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Testosterone and estradiol in men with acute ischemic stroke: A North Indian case control

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Abstract

Background: One intriguing aspect of stroke is its higher incidence in men as compared to women. Endogenous sex hormones, testosterone and estradiol, may be responsible for this difference. This research aims to study serum testosterone and estradiol levels in men with acute ischemic stroke (AIS) and to correlate these levels with National Institutes of Health Stroke Scale (NIHSS) score and infarct size in computed tomography (CT).

Methods: 100 male patients with AIS and 100 age-matched controls were included in this case-control study. Patients with hemorrhagic stroke, taking hormonal preparations, or suffering from chronic illnesses like tuberculosis (TB), cancer, etc. were excluded. Complete history was obtained including presence of established risk factors and physical examination was done in cases and controls with informed written consent. Severity of stroke in cases was assessed by the NIHSS. CT scan of brain was performed within 72 hours of patient's admission to hospital. The infarct size was measured in centimeters as the largest visible diameter of the infarct on CT scan. Fasting blood samples were obtained for routine investigations and estimating estradiol and testosterone levels.

Results: Mean total testosterone level in cases (223.30 ± 143.44 ng/dl) was significantly lower than that of controls (515.34 ± 172.11 ng/dl) ($P < 0.001$), while estradiol levels had no significant statistical difference ($P = 0.260$). A significant inverse correlation was found between total testosterone levels and stroke severity ($r = -0.581$, $P < 0.001$) and also, total testosterone levels and infarct size ($r = -0.557$, $P < 0.001$). Estradiol levels in patients had no significant correlation with stroke severity ($P = 0.618$) or infarct size ($P = 0.463$).

Conclusion: Low testosterone levels are associated with increased stroke severity and infarct size in men. Further studies are required to establish whether low testosterone is a cause or effect of ischemic stroke and also to explore the potential benefits of testosterone supplementation in men with AIS.

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Introduction

Once considered a disease of the developed world, stroke is now rapidly emerging as one of the chief causes of mortality and morbidity in India. 41% of deaths due to the non-communicable diseases (NCDs) in this country are caused by stroke alone.[1] Besides death, stroke is also a major cause of functional impairments. A World Health Organization (WHO) study reports the disability-adjusted life years (DALYs) due to stroke to be an alarming 6398000.[2] As such, for a developing country like India, stroke is not only an important healthcare problem but also a major socioeconomic burden. Stroke has been classified into two major subtypes, ischemic and hemorrhagic, each having a different pathogenesis. Consistent with the worldwide trend, statistics from Indian studies also show ischemic stroke as the most common subtype.[3]

Despite the extensive studies performed to recognize the etiology and risk factors, one of the less understood aspects is the higher incidence of stroke in men as compared to women. Male sex has long been recognized as an important non-modifiable risk factor. This suggests that the endogenous sex hormones, testosterone and estradiol, might have a contributory role in stroke pathogenesis. Results of studies investigating the role of testosterone and estradiol in development of stroke have been conflicting so far. Besides, there is a dearth of Indian studies exploring this aspect of stroke pathogenesis.

Keeping this in mind, the objective of the present study was to evaluate the serum testosterone and estradiol levels in men with acute ischemic stroke (AIS) and to correlate the levels with stroke severity and infarct size.

Materials and Methods

Subjects: A case-control study was carried out on 100 adult male patients (> 18 years) with AIS admitted to Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, India, and 100 age-matched healthy controls. A stroke, or cerebrovascular accident (CVA), was defined by the abrupt onset of a neurologic deficit that is attributable to a focal vascular cause in brain, spine, or retina.[4] Patients with hemorrhagic stroke, taking hormonal preparations, or suffering from chronic illnesses like tuberculosis (TB), cancer, etc. were excluded from the study. Moreover, excluded were patients who had onset of stroke more than 72 hours before admission to the hospital. Each participant was enrolled in the study with an informed written consent. The study was approved by Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences. Detailed history was taken for every patient. Presence of known risk factors like dyslipidemia, diabetes mellitus (DM), smoking, atrial fibrillation (AF), history of ischemic heart disease (IHD), and previous stroke were noted from previous health records or by asking the patients or relatives. Complete physical examination was done in cases and controls according to standardized procedure.

Neurological evaluation: The National Institutes of Health Stroke Scale (NIHSS) was used to assess the initial severity of stroke in cases. The level of stroke severity is measured as 0 (no stroke), 1-4 (minor stroke), 5-15 (moderate stroke), 15-20 (moderate to severe stroke), and 21-42 (severe stroke).

Imaging and biochemical tests: Computed tomography (CT) scan of brain and electrocardiography (ECG) were performed on patient's admission to hospital. The infarct size was measured in centimeters as the largest visible diameter of the infarct on CT scan.

5 ml of fasting venous blood was collected under all aseptic conditions on the day after admission. Serum separation was done by centrifugation and the sample was analyzed in Erba XL30i autoanalyzer (Transasia Biomedicals ltd.) for routine biochemical parameters including fasting blood glucose (FBG), uric acid, and lipid profile. Total testosterone and estradiol levels were estimated by a chemiluminescence method on ADVIA Centaur CP system from Siemens (Germany).

Assumption of normal distribution for continuous variables was tested by the Kolmogorov-Smirnov statistics. Data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Comparison between two groups was done by independent samples t-test in normally-distributed variables and Mann-Whitney U test in non-normally-distributed variables. Pearson and Spearman tests were used for correlation as applicable; "r" was reported for correlation tests. Level of significance was considered as $P < 0.05$.

Results

The demographic and clinical characteristics of cases and controls are shown in table 1.

More than one risk factor was present in some of the cases. Among the risk factors, highest prevalence was of smoking (76%) followed by alcohol consumption (50%), DM and hypertension (HTN) (48% each), history of previous stroke (30%), IHD (18%), and AF (4%).

Table 1. Comparison of demographic and clinical characteristics of cases and controls

Characteristic	Case	Control	P
Age (year)	59.28 ± 12.31	59.88 ± 12.06	0.806
SBP (mmHg)	130 (33)	120 (10)	0.018
DBP (mmHg)	119 (20)	80 (7)	0.124
FBG (mg/dl)	119.5 (58)	89.5 (26)	< 0.001
TG (mg/dl)	191.90 ± 67.31	102.06 ± 31.32	< 0.001
TC (mg/dl)	173.03 ± 50.85	123.40 ± 39.56	< 0.001
HDL (mg/dl)	34.89 ± 8.70	36.93 ± 9.16	0.280
LDL (mg/dl)	131.31 ± 46.83	81.90 ± 33.12	< 0.001
VLDL (mg/dl)	37.72 ± 13.57	20.32 ± 6.23	< 0.001

Data are presented as mean ±standard deviation (SD) or median and interquartile range (IQR)
SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very-low-density lipoprotein

The percentages of cases having mild, moderate, moderate to severe, and severe stroke according to NIHSS were 8%, 32%, 42%, and 18%, respectively (Table 2).

A significant inverse correlation was found between total testosterone levels and stroke severity. Total testosterone was also significantly inversely associated with infarct size. Estradiol levels in patients had no significant correlation with stroke severity or infarct size.

Patients with DM had lower levels of total testosterone than non-diabetics, mean levels being 167.17 ± 68.77 ng/dl and 273.18 ± 174.25 ng/dl, respectively (P = 0.011). A history of IHD in patients was found to be associated with significantly lower testosterone levels (P = 0.027). Serum total testosterone was significantly inversely associated with age in both patients (r = -0.275, P = 0.045) and control subjects (r = -0.651, P < 0.001). FBG, triglyceride (TG), and very-low-density lipoprotein (VLDL) had an inverse correlation with serum total testosterone in both cases and controls, whereas high-density lipoprotein (HDL) demonstrated a positive association (Table 3).

To evaluate the role of testosterone as an independent predictor of stroke, multiple linear regression analysis was performed. NIHSS score on admission was taken as the dependent variable and total testosterone, age, AF, DM, HTN, IHD, alcohol consumption, previous stroke, smoking, serum TG, total cholesterol (TC), low-density lipoprotein (LDL), and VLDL were the independent variables. Testosterone was found to be significantly associated with NIHSS score on admission (P < 0.05).

Table 3. Correlation of hormone levels with stroke severity and infarct size

	r	P
Testosterone and stroke severity	-0.581	< 0.001
Testosterone and infarct size	-0.557	< 0.001
Estradiol and stroke severity	0.072	0.618
Estradiol and infarct size	0.106	0.463

Discussion

In our study, the mean total testosterone level in cases (223.30 ± 143.44 ng/dl) was significantly lower than the mean testosterone level of controls (515.34 ± 172.11 ng/dl) with a P < 0.001. Similar findings were observed by Jeppesen et al. in their study on men with AIS, where the serum total testosterone concentration in patients was 18% lower than that in the healthy control subjects.[5] This association of low testosterone levels with AIS was also validated by the studies done by Zeller et al.,[6] Yeap et al.,[7] and Hollander et al.[8] In contrast to these, are the studies done by Srinath et al.,[9] Shores and Matsumoto,[10] and Abbott et al.,[11] where no significant difference in testosterone level was found between the patients of ischemic stroke and the control subjects.

Serum total testosterone was significantly inversely associated with age in both patients (r = -0.275, P = 0.045) and control subjects (r = -0.651, P < 0.001) of our study. The age-related decline in testosterone has also been observed by previous researchers.[12]

Table 2. Comparison of testosterone and estradiol levels in cases and controls

Parameter	Case	Control	P
Testosterone (ng/dl)	223.30 ± 143.44	515.34 ± 172.11	< 0.001
Estradiol (pg/ml)	22.26 ± 15.13	21.91 ± 9.23	0.260

Data are presented as mean ±standard deviation (SD)

The ability of luteinizing hormone (LH) to stimulate testicular production of testosterone decreases with age, the steroidogenic capacity of Leydig cells being reduced by approximately 50% with aging. There is evidence that reactive oxygen species (ROS), derived from the mitochondrial electron transport chain (ETC), steroidogenesis, and/or macrophages affects cyclic adenosine monophosphate (cAMP) production and cholesterol transport into the mitochondria by altering the redox environment of the aging Leydig cells. This change results in relative insensitivity to LH signaling and the consequent reduced testosterone levels.

In our study, patients with DM had lower levels of total testosterone than non-diabetics, mean levels being 167.17 ± 68.77 ng/dl and 273.18 ± 174.25 ng/dl in diabetics and non-diabetics, respectively (P = 0.011). A history of IHD in patients was found to be associated with significantly lower testosterone levels (P = 0.027). Besides, FBG, TG, and VLDL had an inverse correlation with serum total testosterone in both cases and controls, whereas HDL demonstrated a positive association. These results are in agreement with previous studies which have demonstrated a significant association between reduced testosterone levels and risk factors for ischemic stroke.[13-16]

Cases in our study were categorized as having mild (n = 8), moderate (n = 32), moderate to severe (n = 42), and severe (n = 18) strokes according to NIHSS. A significant inverse correlation was found between total testosterone levels and stroke severity (r = -0.581, P < 0.001). Total testosterone was also significantly inversely associated with infarct size (r = -0.557, P < 0.001). These findings are supported by the study of Jeppesen et al., where lower levels of testosterone were associated with increased stroke severity, greater initial loss of neural function, and increased size of cerebral infarct.[5] However, different results were elicited by certain other studies in which testosterone was found to increase the cerebral infarct size.[17,18]

Estradiol levels in our study did not vary significantly between the cases and controls, the mean levels being 22.26 ± 15.13 pg/ml and 21.91 ± 9.23 pg/ml, respectively (P = 0.260). In addition, the estradiol level in patients had no significant correlation with stroke severity (P = 0.618) or infarct size (P = 0.463). This differs from the observation by Abbott et al., where high levels of serum estradiol were found associated with elevated stroke risk in men.[11] Our results, however, agree with those by Jeppesen et al. who found no significant difference between estradiol levels in male patients of ischemic stroke and controls.[5]

The hypothesis that low testosterone is a causative factor for ischemic stroke in men is supported by our findings which demonstrate significant negative correlations between testosterone and surrogate markers of cardiovascular disease (CVD) like age, DM, dyslipidemia, and history of previous IHD. The positive correlation found between testosterone and HDL in our study also favours this hypothesis. Older age, DM, and derangements in lipid profile have all been implicated in the development of atherosclerosis.[4] Since a major etiology in development of ischemic stroke is embolisation or thrombosis of atherosclerotic blood vessels, it can be postulated that lower testosterone levels in men can act as a predisposing factor for ischemic stroke. It has been found that low testosterone levels can result in increased thrombus formation which can consequently lead to ischemic stroke.[19] Tendency of increased thrombus formation at low testosterone levels can be explained by the negative correlation of testosterone with fibrinogen and plasminogen activator inhibitor-1 (PAI-1) and positive correlation with total plasminogen activator activity.

Jeppesen et al. found an association of low testosterone levels with increased stroke severity and infarct size,[5] which is similar to our study results. This observation is supported by various studies which suggest a neuroprotective role of testosterone.[20-22] Testosterone makes neuronal cells less vulnerable to oxidative stress-induced cell death, via an androgen receptor mediated pathway. An upregulation of the cellular antioxidant defenses including catalase and superoxide dismutase (SOD) occurs. Besides neurons, testosterone also acts on other cells of neurovascular unit like astrocytes and can thus, indirectly influence cerebrovascular functions. Aquaporin-4 (AQP4) is located on astrocyte end-feet that face blood vessels in the brain and in the pia, and is thought to play a crucial role in the development of brain edema following cerebral insults like trauma, ischemia, etc. Testosterone significantly upregulates AQP4 at the level of both protein and messenger ribonucleic acid (mRNA), and ameliorates the osmotic fragility of astrocytes from hypoosmotic stress. This subsequently reduces the extent of edema and hence, the severity of cerebral damage.

Another explanation for the low levels of testosterone in our case group could be an acute stress response induced by cerebral infarct which leads to a fall in testosterone. This is known to occur in several forms of stress, including myocardial infarction (MI), congestive cardiac failure, diabetic ketoacidosis (DKA), respiratory failure, renal failure, surgery, and head trauma. This decrease in testosterone levels was attributed to a temporary hypogonadotropic gonadal insufficiency of hypothalamic origin which occurred as a metabolic adaptation to stressful situation.[23]

However, even if the decrease in testosterone is a result of ischemic stroke, lower levels of testosterone can lead to greater stroke severity by increasing osmotic fragility and neuronal susceptibility to oxidative stress. Low testosterone can also cause delay in lysis of thrombus due to decreased fibrinolytic activity and hence, prolong the recovery from stroke.

Conclusion

Low testosterone levels are associated with increased stroke severity and infarct size in men. However, further studies are required to establish whether low testosterone is a cause or effect of ischemic stroke and also, to explore the potential benefits of testosterone supplementation in men with AIS.

Limitations: The present study has a number of limitations. Firstly, our study was an observational cross-sectional study, so we could only observe the prevalence and temporarily associated factors. Further prospective analytical studies are required for determination of the pathogenesis behind higher incidence of ischemic stroke in men. More analytical or experimental studies are also warranted for determination of therapeutic effects of testosterone supplementation in these patients. Secondly, the present study collected just single serum samples of hormones without assessment of LH, prolactin, and follicle stimulating hormone (FSH). The lack of results on other gonadotropins limited the ability of our study to establish the cause of hypogonadism. Finally, the present study could not determine the free testosterone level, which is the ideal method, because it is not available in our laboratory and is very costly.

Conflict of Interests

The authors declare no conflict of interest in this study.

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