Study of Risk Factors and Assessment of Hematotoxicity and Oxidative Stress Among Women Stroke Patient with Metabolic Syndrome

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ABSTRACT : This study aims at evaluating some risk factors and the variation of certain hematological and oxidative stress markers in women with stroke disease. The observation (clinical study) was done on 130 voluntary women, divided into metabolic syndrome patients with and without stroke disease. Risk factors were estimated by Chi-square test. Results demonstrated that the high levels of family stress, self-medication and anticoagulants treatment were the most dangerous risk factors to stroke diseases, whereas brushing of teeth daily had an important protective factors against participants from disease. From biological study, results showed that RBC, hemoglobin, GSH and total thiol levels were significantly decreased ($p<0.05$) while WBC, neutrophile, platelet, MDA, SOD, ORAC and vit C levels were significantly increased ($p<0.05$) in women with metabolic syndrome (MS) + stroke patients as compared to MS patients without stroke. In conclusion, several socioeconomic and clinical factors contributed to the dispersion and development of the stroke. In addition, oxidative stress during metabolic syndrome contributes to the development of stroke disease.

Key words: Metabolic syndrome, stroke, risk factors, oxidative stress, ROC.

INTRODUCTION

Cerebrovascular accident (stroke) is a disease that affects the system of brain vasculature.[1] Stroke is one of the most devastating worldwide neurological disorders[2] as the increase in the number of people having stroke threatens health and the global economy[3] through the occurrence of some neurological complications that often lead to death.[4] In addition to gender, age and genetic factors, the rest risk factors of stroke are closely related to people's daily habits and behaviors.[5] Routine of life plays an essential role in the etiology of this disease, including diet.[6] Cardiovascular disease, obesity, diabetes mellitus, smoking and dyslipidemia are the main risk factors for stroke.[7] Among the indicators that show the symptoms of stroke, there are some inflammatory indicators such as Facteur de nécrose tumorale (TNF)-alpha and interleukin (IL)-6, as well as lipid parameters such as low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c).[8] On the other hand, with most treatment methods, the treatment approved by the Food and Drug Administration is tissue plasminogen activator (TPA) with duration of 4.5 hours in a treatment period.[9] Many of recent landmarks in scientific research have shown that in human beings, oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body.[10] Oxidative stress is an abnormal condition caused by an excess production of oxidants compared to the antioxidant.[11] It has been considered as the main cause of several pathologies

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including acute ischemic stroke.[12-13] The main reason for the aggravation of cerebral ischemia-induced brain injury is the abnormal increase of oxygen free radicals, and the enhancement of oxygen free radical reactions is an important reason for secondary cerebral edema.[14] Faced with these problems, this study aims to identify the risk factors of stroke disease and evaluate some biological parameters, the sensitivity & specificity of oxidative stress markers in stroke women patients of Touggourt (Algeria) region.

MATERIALS AND METHODS

Study design. We randomly enrolled 200 volunteer women patients aged between 45-62 years old who visited the Hospital, Touggourt (Algeria) from October 10, 2020 until April 15, 2021. These women reported to the clinical study service (NCT00000501). According to the official dates approved by the Hospital (first visit), total of 60 women patients consented and were randomly assigned who had been metabolic syndrome without stroke (group1) (n=30) or who had been metabolic syndrome with stroke (group 2) (n=30). Contact information was received for 200 women initially interested in participating in the study. After we lost contact with each other (23 cases) and others not interested to participate (31 cases) or did not complete medical visits (16 cases), the number reached 130 women who were enrolled in the study (risk factors and biological study). Out of 130 accepted cases, we took 60 cases that we used in the biological study by equally dividing into two groups having metabolic syndrome with or without stroke groups (Figure 1). This is in order to study the change of some biochemical parameters (blood glucose and lipid profile), hematological and oxidative stress (MDA, GSH, vitamin C, SOD, ORAC and total thiol).

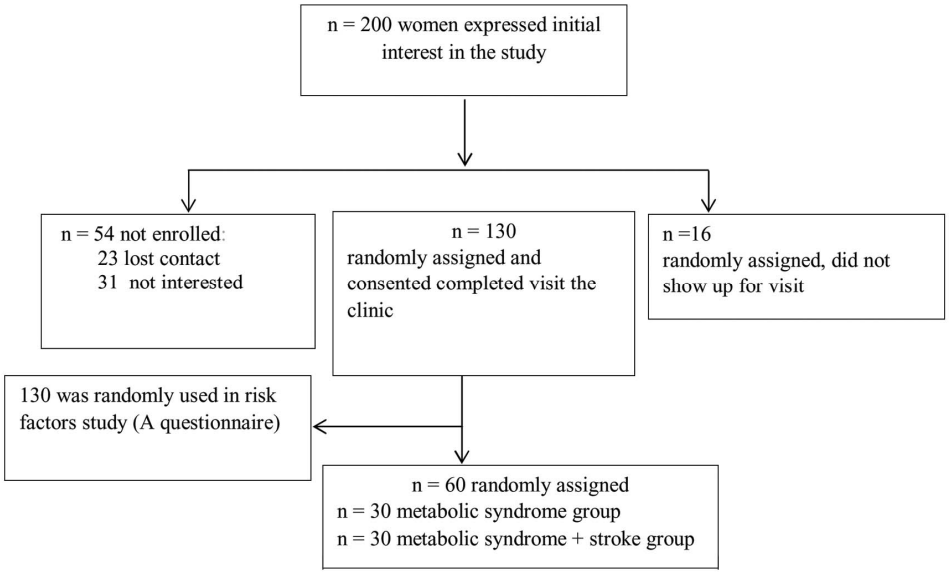

Figure 1. Flowchart of subject recruitment, enrollment and retention from women patients.

Subjects of study. Ethical approval was obtained from the ethics committee (36 EC/DCMB/FNSL/EU2020) of the Department of Cellular and Molecular Biology, University of El Oued. All of the volunteers (all patients) in this study live in the Touggourt area located in the south east of Algeria. Therefore, their social and clinical information including age, sex, BMI, social case, job and blood group were collected by completing the questionnaires from their medical records or through a direct discussion with patients. As inclusion criteria, metabolic syndrome with or without stroke disease patients were selected based on clinical diagnosis by specialist doctors who confirmed stroke

suffering at least for one month. In addition, patients having no other chronic disease treatment for 30 days were included. All other types of diseases were excluded.

Sample collection. Blood sampling for both groups were done at morning after fasting of whole night. It is performed in the vein of the end of the elbow. Blood samples are collected in three tubes. Dry tubes are centrifuged at 3000 rpm for 10 min and then obtained the serum to achieve the dosage of oxidative stress (MDA, GSH, vitamin C, SOD, ORAC and total thiol) parameters. The anticoagulant tube (EDTA) is mixed well and then assays the hematological and oxidative stress (MDA, GSH) parameters. Hematological analysis (FNS) is done by the hematology auto analyzer. Determinations of biochemical parameters (blood glucose and lipid profile) were done by automatic analyzer.

Preparation of erythrocyte and leukocyte homogenate. Tubes having blood mixed with EDTA (ethylenediaminetetra-acetic acid) were centrifuged at 2000 rpm for 10 min and removed the plasma. To obtain a homogenate of red blood cells, we added to the EDTA tube an amount of 50 ml of TBS (tris buffered saline) buffer solution consisting of (EDTA 2.92M; Tris 1.21M; pH = 7) to leave the mixture in the freezer for half an hour, then centrifuge at 2500 cycles for 10 min and the supernatant we put it in a test tube for the realization of the appropriate biological parameters.[15] After removing the plasma and separation of erythrocyte, the rest of EDTA tube contents centrifuged at 2000 rpm for 10 min. After obtaining the homogeneity of red blood cells, we isolated the precipitated solution to be washed and frozen several times with the lysis solution and then we centrifuged at 2500 rpm for 10 min, then isolated the precipitated solution and kept it until it is used in the oxidative stress parameters.[15]

Oxidative markers measurement. The method of malondialdehyde (MDA) assay is founded on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid according to the method of Yagi.[16] The level of reduced glutathion (GSH) was determined based on the formation of 2-nitro-5-mercocapturic acid from the reduction of dithio-bis-2-nitrobenzoic acid according the Weak and Cory method.[17] The analysis method of superoxide dismutase (SOD) activity using the NBT (nitroblue tetrazolium) by the superoxide anion ($O2^{\cdot}$) is used as a basis for detecting the presence of SOD by measuring the absorbance at 560 nm.[18] The total antioxidant power (ORAC: Oxygen radical absorbance capacity) and total thiol of the serum are estimated according to the method of Oyaizu[19] and Ellman[20] respectively. Vitamin c in serum is measured according to the method of Jacota and Dani[21] using the Folin reagent and a range of ascorbic acid.

Statistical analysis. Values were expressed as percent per population or as the mean ± standard deviation (SD). Data were presented using frequency tables. The statistical evaluation is carried out by the student's T test for comparison between groups. For categorical variables, a number (percentage) was used to express them; they were compared using the Chi-square ($\chi 2$) or Fisher's exact test. Chi-square test was used also to test the association between risk factors and stroke. Odds ratios test were calculated by statistics of Cochran's and Mantel Haenszel by using SPSS 20 software. Odds ratios >1 and $P<0.05$ indicate a significant risk factor. $OR<1$ and $P<0.05$ indicate a significant protective factor.

RESULTS AND DISCUSSION

Description of the study population. The general data of demographic characteristics of the two groups of subjects include age, body weight, BMI, number of children, social case, job, educational level and blood group. Patients with metabolic syndrome and stroke had an HbA1c value of 8.92% versus 8.52% in MS patients without stroke. Serum total cholesterol was higher in group 2 ($p=0.048$) compared to group 1. Fasting blood glucose was lower in group MS + stroke compared to group MS. Other baseline characteristics between the two main groups are summarized in table 1.

Table 1. Demographic, clinical and laboratory features between the study groups (n=130).

Parameters		Group 1 MS	Group 2 MS + stroke	p -value
Age (ys)		48.75±1.46	50.43±1.54	0.23
Number of children		4.30±0.36	4.763±0.314	0.54
Body weight (kg)		75.37±1.83	73.68±1.73	0.93
Body mass index, kg/m2		28.02±0.69	27.93±0.66	0.45
Triglycerides, mg/dl		271±47	92±18	0.023
High-density lipoprotein cholesterol, mg/dl		65±2.8	58±2.6	0.068
Low-density lipoprotein cholesterol, mg/dl		136±30	133±23	0.084
Total cholesterol, mg/dl		190±12	212±19	0.048
TG/HDL		4.16±0.53	1.58±0.025	0.006
Fasting blood glucose, mg/dl		289±60	151±25	0.000
Job	Worker (%)	21.54	23.08	0.36
	Retired (%)	3.08	7.69	0.15
	Hero (%)	25.38	19.23	0.63
Educational level	Illiterate (%)	11.54	33.08	0.028
	Educated (%)	31.54	16.15	0.008
	High school (%)	6.92	0.77	0.000
Blood type	A (%)	8.62	9.48	0.96
	B (%)	15.52	6.90	0.002
	AB (%)	3.45	0.86	0.000
	O (%)	28.45	26.7	0.87
Social case	Married (%)	46.15	50	0.64
	Single (%)	3.85	00	0.000
Stroke type	Ischemic (%)	-	61.54	-
	Hemorrhagic (%)		16.92	-
	TIA (%)		21.54	-

Study of socioeconomic and clinic factors. Odds ratio (OR) values for socioeconomic and clinic-pathological factors (Table 2) show that hyper cholesterol, eating of the red meat, diabetes, atrial fibrillation and hypertension were shown to be significant risk factors for the cerebrovascular accident (OR = 2.633; p = 0.045, OR=2.739; p=0.013, OR = 3.765 ; p = 0.006 , OR= 4.636 ; p = 0.004 and OR= 6.682 ; p = 0.000) respectively. In addition smoke, excess treatment with anticoagulants, self-medication and exposed to high levels of family stress also shown to be significant risk factors for stroke in the study population (OR = 8.071; p = 0.000, OR=12.310; p=0.000 and OR = 12.687 ; p = 0.000) respectively. In contrast brush of teeth daily and anemia safety were protective factors (OR = 0.337, p = 0.007; OR = 0.190, p = 0.026) respectively. Obesity, heart attack, hereditary, cancer treatments, self-medication, excess of analgesics, migraines, fast food, spicy foods, salt, sugars, soft drinks, excess of water, herds for medication, exercise regularly, exposed to the sun continuously and exposed to work stress were not considered as predictors of stroke in our population since the OR values obtained are not significant .

Hematological and oxidative stress markers. The results of the hematological and oxidative stress analysis for metabolic syndrome with or without stroke patients were illustrated in table 3. The results show that the leukocytes lineage (WBC and Neutrophile) and platelets (PLT) were significantly increased ($P < 0.05$ and $P < 0.01$) in the group 2 as compared to the group 1. Erythrocyte line is significantly decreased (HGB; $P < 0.01$ and RBC; $P < 0.001$) in group 2 compared to group 1. Lymphocyte levels showed no significant differences ($P > 0.05$). The analysis of oxidative stress markers in the blood of patients were shown in table 3. Our results explained that a significant increase ($P < 0.05$) of MDA level and SOD activity in leukocytes, erythrocytes and serum ($P < 0.01$) and of ORAC activity ($P < 0.05$), also a significant decrease of GSH

($P < 0.001$) and total thiol ($P < 0.01$) in serum of group MS + stroke compared to group MS. However, there was no significant change ($P>0.05$) in serum MDA, erythrocyte and leukocyte GSH and in serum vitamin C concentration.

Table 2. Comparison of the clinic factors of patients groups (N=130).

Factors	MS (%)		MS + stroke (%)		OR*	95% CI**	p-value
	Positive	Negative	Positive	Negative			
Hypertension	8.46	41.54	27.69	22.31	6.682	2.61-17.10	0.000
Diabetes	6.92	43.08	19.23	30.77	3.765	1.41-10.05	0.006
Hyper cholesterol	6.92	43.08	14.62	35.38	2.633	0.96-7.17	0.045
Atrial fibrillation	5.38	44.62	16.92	33.08	4.636	1.55-13.84	0.004
Anemia safety	9.23	40.77	2.31	47.96	0.190	0.03-0.92	0.026
Obesity	10	40	8.46	41.54	0.762	0.27-2.12	0.398
Migraines	5.38	44.62	6.15	43.85	1.227	0.35-4.31	0.500
Family history	5.38	44.62	12.31	37.69	2.842	0.92-8.79	0.054
Excess of analgesics	20	30	11.54	38.46	0.474	0.20-1.12	0.066
Self-medication	13.85	36.15	6.92	43.08	0.419	0.15-1.15	0.040
Anticoagulants treatment	3.08	46.92	21.54	28.46	12.310	3.37-44.89	0.000
Smoking	3.08	46.92	16.92	33.08	8.071	2.18-29.78	0.000
Fast food	9.23	40.77	8.46	41.54	0.868	0.30-2.46	0.500
Salt	5.38	44.62	9.23	40.77	1.976	0.61-6.38	0.194
Sugars	11.54	38.46	10.77	39.23	0.893	0.35-2.26	0.500
Red meat	15.38	34.62	26.92	23.08	2.739	1.20-6.23	0.013
Soft drinks	16.92	33.08	26.15	23.85	2.103	0.93-4.71	0.053
Exercise regularly	12.31	37.69	5.38	44.62	0.352	0.11-1.08	0.054
Brushing teeth daily	27.69	22.31	15.38	34.62	0.337	0.14-0.76	0.007
Exposed to the sun	21.54	28.46	15.38	34.62	0.545	0.23-1.24	0.107
Exposed to family stress	20	30	38.46	11.54	12.687	4.90-32.73	0.000

*OR: Odds ratio. **CI: Confidence interval (95%).

Table 3. Haematological and oxidative stress parameters in the blood of patients groups.

Parameter		Group 1 MS	Group 2 MS + stroke	p-value
White blood cells (10*9/l)		5.91±1.39	7.91±1.10	0.023
Lymphocyte (10*9/l)		1.92±0.35	2.69±0.56	0.069
Neutrophil (10*9/l)		2.84±1.28	4.80±1.05	0.021
Red blood cells (10*12/l)		5.02±0.31	4.16±0.08	0.000
Hemoglobin (g/dl)		14.9±0.88	12.42±0.83	0.002
Platelets (10*9/l)		216.8±51.3	313.8±26.3	0.001
MDA (nmol/mg Hb)	Erythrocytes	3.36±0.92	9.59±2.74	0.042
	Leukocytes	3.96±1.38	7.78±2.65	0.032
	Serum	6.46±1.45	8.59±2.40	0.062
GSH (µmol/l)	Erythrocytes	0.30±0.07	0.83±0.09	0.056
	Leukocytes	0.13±0.03	0.09±0.03	0.059
	Serum	0.21±0.06	0.14±0.01	0.000
SOD (U/mg Hb)	Leukocytes	13±0.83	14.6±0.92	0.02
	Serum	11.82±1	14.4±1.12	0.006
ORAC (U/l)	Serum	0.92±0.06	1.24±0.16	0.03
Vit C (mmol/l)	Serum	6.40±1.59	10.88±8.44	0.061
Total thiol (mol /l)	Serum	0.62±0.06	0.23±0.09	0.006

Correlation between biological markers and risk factors. In women stoke group, there was a significant correlation (P<0.001) between serum thiol and each of hyper cholesterol, red meat, diabetes, in addition between serum ORAC and each of atrial fibrillation, hypertension, excess of anticoagulants, high levels of family stress (P<0.001). We found a significant correlation (P<0.01) between serum SOD and each of atrial fibrillation, hypertension, excess of anticoagulants, high levels of family stress. There was no correlation (P>0.05) between the rests of correlation test in patients' stoke groups (table 4). In contrast, all of correlation test was no significant (P>0.05).

Table 4. Multivariate analysis to multiple predictors (risk factors) and oxidant status in the population study.

Independent variable	Dependent variable					
	Erythrocytic MDA	Leukocytic MDA	Leukocytic SOD	Serum SOD	Serum ORAC	Serum thiol
Hyper cholesterol						
P	0.617	0.981	0.461	0.252	0.104	0.000
R^2	0.04	0.0001	0.06	0.16	0.29	0.89
Red meat						
P	0.907	0.412	0.282	0.312	0.595	0.000
R^2	0.002	0.08	0.14	0.12	0.03	0.89
Diabetes						
P	0.617	0.981	0.461	0.252	0.104	0.000
R^2	0.04	0.0001	0.06	0.16	0.29	0.89
Atrial fibrillation						
P	0.076	0.257	0.767	0.005	0.000	0.624
R^2	0.43	0.15	0.01	0.64	0.99	0.03
Hypertension						
P	0.076	0.257	0.767	0.005	0.000	0.624
R^2	0.43	0.15	0.01	0.64	0.99	0.03
Excess anticoagulants						
P	0.076	0.257	0.767	0.005	0.000	0.624
R^2	0.43	0.15	0.01	0.64	0.99	0.03
Family stress						
P	0.076	0.257	0.767	0.005	0.000	0.624
R^2	0.43	0.15	0.01	0.64	0.99	0.03

Results of our study revealed that hyper cholesterol and excessive consumption of the red meat are associated with increased risk of stroke. Study of Wang[22] showed a significant relationship between the ischemic stroke risk and the serum total cholesterol level for the general population. Red meat contains a large amount of saturated fats that can raise the level of plasma cholesterol, low-density lipoprotein cholesterol and triglycerides.[23] In our study the results show that the diabetes mellitus is significantly associated with stroke risk. This result is in agreement with study of Mohamed.[24] Diabetes is an important cause of stroke complications as it causes vascular endothelial fragility, systemic inflammation and hypertension.[25] Diabetes mellitus characterized by insulin resistance plays a major role in the pathology of cardiovascular disease including stroke.[26] Our study shows that hypertension is as an important risk factor to having a stroke. This was in accordance with some other studies which demonstrated that hypertension is the main risk factor of stroke disease[27] with prevalence at around 60% to 70% for all studies.[28] High blood pressure is also one of the main causes of stroke, as it hardens or narrows the arteries of the brain which leads to brain tissue damage.[29] Our study also shows that atrial fibrillation

(AF) and excess anticoagulants are risk factors for stroke. Study of Elkhatib[30] showed that AF constitute an important risk factors for ischemic stroke. On the other hand, Budincevic[31] said that the oral anticoagulant therapy is a risk factor for hemorrhagic stroke. Our results showed a significant correlation between smoking and stroke disease, which is in agreement with another study[32], who stated that cigarette smoking, an important risk factor for cardiovascular disease doubles the risk of stroke**.** Our study showed that high levels of family stress is largely associated with a risk of stroke, which is in agreement with other studies.[33] In our study we have found that good oral health (brush of teeth daily) is a protective factor from stroke. The two most common diseases affecting oral health are dental caries and periodontitis.[34] Choe[35] found a positive correlation between periodontal disease and high risk of ischemic stroke compared with persons with no periodontitis, gingivitis or tooth loss. Our study showed that WBC and neutrophil are significantly increased in the stroke patients as compared to control both in men and women. Our results are in the line with the results of Laridan[36] who found a neutrophil levels in stroke thrombi are considerably high, because of being the first cell to be recruited after stroke to the injured area and contributes the increased expression of adhesion molecules, cytokines/chemokines, proteases and reactive oxygen species.[37] These molecules contribute to post ischemic inflammation and endothelium damage and tissue necrosis.[38] The immune response of the patients with an ischemic stroke leads to inflammatory response and the accumulation of white blood cells, and thus their number increases.[39] Moreover, our results illustrated a significant decrease of RBC and HGB in women with stroke when compared with healthy individuals which is in agreement with study of Santos-Silva.[40] Ischemic stroke is related to the central mechanisms of erythropoiesis regulation.[41] In our study, experimental data shows that MDA level is significantly increased in women with stroke compared to controls. Stroke is enhanced by high level of free radicals.[42] The brain is mainly sensitive to the oxidative injury because of the high content of polyunsaturated fatty acids.[43] The increase of MDA level because of lipid peroxidation products are a key mediator of apoptosis induced by oxidative stress.[44] In our study, the experimental results show a significant decrease in glutathione (GSH) and in total thiol levels (SHt) in patients with MS + stroke women as compared to MS. Ischemic stroke causes brain damage and alterations in GSH redox status[45] by post stroke hypoxic episode which reduced the cysteine uptake and a concomitant decrease in GSH.[46] Our study demonstrated a significant rise in SOD activity in patient with MS + stroke women as compared to MS. The results found were in agreement with study realized by Abdullah[47] who found that patients with ischemic stroke had higher levels of serum SOD activity and MDA concentration. Our data revealed that the total antioxidant activity (ORAC) was increased in serum of MS + stroke patients than in MS women patients. Studies of Lorente[48] described a similar results to ours apropos higher levels of ORAC in patients suffered with ischemic stroke. As a kind of response to ischemia and increased production of reactive oxygen species in patients with AIS, the body works to increase the ability to produce antioxidants.[49]

CONCLUSION

In this study, it was found that hyper cholesterol, eating of red meat, diabetes, atrial fibrillation, hypertension, long term treatment with anticoagulants and high levels of family stress are major risk factors for stroke. On the other hand, during metabolic syndrome oxidative stress was a major cause or a development factor for stroke disease. Finally, ROC analysis results that MDA, SOD and ORAC are considered to be the most important markers which contribute to early detection of stroke diseases in metabolic syndrome women patients.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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