent prior to their inclusion in the study.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared that they have no conflicts of interest.

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