

ORIGINAL ARTICLE

Serum C-Reactive Protein and Sex Hormone Levels in the Early Hyperacute Phase of Stroke

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SUMMARY

Background: To see the relationship of early admission parameters with the type of stroke and/or with the 30-days mortality from this disease.

Methods: Stroke patients at their early hyperacute phase (n = 180) were enrolled in this study (156 ischemic strokes and 24 hemorrhagic strokes). Blood levels of C-reactive protein (CRP), testosterone, and estradiol were determined at admission, before any specific intervention. Patients' clinical data, including the above-mentioned laboratory parameters, were compared between the above two stroke types (in total and between sexes).

Results: The mean age of the patients was 69.55 ± 12.03 years old (69.92 ± 11.94 years old in ischemic stroke and 67.12 ± 12.54 years old in hemorrhagic stroke). Serum estradiol levels of both males of ischemic stroke and females of hemorrhagic stroke patients were significantly higher than the females of the ischemic stroke. Serum CRP levels of both females and males of the hemorrhagic group were higher than their peers of the opposite group. Early admission serum CRP level ≥ 0.74 mg/dL in males helped predict hemorrhagic stroke while a serum estradiol level ≥ 14.07 ng/mL helped predict the same type of stroke in females.

Conclusions: Our study results show that simple early laboratory measures (such as CRP and estradiol) may help in the early phase management of stroke. Further studies are needed to confirm our findings.

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KEY WORDS

C-reactive protein, estradiol, testosterone, stroke, outcome

INTRODUCTION

Stroke is one of the leading causes of morbidity and mortality worldwide. Each year 15 million people suffer a stroke. About 5.8 million of these die from it. The etiology, cause, and outcome of a stroke may differ between females and males [1]. Ischemic strokes are more common than hemorrhagic strokes [2]. One of the interesting points is that the women get the 1st attack of a

stroke at older ages (in comparison to men) [1]. Another interesting point is that the risk of atherosclerotic cardiovascular disease (CVD [including ischemic stroke]) increases dramatically in women after the menopause. This increased CVD risk could be attributed to sex hormone changes with menopause. In a study by Zhao D, et al. [3], a higher testosterone/estradiol ratio was associated with an elevated risk for CVD in postmenopausal women. In hemorrhagic stroke, unlike the ischemic one, patients' gender does not affect age-related 90 days outcomes [4]. Some protein biomarkers and CRP have been studied in the early phase of stroke [5-7]. Researchers from Greece have studied sex hormone levels in the acute phase (within 2 - 3 days of admission) in postmenopausal women (but not in men) [8]. Increased CRP levels are associated with an increased risk of further ischemic events in patients with symptomatic intracranial atherosclerotic disease. Additionally, Arenillas JF, et al. found that the CRP gene C1444T polymorphism is associated with this increased risk [9]. As far as we know, serum CRP and sex hormone levels had not been studied at the early hyperacute phases of stroke. So, we aim to determine and study these biomarkers in this phase of the stroke.

MATERIALS AND METHODS

This prospective study has been approved by Bakirkoy Dr. Sadi Konuk Training & Research Hospital's ethical committee. It was conducted according to The Declaration of Helsinki. Written informed consent was taken from patients or their family members before enrollment. Patients with first-episode stroke (ischemic or hemorrhagic) admitted to Bakirkoy Dr. Sadi Konuk Training & Research Hospital's Neurology department were enrolled in this study within 12 hours of symptom onset. At the time of suspicion of a stroke at first visit, blood samples for routine blood tests were obtained. Due to quality and safety purposes, all blood samples are routinely stored for 56 hours in our hospital's laboratory. Simultaneously, a noncontrast CT (NCT) was performed in order to confirm the diagnosis and type of stroke. At this stage, patients (or their family members) are asked to participate in this study. The above-mentioned study blood tests were determined from the kept blood samples of patients that accept to participate in this study. The researchers did not influence treatment strategies. Management and follow-up of the participants were undertaken by the managing neurologist according to the 2018 American Heart Association (AHA)/American Stroke Association (ASA) Guidelines [10]. At early admission, before starting any specific treatment for stroke, blood samples for determining serum CRP, testosterone, and estradiol were drawn. Detailed clinical and laboratory data of patients were collected as well. According to stroke types, patients were divided into 2 groups; group 1 consisted of ischemic stroke patients, and group 2 consisted of hemorrhagic

(intracerebral hemorrhage) stroke patients. Inclusion and exclusion criteria were as follow:

Inclusion criteria:

- The ability of patients or their family members to give written informed consent (both groups)
- Age more than 50 years old (both groups)

Exclusion criteria:

- History of previous stroke attack
- Transient ischemic attack
- Patients already on sex hormone replacement therapy at the time of diagnosis of stroke or preceding 3 months
- Using other medications that may affect the sex hormone profiles of the patient (such as steroids).

The primary aim of this study was to evaluate and compare the early admission serum sex hormones and CRP levels between these two groups as a whole and in relation to their sex (females and males separately). Other study parameters were also compared as a whole and in relation to gender. The secondary aim was to see the relation of sex hormones and CRP levels with 30-day outcomes of the patients. Patient outcomes were followed up by the hospital and official reports of General Health Insurance, Turkish Ministry of Family, Labor and Social Services.

Laboratory parameter measurements

Venous blood samples for the below laboratory measurements were determined from admission routine blood tests samples, i.e., before starting any specific treatment for stroke. Laboratory measurements were done in our hospital's central laboratory. CRP (in mg/dL) determination, complexes formed during the immunological reaction of rabbit anti-CRP antibodies coated on patient serum and latex particles were determined by the immunoturbidimetric method in Unicel DXI 800 analyzer (Beckman Coulter, USA). Its detection limit was ≤ 0.15 mg/dL. Estradiol (detection limit 15.00 - 5,200 pg/mL), and testosterone (detection limits 0.10 - 16.00 ng/mL) analysis from venous blood samples were performed by chemiluminescence method and magnetic particle-separation technology in UniCel DXI 800 Immunoassay system (Beckman Coulter, USA). T/E ratio was computed by dividing serum testosterone (ng/mL) by serum estradiol (ng/mL).

Data availability

Data are available to researchers (for research purpose only) on request by directly contacting the corresponding author.

Statistical analyses

Statistical analyses were performed using SPSS 22.0 statistical package for Windows. Description of data was expressed by mean, standard deviation, median, and interquartile range, as appropriate. The distribution of variables was checked with the Kolmogorov-Smirnov test. For a comparison of variables of a normal dis-

Table 1. Comparison of study parameters between ischemic and hemorrhagic stroke groups.

Parameter	Ischemic stroke		Hemorrhagic stroke		p-value
	Female (n = 74) ^{1,3}	Male (n = 82) ^{2,3}	Female (n = 11) ^{1,4}	Male (13) ^{2,4}	
Age (years)	<u>73.58 ± 1.22</u>	66.62 ± 1.08	<u>73.54 ± 1.28</u>	<u>61.69 ± 9.81</u>	NS ¹ , NS ² , < 0.001 ³ , NS ⁴
BMI (kg/m ²)	27.34 (17.30 - 44.44)	26.03 (20.76 - 40.12)	<u>27.17</u> (<u>21.61 - 32.37</u>)	<u>24.91</u> (<u>21.61 - 34.73</u>)	NS ¹ , NS ² , 0.005 ³ , NS ⁴
C-reactive protein (mg/dL)	0.81 (0.00 - 18.60)	0.65 (0.00 - 20.70)	4.00 (0.10 - 64.00)↑	4.00 (0.10 - 64.00)↑	0.018 ¹ , < 0.001 ² , NS ³ , NS ⁴
Testosterone (ng/mL)	0.34 (0.01 - 4.85)	2.49 (0.21 - 65.10)	<u>0.46</u> (<u>0.00 - 1.30</u>)	<u>1.79</u> (<u>0.754,79</u>)	NS ¹ , NS ² , < 0.001 ³ , < 0.001 ⁴
Estradiol (pg/mL)	1.30 (0.01 - 1.27)	<u>2.42</u> (<u>2.04 - 59.31</u>)	2.07 (9.92 - 75.42)	<u>2.07</u> (<u>9.92 - 75.42</u>)	0.017 ¹ , NS ² , < 0.001 ³ , NS ⁴
T/E ratio	25.28 (1.72 - 4,666.67)	99.74 (12.00 - 12,122.90)	<u>19.83</u> (<u>0.07 - 52.98</u>)	<u>90.71</u> (<u>25.41 - 152.51</u>)	NS ¹ , NS ² , < 0.001 ³ , < 0.001 ⁴

Underlined: normally distributed. T/E: testosterone (pg/mL)/estradiol (pg/mL).

¹ - Comparing females of both group

² - Comparing males of both group

³ - Comparing ischemic stroke females with their peers of males

⁴ - Comparing hemorrhagic stroke females with their peers of males.

Table 2. Comparison of ischemic stroke patients according to their 30-days outcomes.

Parameter	Survivors		Non-survivors		p-value
	Female (n = 58) ¹	Male (n = 71) ²	Female (n = 16) ¹	Male (11) ²	
Age (years)	<u>67.96 ± 1.40</u>	<u>60.35 ± 1.21</u>	<u>75.50 ± 1.08</u>	<u>72.00 ± 1.2</u>	NS ¹ , NS ²
BMI (kg/m ²)	26,23 (17.30 - 40.40)	25,95 (21.10 - 29.96)	<u>27.45</u> (<u>20.45 - 40.40</u>)	<u>24.69</u> (<u>21.09 - 27.68</u>)	NS ¹ , NS ²
C-reactive protein (mg/dL)	0.95 (0.22 - 17.40)	0.50 (0.10 - 5.50)	0.90 (0.22 - 17.40)	0.64 (0.10 - 5.50)	NS ¹ , NS ²
Testosterone (ng/mL)	0.38 (0.07 - 4.85)	2.38 (0.60 - 65.10)	0.35 (0.08 - 4.85)	2.33 (0.71 - 65.10)	NS ¹ , NS ²
Estradiol (pg/mL)	1.55 (0.01 - 1.27)	2.42 (2.04 - 57.17)	1.50 (7.60 - 49.60)↑	2.42 (2.04 - 57.17)	0.006 ¹ , NS ²
T/E ratio	28.62 (1.72 - 4,666.67)	1.03 (12.00 - 293.8)	17.70 (3.18 - 104.35)	77.10 (40.76 - 12,122.90)	NS ¹ , NS ²

Underlined: normally distributed. T/E: testosterone (pg/mL)/estradiol (pg/mL).

¹ - Comparing females of both group

² - Comparing males of both group

tribution, the *t*-test for independent samples was used and the Mann-Whitney U test was used for the comparison of variables with non-normal distribution. Pearson's and its nonparametric equivalent (Spearman's) tests were also used for evaluation of the correlation between quantitative variables. Regression analysis was done by putting types of stroke (ischemic = 0/hemorrhagic = 1, age (female ≥ 75, and male ≥ 65 years old), BMI (± or < 24.99k g/m²), DM (present or absent), ischemic heart

disease (present or absent). Also by putting laboratory parameters (median); CRP (females ≥ 0.90/males ≥ 0.74 mg/dL), estradiol (females ≥ 14.07/males ≥ 24.27 ng/mL), testosterone (females ≥ 0.35/males ≥ 2.44 pg/mL) into two different logistic regression models (Model: Forward LR) (adjusting odds ratio at 95% confidence interval). A p-value < 0.05 was accepted as significant for all others.

RESULTS

A total of 180 (85 female and 95 male) stroke (24 hemorrhagic and 156 ischemic) patients' data were included in analysis. The mean age of the patients was 69.5 ± 12.0 years old (69.9 ± 11.9 years old in ischemic stroke and 67.1 ± 12.5 years old in hemorrhagic stroke, $p > 0.05$). There were no significant differences in gender distribution between both groups (47.4% (74/156) of the ischemic strokes and 45.8% (11/24) of the hemorrhagic strokes were females, $p > 0.05$). Other study parameter comparisons are also shown in Table 1. Females of the ischemic group were older and heavier than the males of the same group (73.5 ± 1.2 versus 66.62 ± 1.0 years and 28.3 ± 4.7 versus 26.4 ± 3.3 kg/m², $p < 0.05$ for both). CRP levels of both females and males of the hemorrhagic group were higher than their peers of the opposite group (4.0 versus 0.8 and 4.0 versus 0.6 mg/dL, $p < 0.005$ for both). Serum testosterone levels of males of both groups were significantly higher than their female peers (2.5 versus 0.3 and 1.8 versus 0.5 ng/mL, $p < 0.05$ for both). But comparing the same of the two groups yielded no significant difference ($p < 0.05$ for both). On the other hand, serum estradiol levels of both males of ischemic stroke and females of hemorrhagic stroke patients were significantly higher than the females of the ischemic stroke (2.4 versus 1.3 and 2.1 versus 1.3 ng/mL, $p < 0.05$ for both). The T/E ratio of males of both groups was higher than the females of the same group (99.7 versus 25.3 and 90.7 versus 19.8, $p < 0.05$ both). The logistic regression analysis showed that only serum CRP in males and serum estradiol in females had significant results. When serum CRP in male patients was ≥ 0.74 mg/dL, the probability of being a hemorrhagic stroke was 17-fold higher ($p < 0.001$). On the other hand, when the serum estradiol level was ≥ 14.07 ng/mL (median) in female patients, the probability of being a hemorrhagic stroke was 4.4-fold higher. The 30-day mortality rate of ischemic stroke group was 17.31% (27/156 (16F/11M)) and of the hemorrhagic stroke group was 16.67% (4/24 (2F/2M)). Further comparison of the study parameters of the ischemic stroke group according to 30-day outcome is shown in Table 2. Only serum estradiol levels of the surviving females were significantly higher than non-survivors ($p = 0.006$). T/E ratio was higher in the surviving patients, but it did not reach a statistical significance ($p > 0.05$). Also, in female patients, there was a negative correlation between 1st 30-day survival and serum estradiol levels (correlation coefficient -0.323, $p < 0.05$). Regression analysis did not show estradiol as an independent risk factor of mortality ($p > 0.05$). Regression analysis results were not significant in determining the 1st 30-day outcome ($p < 0.05$).

DISCUSSION

Gender and sex hormones are implicated in the occurrence of atherosclerotic cardiac and cerebral events [3, 11,12]. But the results of sex hormone treatments (especially estrogen replacement therapy) is conflicting [11, 12]. A study by Pappa T and colleagues assessed the early phase (within 2 - 3 days) of stroke in postmenopausal women and found endogenous estrogen as an independent predictor of stroke severity [9]. Our study's hyperacute phase blood sampling was not fasting. Still, serum estrogen levels correlated with 30-day mortality of female ischemic stroke patients. So these two independent study results show that serum estrogen levels are not affected by the day time and/or prandial status of the patients. In our study, study parameters were taken before any intervention (thrombolytic, thrombectomy, etc.), while in the other study, although not mentioned, some patients' blood samples probably were taken after such emergency treatment interventions [8]. Because of the similarity of sex hormone analysis results in these two studies, we can expect that these interventions do not affect profile of the sex hormones very much. Still, further studies are needed to ascertain this. In our preliminary study, serum estradiol levels of ischemic stroke females were significantly lower than the males of the same group and from females of the opposite group as well. Comparing the T/E ratios showed that females of both groups had lower ratios than males of the same groups. This was mostly due to the associated high serum testosterone levels of the mentioned male group (Table 1). Although they did not reach statistical significance, this ratio was also high in ischemic stroke female patients that survived (comparison to non-survivors). But serum estrogen levels were higher in the female ischemic stroke patients did not survive (Table 2). Maybe the failure of preventing stroke with estrogen replacement therapies is due to changing this T/E ratio with such treatments [8]. Considering that this T/E ratio in such hormonal therapy studies may affect the occurrence of stroke and/or its outcome needs to be studied. Serum CRP, which is in daily routine use, was significantly correlated with the type of stroke in men and serum estradiol in women. This if ascertained by other studies, will help us in daily practice by planning the first imaging study of stroke patients accordingly. Because of the lack of necessary funding, we were able to measure serum estradiol and testosterone at the hyperacute phase (early admission) of the stroke only. The serial measurement of these study parameters might be more informative. But in Pappa T, et al.'s study results (where serum sex hormones were determined after 2 - 3 days of acute stroke) are not so different from ours [8]. Another point to mention is that the measured CRP was not high-sensitivity CRP (hs-CRP). As we know, hs-CRP is more accurate than the classical CRP assay [13]. Nevertheless, our widely used classical CRP levels were useful in predicting the type of stroke in men. Whether using hs-CRP could add more to this study's findings

needs to be further studied.

CONCLUSION

In this study, serum CRP ≥ 0.74 mg/dL was useful to predict hemorrhagic stroke in men while the serum estradiol level ≥ 14.07 ng/mL was helpful in females. Further studies are needed to confirm our findings.

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Declaration of Interest:

The authors have no conflict of interest.

Authors' Contributions:

GSE: Conceived and designed the study, participated in data collection, data analysis, and data interpretation, helped in reviewing the 1st draft of the manuscript, and approved the last version of the manuscript. MH: Conceived and designed the study, participated in data collection, statistical analysis, and data interpretation, and wrote the first draft of the manuscript. HI: Helped the first author in statistical analysis and revising the written first draft and approved the last version of the manuscript. All other authors: Participated in data collection, data interpretation, and revising and approval of the final version of the manuscript.

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