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**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

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**SEX HORMONE LEVELS IN PATIENTS WITH TRANSIENT ISCHEMIC
STROKE**

Diploma thesis

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TABLE OF CONTENTS

1. INTRODUCTION.....	1
1.1. TIA.....	3
1.1.1. DEFINITION.....	3
1.1.2. ETIOLOGY.....	3
1.1.3. EPIDEMIOLOGY.....	4
1.1.4. PATHOPHYSIOLOGY.....	4
1.1.5. CLINICAL PRESENTATION.....	4
1.1.6. IMAGING AND DIAGNOSIS.....	5
1.1.7. TREATMENT.....	7
1.1.7.1. <i>Blood Pressure</i>	7
1.1.7.2. <i>Hyperthermia, Hypoxia, Hypoglycemia</i>	7
1.1.7.3. <i>Anticoagulation</i>	7
1.1.7.4. <i>Statins</i>	8
1.1.7.5. <i>Platelet Inhibitors</i>	8
1.1.7.6. <i>Secondary Prophylaxis</i>	8
1.1.7.7. <i>Tissue Plasminogen Activator</i>	9
1.1.7.8. <i>Mechanical Thrombectomy</i>	9
1.2. HORMONES AND STROKE.....	9
1.2.1. TESTOSTERONE.....	9
1.2.2. LUTEINIZING HORMONE.....	9
1.2.3. FOLLICLE STIMULATING HORMONE.....	10
1.2.4. HYPOTHALAMIC-PITUITARY-GONADAL AXIS.....	10
1.2.5. DEHYDROEPIANDROSTERONE.....	11
1.2.6. IMPACT OF DHEA ON NEURONAL AND CNS CELLS.....	12
1.2.7. ROLE OF HORMONES IN STROKE PATHOPHYSIOLOGY.....	12
1.2.8. POTENTIAL INFLUENCE ON STROKE RISK AND SEVERITY.....	12
2. OBJECTIVE.....	13
3. MATERIALS AND METHODS.....	15
3.1. STUDY DESIGN.....	16
3.2. STUDY POPULATION.....	16
3.3. METHODS OF COLLECTING AND ANALYZING DATA.....	16
4. RESULTS.....	18
4.1. BASELINE ANALYSIS.....	19
4.2. CONTROL GROUP AND TIA GROUP COMPARISON.....	20
4.3. CORRELATION ANALYSES.....	22
4.3.1. LINEAR REGRESSION.....	22
4.3.2. SPEARMAN CORRELATION.....	25
5. DISCUSSION.....	29
6. CONCLUSION.....	36
7. REFERENCES.....	38
8. SUMMARY.....	43
9. SUMMARY IN CROATIAN.....	46

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LIST OF ABBREVIATIONS

TIA – Transient ischemic attack

MRI – Magnetic Resonance Imaging

CT – Computed Tomography

SPECT – Single Photon Emission Computed Tomography

tPA – Tissue plasminogen activator

MT – Mechanical Thrombectomy

ASA - Acetylsalicylic acid

LDL – Low Density Lipoproteins

HDL – High Density Lipoproteins

HPG axis – hypothalamic-pituitary-gonadal axis

HPA axis – hypothalamic-pituitary-adrenal axis

GnRH – Gonadotropin releasing hormone.

LH – Luteinizing hormone

FSH – Follicle stimulating hormone.

aPTT - Partial thromboplastin time

ESUS - embolic stroke of unknown source.

DHEA - Dehydroepiandrosterone

17OH P - 17 Hydroxyprogesterone

DOACs – Direct oral anticoagulants

N - Number

GABAA - γ -aminobutyric-acid type A

NMDA - N-methyl-d-aspartate

1. INTRODUCTION

In the United States, stroke ranks as the fifth leading cause of death, after heart disease, cancer, chronic lung disease, and injuries/accidents [1]. Currently, strokes are the most prevalent and disabling neurological disorder that exists today [1]. TIA (transient ischemic attack), commonly referred to as a “mini stroke”, is characterized by a short-lived occurrence of neurological symptoms resulting from cerebral, retinal or spinal ischemia [6]. These symptoms usually persist for seconds to minutes and typically less than an hour [2]. Approximately 20% of individuals who experience TIA will suffer a stroke within the initial months, while approximately half of these subsequent strokes will occur within the first few days after TIA [2]. Therefore, prompt evaluation to identify risk factors and initiate preventative strategies are crucial not only for strokes, but TIA’s as well [3].

Available treatment options today for TIA and strokes are tissue plasminogen activator (tPA) and mechanical thrombectomy (MT) for strokes [4]. Nonetheless, their effectiveness is constrained by several factors, most notably a narrow treatment-time window: 4.5 to 9 hours since first symptom onset for tPA and up to twenty-four hours since first symptom onset for MT [4]. Thus, demonstrating a greater necessity for identifying risk factors and implementing preventative measures before TIA or stroke occurs.

Research into hormones and their impact on ischemic strokes has shown promising signs of potential because hormone levels have been observed to change throughout the progression of TIA [5]. There has been an increased focus on examining the relationship between testosterone and stroke in recent times. Previous studies have found that lower levels of total testosterone are associated with an elevated likelihood of having a stroke [5].

1.1. TIA

1.1.1. DEFINITION

A serious medical illness characterized by a transient period of neurological dysfunction without immediate tissue destruction, caused by a localized decrease in blood flow to the brain, spinal cord, or retina (Figure 1) [1]. The duration of TIA is typically for a short period of time, often resolving within one hour [1], and frequently lasts for just a few minutes [6]. The characterization of TIA has transitioned away from a reliance on the duration or timing of the neurologic dysfunction to rather focus on tissue involvement [6].

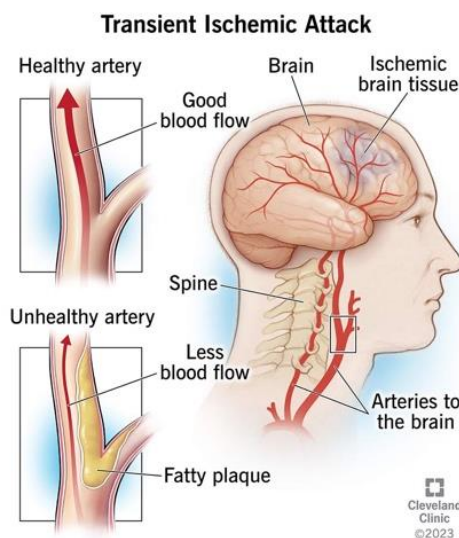


Figure 1. Visual representation of how a transient ischemic attack (TIA) occurs and subsequently effects the brain [7].

1.1.2. ETIOLOGY

The primary cause of TIA is neurologic dysfunction, which is usually brought on by temporary disturbances in blood flow to the brain [1, 6]. These temporary disturbances in blood flow can be caused by various factors such as atherosclerosis, embolism, small vessel disease linked to conditions like hypertension or diabetes, specific cardiac disorders like atrial fibrillation and abnormalities in heart valves that increase the risk of blood clot formation and migration to the brain [1, 2, 6]. Other contributing factors may include vasospasm (abnormal narrowing of blood vessels), inflammation, or hypercoagulable conditions (elevated tendency for blood clotting) [6].

1.1.3. EPIDEMIOLOGY

It has been challenging for clinicians and researchers to accurately calculate the epidemiology of a TIA because the presentation could differ based upon present neurologic comorbidities and TIA has been mimicked by other syndromes, such as hypoglycemia [8]. In the United States, the prevalence of TIA is estimated to be around 1.1 per 1000 people and the overall frequency is roughly 2% [6]. It is important to note that a history of previous stroke has been found to elevate the likelihood of TIA occurrences. [6].

1.1.4. PATHOPHYSIOLOGY

The pathophysiology of TIA varies across its subtypes, but a common characteristic is the temporary interruption of arterial blood flow to a specific area of the brain supplied by the affected artery [1, 2, 6]. TIA typically presents with a manifestation of cortical ischemia without identifiable large artery thrombosis or heart-originating emboli; this condition has more recently been termed ESUS (embolic stroke of unknown source) [6]. Other less common causes include hypercoagulable states or arterial dissection [1, 6]. Possible identifiable causes of a TIA include large artery atherothromboses, which could lead to a temporary decrease in blood flow due to stenosis or embolism [1].

One of the most common causes of an embolism resulting in TIA, originates from the heart where an embolus forms within a cardiac chamber during atrial fibrillation and later travels to the cerebral arteries [6]. Small vessel ischemic diseases are primarily associated with lipohyalinosis (vessel wall thickening and buildup plaque buildup) or small vessel arteriosclerosis, which can be caused by hypertension, diabetes, or increased age [6].

1.1.5. CLINICAL PRESENTATION

A TIA is identified by the presence of focal cerebral or retinal symptoms that start suddenly and are brief (seconds or minutes, usually less than sixty minutes) [9].

The presentation of a “Definite TIA” includes paralysis in one or two extremities (*hemi*) including the face (*facialis*), sensory changes in one or two extremities (*hemi*) including the face, visual field loss such as homonymous hemianopsia or monocular vision loss like *amaurosis fugax*, aphasia, or dysarthria [3].

The presentation of a “Possible TIA” is indicated if at least two of the following are present; unsteady gait, diplopia, vertigo/dizziness, or dysphagia [3, 9]. Although, a “Possible TIA” could still be diagnosed if only one of these symptoms are present [9]. Patient presentations that are usually not considered to be symptom of a TIA include amnesia/confusion; lack of coordination; partial sensory loss affecting only one limb or just the face; typical cortical visual symptoms like isolated bilateral vision loss and positive bilateral visual phenomena such as flashes of light and colored dots among others listed below along with alteration of consciousness, headache, photopsias, complex visual hallucinations, palinopsia hearing impairment [9].

Recurrent symptoms otherwise known as recurring TIA’s are usually caused by carotid emboli in the opposite hemisphere or eye [9]. When an embolus originates from a narrowed blood vessel within the brain, distinct and repetitive symptoms could occur. Hemodynamic TIAs’ are also characterized by predictable involuntary choreatic movements (“limb-shaking TIA”), and may be associated with an upright posture, particularly affecting watershed areas [10]. Additionally, emboli originating from the heart or aortic arch can induce symptoms related to various cerebral vascular territories. [10].

1.1.6. IMAGING AND DIAGNOSIS

Prompt and accurate identification of a stroke and early detection of a prior TIA are essential for positive patient outcomes. Increased public education on the indicators of TIA and stroke, along with advancements in diagnostic methods, could potentially reduce the debilitating effects of a stroke [11]. Diagnostic approaches like magnetic resonance imaging (MRI), computed tomography (CT) scans, and single photon emission computed tomography (SPECT) play a key role in diagnosing cerebral infarction. Although MRI is the preferred diagnostic option, its availability is restricted by cost considerations [11-14].

Consequently, CT is frequently used, although it has its limitations in detecting small infarctions, particularly in the posterior fossa [11]. Ultrasound serves as a safe and cost-effective diagnostic tool that can be found at the bedside of most hospitals worldwide (Figure 2) [13, 14]. However, effectiveness in patients following TIA or stroke may face challenges such as inadequate conditions for ultrasound beam transmission through the skull or deep-seated vessels within the brain [14]. Additionally, imaging arteries with slow blood flow presents further difficulties [14]. Advancements in ultrasound technology such as two-dimensional transcranial color-coded sonography have expanded ultrasound's usefulness by providing both anatomical and functional insights into major cerebral vessels [14].

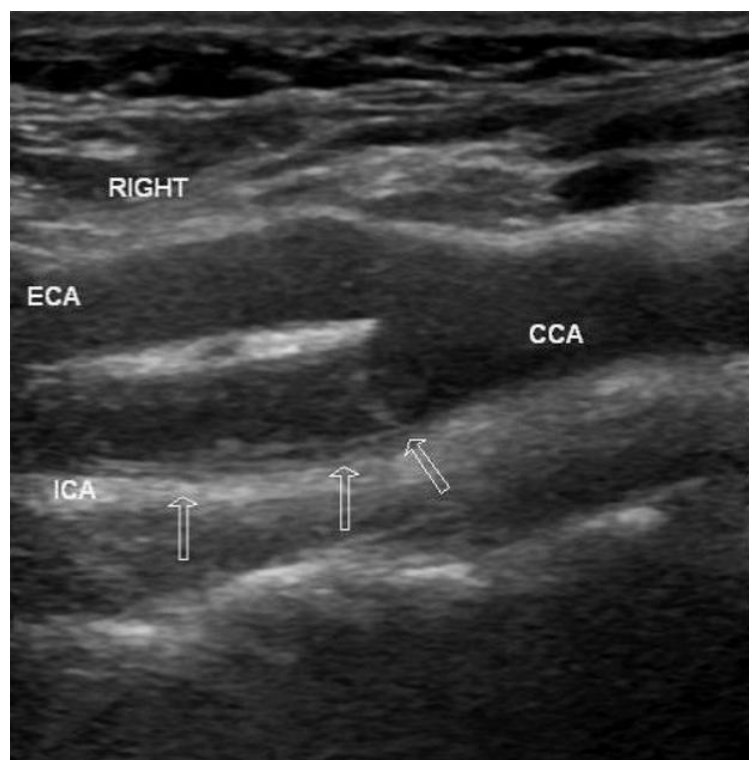


Figure 2. Ultrasound of an echogenic thrombus in the right internal carotid artery [15].

Abbreviations: ICA – Internal carotid artery ECA - external carotid artery, CCA - common carotid artery.

1.1.7. TREATMENT

Patients experiencing TIA should undergo prompt examination by a stroke specialist within twenty-four hours of first symptoms to identify risk factors and begin preventive actions [6]. Those who have had a TIA in the past forty-eight hours, recurrent TIAs', or high ABCD2 scores are at significantly higher risk of another stroke in the initial days if assessment and treatment are postponed [4]. Before potential medical treatment of a stroke or TIA with tPA or MT can begin, patients should be stabilized and other causes of similar neurological deficient should be ruled out, as follows.

1.1.7.1. Blood Pressure

High blood pressure has been noted in the literature as a cause of TIA and stroke [1]. Acute lowering of blood pressure is typically not advisable, unless it is necessary for patients who have had an acute ischemic stroke, and their blood pressure exceeds certain levels (>185 mm Hg systolic or >110 mm Hg diastolic) [1]. In these circumstances, intravenous labetalol or nicardipine are recommended as a suitable option for antihypertensive treatment [1].

1.1.7.2. Hyperthermia, Hypoxia, Hypoglycemia

Hypoglycemia, defined by a blood glucose level <3.3 mmol/L, could resemble a stroke and therefore should be corrected as soon as possible upon hospital admission and ruled out before stroke treatment can begin [1]. Hyperthermia and potential infectious causes of neurologic deficient resembling a TIA or stroke should be ruled out as well because these may adversely affect patient outcomes [1]. Furthermore, hypoxia (oxygen saturation \leq 94%) can also cause neurologic deficits and thus requires treatment with supplemental oxygen to [1].

1.1.7.3. Anticoagulation

Anticoagulation therapy involves the use of heparin delivered through a continuous intravenous infusion to achieve an activated partial thromboplastin time (aPTT) that is 1.5 to 2.5 times the normal range, followed by oral administration of warfarin or another oral anti-

coagulant, daily with the goal of achieving an international normalized ratio of 2.5 ± 0.5 [1]. This treatment approach is recommended when TIA or acute ischemic stroke appears to be caused by a cardiac embolic source such as atrial fibrillation, mitral stenosis, or mechanical valve replacement [1].

1.1.7.4. Statins

Patients receiving long-term statin treatment should continue taking statins during their TIA and/or stroke treatment [1].

1.1.7.5. Platelet Inhibitors

A prompt administration of Acetylsalicylic acid (ASA) should follow a TIA or stroke diagnosis to potentially prevent further increase in pathologic clot size [1]. Occasionally, bleeding may lead to transient symptoms, therefore a CT scan should be performed before administering a loading dose of ASA to rule-out hemorrhagic stroke [16]. However, if there is significant travel time to the hospital, and severe ($ABCD_2 > 3$) or recurring TIAs, a loading dose of ASA can still be considered [16].

TIA patients on anticoagulation with warfarin or direct oral anticoagulants (DOACs) may be given an ASA loading dose after assessing the individual benefit and risk of bleeding. [17]. TIA patients at high risk of cerebral infarction ($ABCD_2 \geq 4$) can receive clopidogrel in addition to ASA as a bolus dose contribution [18 - 20]. The impact and safety of administering clopidogrel with ASA as a bolus dose has not been studied in TIA patients with an elevated risk of bleeding, including those who are on anticoagulation treatment [18, 20].

1.1.7.6. Secondary Prophylaxis

Recommended to use double platelet inhibition with ASA and clopidogrel for three to six weeks after undergoing TIA [18 - 20].

1.1.7.7. Tissue Plasminogen Activator

If the patient experiences new symptoms of an acute brain infarction following a TIA, it is important to promptly conduct a new cerebral CT scan and consider thrombolysis if the symptoms do not resolve rapidly [17].

1.1.7.8. Mechanical Thrombectomy

Endarterectomy of the internal carotid artery or thrombectomy in large blood vessels within the brain may be considered effective for treating blockages of major blood vessels, although it is unlikely to benefit patients who are experiencing TIA [9].

1.2. HORMONES AND STROKE

1.2.1. TESTOSTERONE

Testosterone is a crucial sex hormone with significant functions within the body. In males, it is believed to control sexual desire (libido), bone density, distribution of fat, muscle mass and strength, as well as the generation of red blood cells and sperm [21]. A small percentage of testosterone circulating in the body gets transformed into estradiol. As men grow older, their production of testosterone tends to decrease along with a reduction in estradiol levels [22]. Consequently, alterations commonly linked to low testosterone may be partially or entirely influenced by a simultaneous decrease in estradiol [22].

1.2.2. LUTEINIZING HORMONE

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are both pituitary gland-producing hormones involved in sexual development and reproduction, which are directly involved in influencing function of both ovaries and testicles [21]. LH prompts Leydig cells in males to generate testosterone, which then offers negative input to the anterior pituitary and hypothalamus [21].

1.2.3. FOLLICLE STIMULATING HORMONE

FSH is also a pituitary gland-produced hormone involved in sexual development and reproduction. FSH encourages Sertoli cells to generate androgen-binding protein, promoting the process of spermatogenesis [21]. Additionally, FSH prompts Sertoli cells to produce inhibin, which in turn delivers negative feedback to the anterior pituitary gland, reducing FSH secretion [21].

1.2.4. HYPOTHALAMIC-PITUITARY-GONADAL AXIS

During puberty, the hypothalamic-pituitary-gonadal axis (HPG axis) is essential in controlling testosterone levels and gonadal function [21]. The hypothalamus releases Gonadotropin releasing hormone (GnRH), which travels through the portal system to the anterior pituitary. This triggers the secretion of LH and FSH [21]. LH and FSH are two hormones that affect the gonads after traveling through the bloodstream. LH specifically influences Leydig cells to enhance testosterone production [21]. Testosterone regulates its own release by negative feedback mechanisms [21]. Elevated blood levels of testosterone signal back to suppress GnRH secretion from the hypothalamus and reduce responsiveness to GnRH stimuli at the anterior pituitary level [21].

Throughout the reproductive lifespan of males, the hypothalamus intermittently produces GnRH every one to three hours [21]. Despite this pulsatile secretion, average levels of FSH and LH in the bloodstream remain relatively stable from adolescence when they surge, until around age thirty when they reach their peak and start declining gradually [21]. Before puberty, testosterone levels are low due to minimal release of GnRH and gonadotropins. The onset of puberty triggers a significant increase in GnRH secretion influenced by changes in neuronal input to the hypothalamus and brain activity [21].

Leydig cells in the testes are responsible for transforming cholesterol into testosterone, while LH plays a key role in regulating the initial step [21]. Dehydroepiandrosterone (DHEA) and androstenedione are important intermediates in this process, with androstenedione being converted to testosterone by the enzyme 17-beta-hydroxysteroid dehydrogenase [21].

Most of the produced testosterone is bound to plasma proteins like sex-hormone-binding-globulin and albumin as a reserve for the body's needs [21]. Smaller amounts of free testosterone affect various tissues at a cellular level before it can be transformed into dihydrotestosterone by the enzyme 5-alpha-reductase [21].

1.2.5. DEHYDROEPIANDROSTERONE

DHEA, produced by various organs including the adrenal cortex, gastrointestinal tract, gonads, and brain, along with its sulfated form DHEA-S, are among the most common natural steroid hormones in circulation [23]. Traditionally, DHEA was linked to age-related changes in cardiovascular health, female fertility, metabolism and nervous system functions [23-25]. Early research focused on the impact of DHEA on hormone receptor activation after its conversion into potent sex hormones like testosterone and estradiol (Figure 3). Current research of DHEA and DHEA-S has shown that they act directly as ligands on multiple nuclear receptors in the liver as well as G-protein-coupled receptors [23]. Furthermore, they play a role in immediate cell signaling pathways.

The breakdown of DHEA and its sulfated form DHEA-S contributes to approximately half of the total androgens in men and about three-quarters of the total estrogens in premenopausal women [23]. As individuals age, levels of DHEA and DHEA-S decrease [24], and these changes are linked to overall cardiovascular health [25], metabolism, as well as brain function [27]. In addition to serving as precursors for sex hormones, DHEA also directly interacts with steroid hormone receptors and nuclear receptors while influencing various cellular pathways [27].

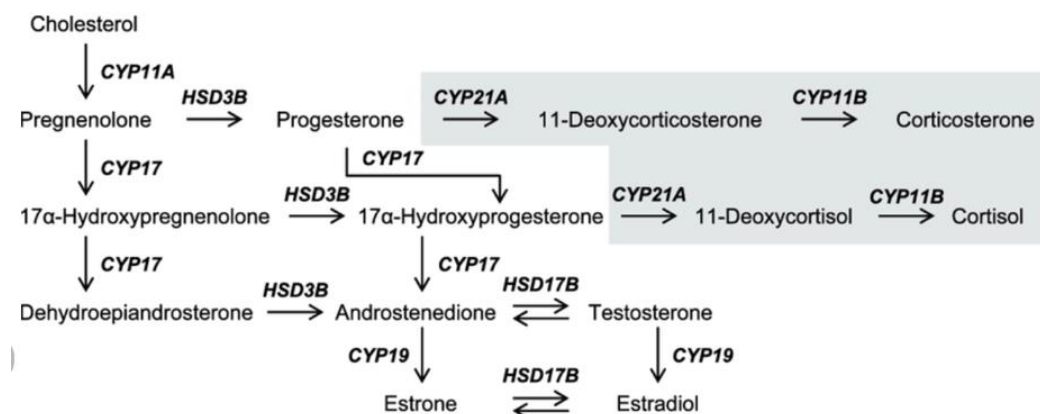


Figure 3. steroid hormone biosynthesis pathway [26].

1.2.6. IMPACT OF DHEA ON NEURONAL AND CNS CELLS

DHEA and DHEA-S are well-known regulators of the neurotransmitter receptors N-methyl-d-aspartate (NMDA) receptor, γ -aminobutyric-acid type A (GABAA), and sigma-1 receptors [28]. Their activation provides evidence for the quick actions of DHEA and DHEA-S and supports the potential beneficial effects of DHEA in addressing various neurological disorders linked to these receptors [29]. The NMDA receptor is a major player in learning and memory processes and has been associated with conditions like autism, epilepsy, as well as depressive and bipolar disorders [28].

1.2.7. ROLE OF HORMONES IN STROKE PATHOPHYSIOLOGY

Since the 1990s, there has been a growing interest in studying hormones in relation to ischemic stroke. Specifically, Insulin, estrogen, progesterone, testosterone, arginine vasopressin, and thyroid hormone have all been studied to find a potential link with TIA and/or ischemic stroke, a previously underserved area of study [5]. Hormone changes have been identified as a potential risk factor for ischemic stroke and ischemic stroke itself has been observed to cause changes in hormone levels [5]. These hormones have also been studied in relation to their effect on secondary brain damage following a stroke [5]. There appears to be a complex interplay of pathophysiological events such as excitotoxicity, oxidative and nitrosative stress, inflammation, and apoptosis that occur after a primary insult to the brain that requires further study and understanding [5].

1.2.8. POTENTIAL INFLUENCE ON STROKE RISK AND SEVERITY

Previous studies have shown that serum levels of testosterone decreased following acute ischemic stroke in men, and there was a negative association between total testosterone and infarct size [5]. The impact of testosterone on ischemic stroke appears to be age specific. Lower testosterone levels have been linked to a higher risk of ischemic stroke in older men as well as increased mortality after acute ischemic stroke [5, 30].

2. OBJECTIVE

The goal of this research was to determine whether there was a significant difference in testosterone levels and other biomarkers in male TIA patients when compared to non-TIA patients. Furthermore, our objective was to evaluate if changes in these biomarkers could be used to predict TIA or subsequent stroke risk in patients who have experienced TIA

The biomarkers investigated in this study were: Age; diabetes status; smoking status; hypertension status; atherosclerotic changes, stenosis >50%, and stenosis >75% of at least one of the internal carotid arteries; and hematological tested parameters: cholesterol, low density lipoproteins (LDH), high density lipoproteins (HDL), triglycerides, FSH, LH, testosterone, DHEA, 17 Hydroxyprogesterone (17OH P), progesterone, and estradiol.

3. MATERIALS AND METHODS

3.1. STUDY DESIGN

This study is a retrospective, case control study. Residents of the dalmatian district of Croatia who were admitted to University Hospital Split, Firule location with a TIA between the years of 2001 and 2002 were randomly chosen for inclusion in the TIA group of this study, according to the traditional time-based definition of TIA. The control group were randomly chosen from residents of the dalmatian district of Croatia who were admitted to the University Hospital Split, at the department of Neurology for any condition, excluding a TIA or stroke during the years of 2001 and 2002. Inclusion in this study required patients to be current residents in the district of Dalmatia at the time of the event and for participants of the control group to have no previous history of stroke or TIA, per their medical record. This study was approved by the University Hospital Split Ethics Committee, registration number: 2181-147/01-06/LJ.Z.-24-02, and because of the retrospective and observational design, no informed consent was required from the study participants.

3.2. STUDY POPULATION

Fifty male patients were included in this study. Average age of all participants was 61 ± 9.42 years, while the average age of the control and TIA groups were 59.4 ± 9.27 years and 62.6 ± 9.50 years, respectively. The patients in this study were residents of the dalmatian province of Croatia, a coastal area of 12 158 km². During the 2001-2002 census, the total resident population was 463 676 [31]. For Croatian residents, medical care is free of charge for hospitalized patients, allowing easy access to medical services, whereas the payment of a fee is required for outpatient visits.

3.3. METHODS OF COLLECTING AND ANALYZING DATA

The blood results of fifty study participants were taken on hospital admission or during their hospital stay at University Hospital Split during the year, 2001 to 2002. These blood parameters were recorded on the University Hospital Split, IBIS health information system and later analyzed by the authors of this study with Jamovi, a statistical software package, version 2.5.3.0.

The statistical analyses of the fifty study participants included Spearman Rank correlation of atherosclerotic changes between the control group biomarkers, age, smoking status, hypertension status, diabetes mellitus status, cholesterol, LDL, HDL, triglyceride, FSH, LH, testosterone, DHEA, 17OH P, progesterone, and estradiol. Also, Spearman Rank correlation was completed between TIA patients with a stenosis level of greater than or equal to 75% and greater than or equal to 50% with the patient biomarkers previously mentioned. A Simple Linear Regression was used to analyze testosterone and DHEA with patient parameters as predictor variables in both the control and TIA groups. Then an Independent sample T test was used to analyze the difference in changes of normally distributed patient biomarkers between the control and TIA groups, as defined by a Shapiro-Wilk test. The non-normally distributed patient biomarkers of the control and TIA groups were analyzed with a Mann Whitney U test. Finally, a Fischer Exact Test was completed between the three categorical patient biomarkers of the control and TIA groups: smoking status, hypertension status, and diabetes status.

The statistical analysis and a minority of figures used in this study were developed and performed with Jamovi, a statistical software package, version 2.5.3.0, manufactured by the Jamovi Project in Sydney Australia. The statistical significance of this study was defined as $P < 0.05$. The majority of figures used in this study were created with Microsoft Excel, version 16.85, manufactured by Microsoft in Redmond, Washington, USA.

4. RESULTS

4.1. BASELINE ANALYSIS

Baseline demographics were taken from fifty volunteer participants. An evaluation of normality was measured with a Shapiro-Wilk test for each patient parameter to determine which parametric group comparison test would be most accurate, Mann Whitney U or Independent Sample T Test. In section 4.2, the results of the Shapiro-wilk tests were utilized to determine the distributional characteristics of the data and thus, ensuring appropriate statistical methodology for the group comparison data.

As seen in Figure 4, the difference in mean values of FSH, LH and testosterone were substantially larger than the differences observed in the other patient parameters when the mean control and TIA groups were compared. In particular, the mean values and standard deviations for TIA group, FSH (5.28 ± 4.89); LH (3.57 ± 1.15); and testosterone (12.4 ± 5.57) were substantially less than that of the control group, (7.61 ± 5.77); (5.42 ± 2.47); (15.9 ± 7.86), respectively (Figure 4).

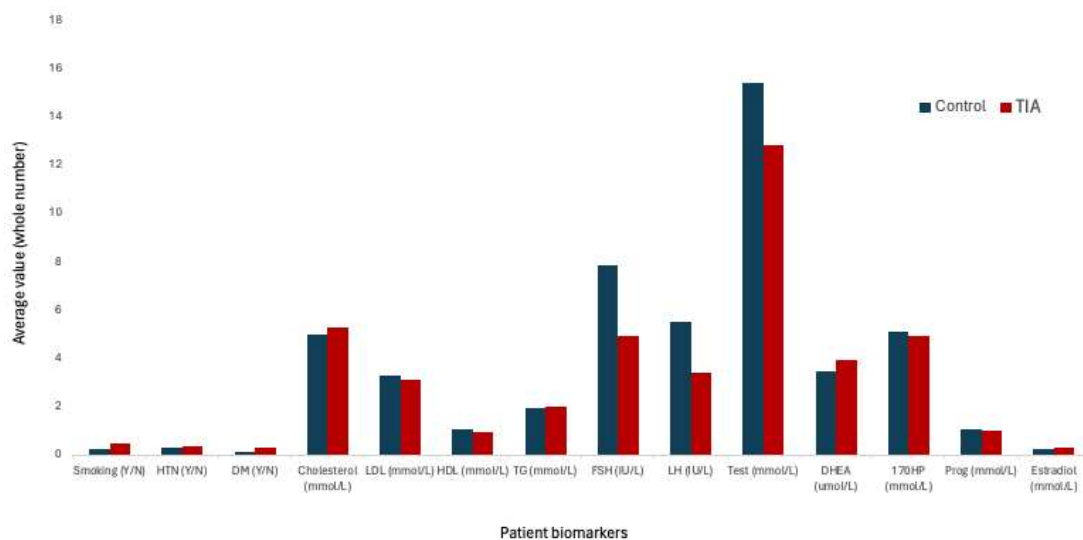


Figure 4. Comparison of means between control and TIA groups.

Data presented as average concentration of measured sex hormones.

4.2. CONTROL GROUP AND TIA GROUP COMPARISON

A Mann Whitney U group comparison was completed between the patient parameters of the control and TIA groups that exhibited non-normally distributed results, as defined by a Shapiro-Wilk P value of <0.05 . These included: HDL; triglycerides; FSH; LH; testosterone; DHEA; 17OH P; progesterone; and estradiol. Although, the Shapiro-Wilk results for triglycerides ($P=0.421$), LH ($P=0.227$), and testosterone ($P=0.060$) produced p values greater than 0.05, they were still deemed non-parametric because the results could be skewed by small sample sizes ($N = 50$), as demonstrated in our study. Additionally, all nine patient parameters were confirmed to hold non-linear relationships and thus exhibited non-normally distributed results when their respective data was compared on a Q-Q plot.

The results of the Mann-Whitney U test indicated a potentially significant difference in LH between the control and TIA groups, $U = 159$, $P = 0.003$. Figure 5 presents a violin plot of LH (IU/L), which demonstrated that the TIA group had a lower median LH score (3.3) and mean (3.57) compared to the control group (5.0) and (5.42), respectively, and a smaller interquartile range and range (4.60) compared to the control group (9.0). As seen in Figure 5, the majority of the data points are concentrated around the median in the TIA group which indicated lower variability in the data compared to a higher variability in the data observed in the control group based upon a larger distribution of data points dispersed from the median.

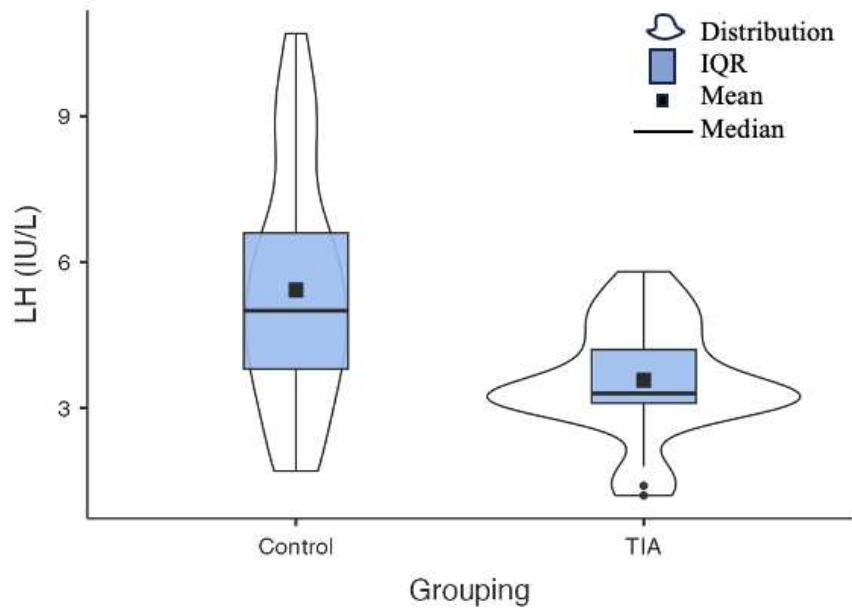


Figure 5. Violin plot of LH (IU/L) levels of control compared to TIA group. Data are presented as mean, median, distribution, interquartile range and whiskers (range) *Mann Whitney U test = 159, $P = 0.003$

An independent samples T-Test was performed on the three parameters that expressed normally distributed data per a Shapiro Wilk test. Age ($P = 0.246$), cholesterol ($P = 0.130$), and LDL ($P = 0.331$) showed no statistically significant differences between the control and TIA groups. A Fischer exact test was utilized for comparing the categorical (yes or no) parameters of the control and TIA groups. As seen in Figure 6, a greater number of participants in the TIA group smoked, had hypertension, and diabetes compared to the control group. Smoking status ($P = 0.062$), hypertension status ($P = 0.072$), and diabetes status ($P = 0.138$) showed no statistically significant differences between the control and TIA groups (Figure 6).

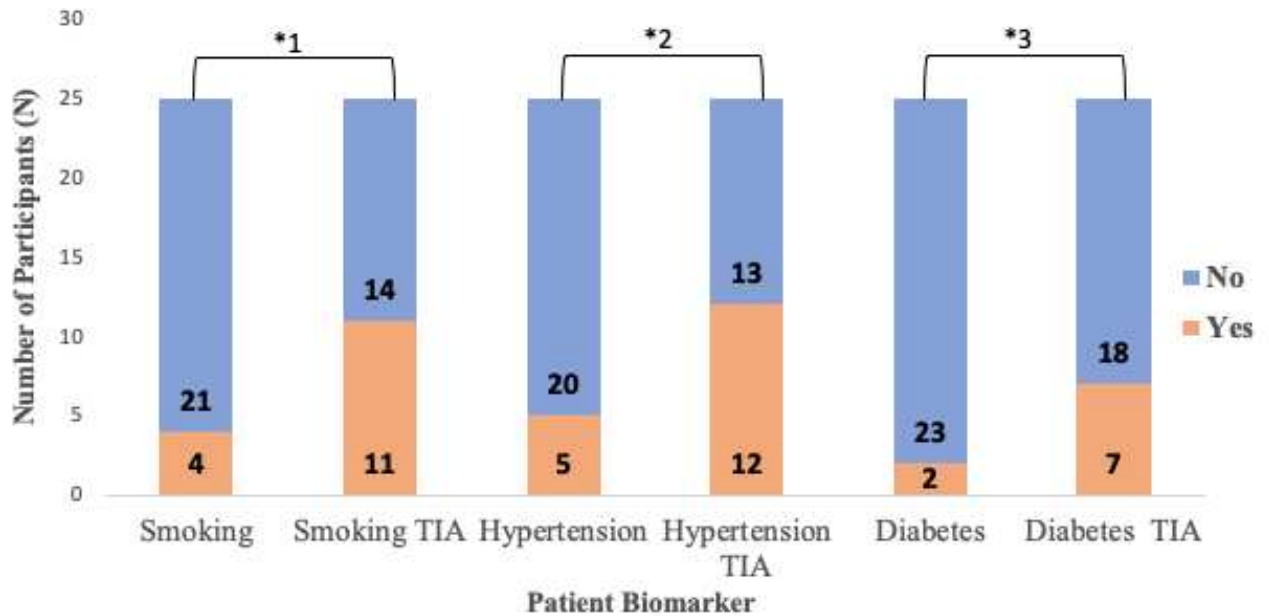


Figure 6. Stacked bar plot of the categorical results comparing the number of participants (N) which smoked, had hypertension, or had diabetes in the control and TIA groups

*Fischer exact test p value: *1 $P = 0.062$; *2 $P = 0.072$; *3 $P = 0.138$

4.3. CORRELATION ANALYSES

4.3.1. LINEAR REGRESSION

The results of the simple linear regression analyses of the control group indicated that HDL had a larger effect on testosterone levels ($b = 12.52$, $P = 0.020$) compared to 17OH P ($b = 2.17$, $P = 0.001$), which showed that a one unit increase in HDL was associated with a 12.5 unit increase in testosterone levels (Figure 7). Further linear regression analysis of the control group revealed that progesterone significantly predicted DHEA levels and for each one unit increase in progesterone there was an increase in DHEA by 1.76 units, $P = 0.008$ (Figure 8).

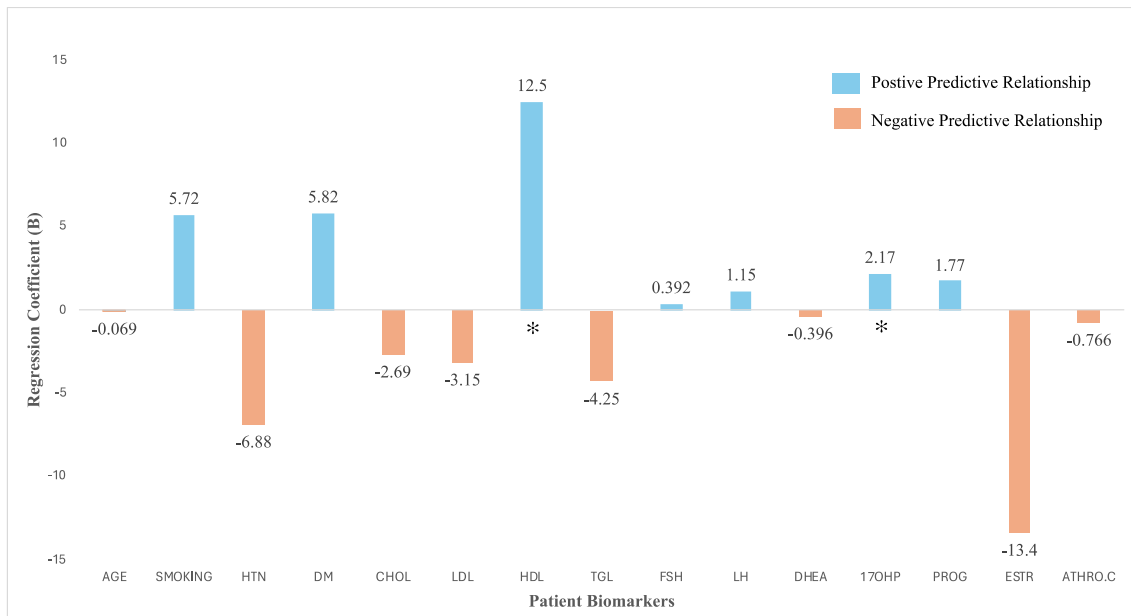


Figure 7. Linear regression analysis of testosterone levels and predictive biomarkers in the control group: AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); DHEA (umol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L); ATHRO.C (y/n)

*Significant predictive values for HDL (B =12.5, $P < 0.05$) and 17OHP (B = 2.17, $P < 0.05$)

Abbreviations: HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol; TGL – Triglycerides; PROG – Progesterone; ESTR – Estradiol; ATHRO.C – Atherosclerotic Changes

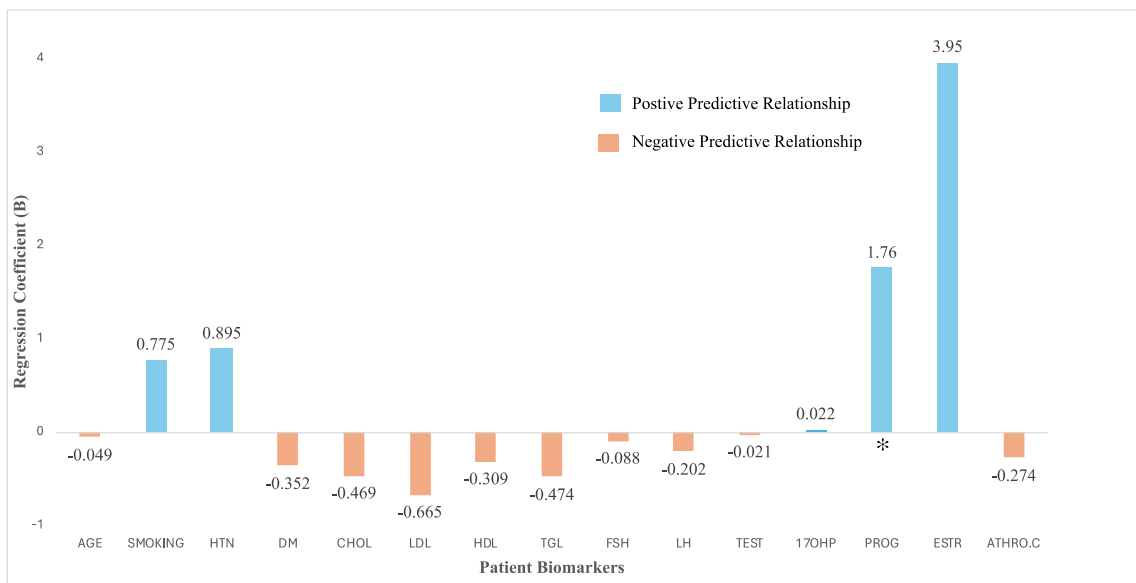


Figure 8. Linear regression analysis of DHEA levels and predictive biomarkers in the control group: AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); TEST (nmol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L); ATHRO.C (y/n)

*Significant predictive value for progesterone ($B = 1.76, P < 0.05$)

Abbreviations: Smoking – Smoking Status; HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol; TGL – Triglycerides; TEST – Testosterone; PROG – Progesterone; ESTR – Estradiol; ATHRO.C – Atherosclerotic Changes

Simple linear regression of the TIA group, as seen in Figure 9, demonstrated that DHEA had a significant positive predictive relationship with testosterone, and for every 1 unit increase in DHEA, testosterone was observed to increase by 1.25 units, $P = 0.002$. DHEA was shown to potentially cause 35% of testosterone variation ($R^2 = 0.350$). Similarly, testosterone significantly predicted DHEA levels, potentially explaining 35% of DHEA variation ($R^2 = 0.350$). Testosterone had a lower positive predictive value on DHEA ($b = 0.281, P = 0.002$), compared to DHEA on testosterone ($b = 1.25, P = 0.002$) (Figure 10).

Based on the results of simple linear regressions conducted in this study, there was no statistically significant, negative predictive link between any patient parameter on DHEA or testosterone in the control or TIA groups (Figure 7-10).

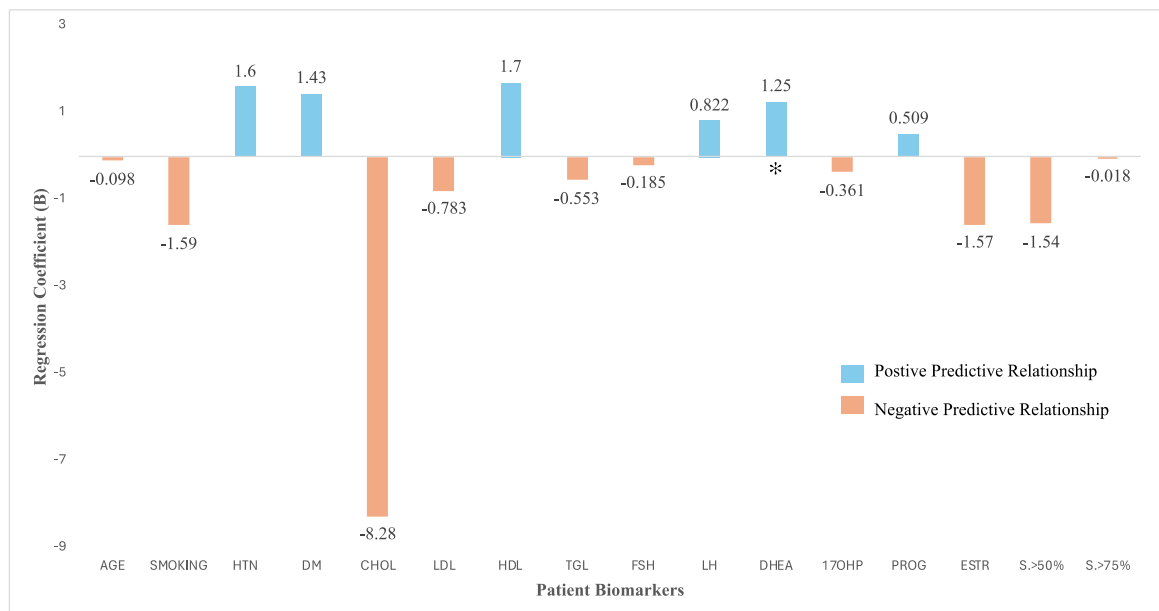


Figure 9. Linear regression analysis of testosterone levels and predictive biomarkers in the TIA group: AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); DHEA (umol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L); S.>50% (y/n); S.>75% (y/n)

*Significant predictive values for DHEA ($B = 1.25, P < 0.05$)

Abbreviations: HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol; TGL – Triglycerides; PROG – Progesterone; ESTR – Estradiol; S.>50% – Stenotic Changes >50%; S.>75% - Stenotic Changes > 75%

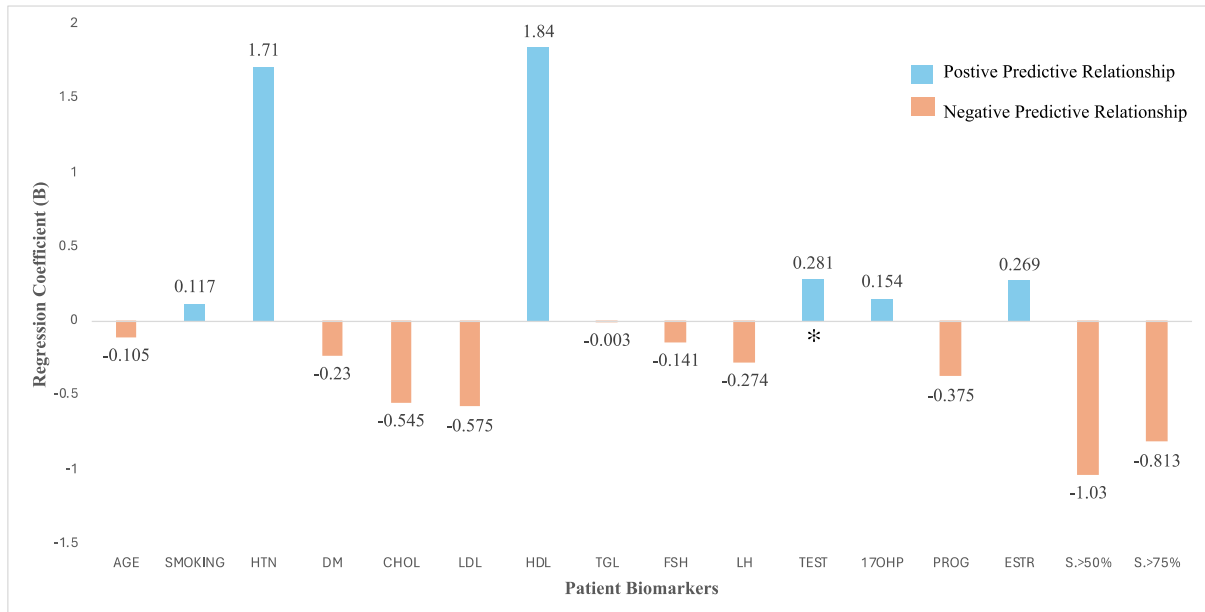


Figure 10. Linear regression analysis of DHEA levels and predictive biomarkers in the TIA group: AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); TEST (nmol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L); S.>50% (y/n); S.>75% (y/n)

*Significant predictive values for testosterone (B = 0.281, $P < 0.05$)

Abbreviations: HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol; TGL – Triglycerides; TEST – Testosterone; PROG – Progesterone; ESTR – Estradiol; S.>50% – Stenotic Changes >50%; S.>75% - Stenotic Changes > 75%

4.3.2. SPEARMAN CORRELATION

A Spearman correlation was completed between atherosclerotic changes and the patient parameters in the control group (Figure 11). There was a potentially significant, positive correlation of age ($P(25) = 0.795$, $P < 0.001$), and hypertension status ($P(25) = 0.480$, $P = 0.015$) with atherosclerotic changes. An increase in age was shown to exhibit a greater association with atherosclerotic changes of the internal carotid artery than those participants who had hypertension the control group.

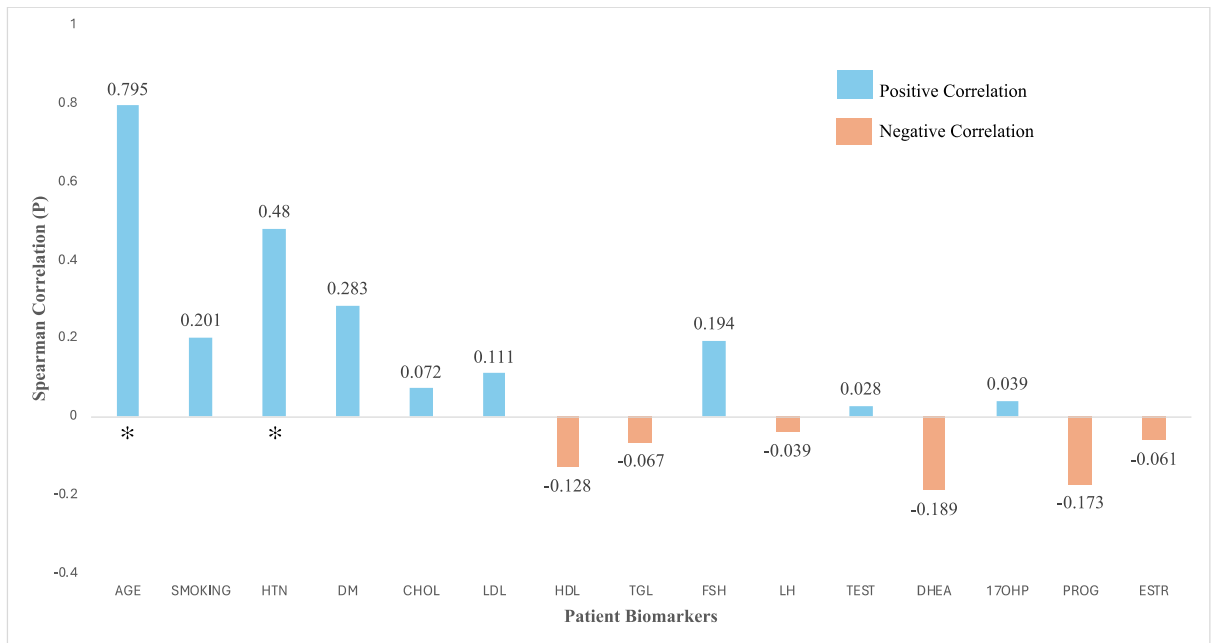


Figure 11. Spearman correlation analysis of atherosclerotic changes in the control group:

AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); TEST (nmol/L); DHEA (umol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L)

*Significant predictive values for age ($P = 0.795$, $P < 0.05$) and hypertension ($P = 0.48$, $P < 0.05$)

Abbreviations: HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol;

TGL – Triglycerides; TEST – Testosterone; PROG – Progesterone; ESTR – Estradiol

Further Spearman correlations were performed between stenosis parameters, $>50\%$ and $>75\%$ with the patient parameters in the TIA group. There was a potentially significant negative correlation between progesterone ($P(25) = -0.437$, $P = 0.029$) and stenosis $>75\%$, and a potentially significant positive correlation between stenosis $>50\%$ ($P(25) = 0.667$, $P < 0.001$) and stenosis $>75\%$ (Figure 12). A TIA patient with stenosis of the internal carotid artery $>50\%$, demonstrated a stronger correlation and thus was more predictive of stenosis $>75\%$ compared to a moderate correlation of decreasing stenosis severity as progesterone levels increased.

There were no statistically significant correlations found between Stenosis $>50\%$ with the other patient parameters in the TIA group.

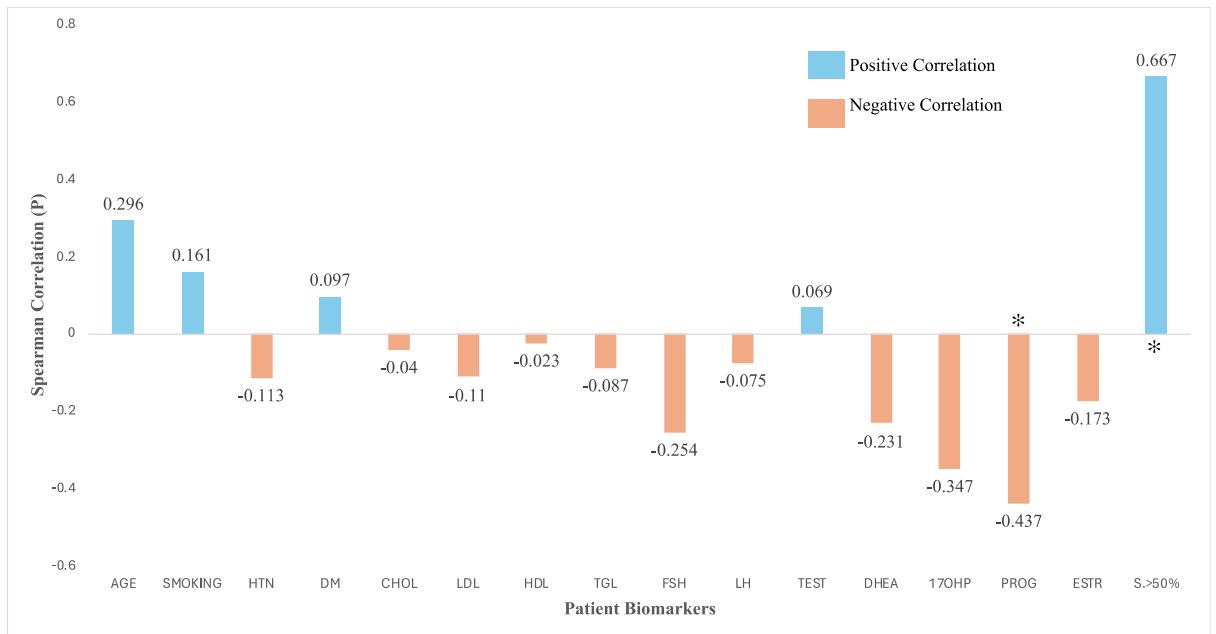


Figure 12. Spearman correlation analysis of stenosis >75% in the TIA group: AGE (years); SMOKING (y/n); HTN (y/n); DM (mmol/L); CHOL (mmol/L); LDL (mmol/L); HDL (mmol/L); TGL (mmol/L); FSH (IU/L); LH (IU/L); TEST (nmol/L); DHEA (umol/L); 17OHP (mmol/L); PROG (mmol/L); ESTR (mmol/L); S.>50% (y/n)

*Significant predictive values for progesterone ($P = -0.437$, $P < 0.05$) and stenosis >50% ($P = 0.667$, $p < 0.05$)

Abbreviations: HTN – Hypertension; DM - Diabetes; CHOL – Cholesterol; TGL – Triglycerides; TEST – Testosterone; PROG – Progesterone; ESTR – Estradiol; S.>50% - Stenotic Changes >50%

As seen in Figure 13, The results of the Spearman correlation test found 52% of the control group participants had atherosclerotic changes of at least one internal carotid artery, while 64% of the TIA group participants had stenosis of at least one internal carotid artery greater than 75%. Atherosclerotic changes and stenosis of the participants carotid arteries were determined by an ultrasound scan.

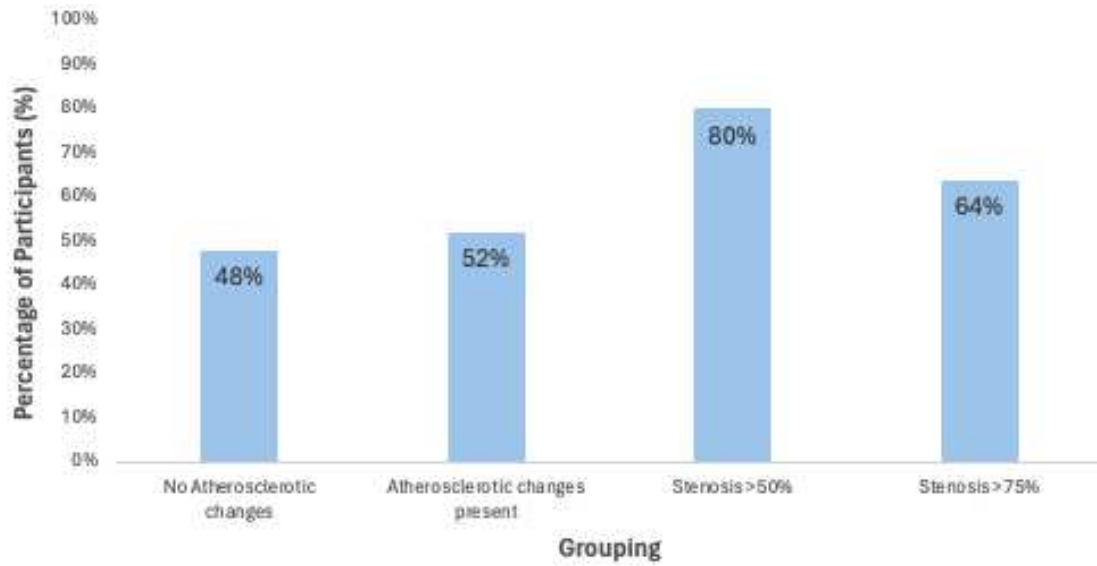


Figure 13. Percentage of patients with atherosclerotic changes and stenosis in the control and TIA groups, respectively, as determined by ultrasound.

5. DISCUSSION

The purpose of this study was to investigate whether there is a significant difference in sex hormone levels and other prominent or novel biomarkers in TIA patients compared to a non-TIA patient control group. Also, the study examined if changes in these biomarkers could be used to predict a TIA or subsequent stroke. There have been many studies published on the potential link between health status, age, atherosclerotic changes, stenosis grading, blood lipid levels and even testosterone with stroke and less so, with risk of TIA. Therefore, we decided to include these prominent biomarkers in our analysis as well as those biomarkers where there has been limited research in relation to TIA or stroke. Specifically, hormones such as DHEA, 17OH P, FSH, LH, estradiol and progesterone.

Because of the low number of participants tested in this study, there is a possibility that all meaningful differences were not detected statistically. When comparing both the control and TIA groups, the only biomarker that showed a significant difference was LH. This finding was unexpected considering previous research did not demonstrate significant variations in LH levels among male ischemic stroke patients. While notable changes in LH levels have been shown among female TIA patients, especially those experiencing menopause [32]. Since our study exclusively included male participants, we were unable to compare our findings with those of the female population in previous studies.

According to Decaroli and Rochira, serum hormone levels, especially sex steroids such as testosterone, decline with aging and can be referred to as late-onset hypogonadism [33]. A relatively common condition in men, usually due to other comorbidities occurring with aging such as hypertension and presents with increased serum LH concentration in response to decreased serum testosterone concentration [34]. Considering the older age group of the participants in this study and more than twice the participants in the TIA group had hypertension or diabetes compared to the control group, we should have observed a significantly higher serum LH concentration in the TIA group. The significantly decreased LH concentration in the TIA group could have been due to dysregulation of the HPG axis and the hypothalamic-pituitary-adrenal (HPA) axis [35]. A potential complication of activating these axes in response to an acute stress such as TIA, is a subsequent release of cortisol after a sudden interruption of blood flow to parts of the brain [36]. If serum cortisol is sustained at higher levels or longer periods of time post TIA or stroke, increased oxidative stress, impaired immune response and exacerbated inflammation in those affected parts of the brain could occur [36].

This cascade of long-term effects following a TIA could chronically dysregulate the HPA and HPG axes, potentially contributing to the observed novel decrease in serum LH of the TIA patients in this study. A possible exception to this theory was found by Veldhuis, when LH levels in older men were not stimulated by compensatory feedback from lower testosterone levels as expected because of a reduced testicular response to LH and an incomplete response from the hypothalamus and pituitary glands, both as a result of age-related changes [37]. However, in our study the mean and median testosterone levels of both groups were within the reference range, albeit the TIA group was more towards the lower end of the reference range. These differences were not large enough to be comparable with the findings of the aforementioned studies on serum LH to suggest reduced testicular response or chronic dysregulation of the HPA or HPG axes. Therefore, the neurohormonal inter-relationship changes in regulating TIA, specifically with LH should be further explored in subsequent research for further understanding and identifying prospective therapeutics.

Previous studies have found a potential link between decreased serum testosterone in men with comorbidities such as obesity and hypertension with a higher risk for ischemic stroke [38]. Holmegard *et al.* also discovered that participants with extremely low testosterone concentrations at or below the 10th percentile were at risk, but they could not determine if the low testosterone was caused by obesity or vice versa [38]. Therefore, is testosterone a risk factor for ischemic stroke or the result of poor lifestyle choices. Even though the participants in the TIA group of our study had a greater number of single comorbidities compared to the control group, we did not observe a significant difference between mean testosterone levels of the TIA and control group or a significantly lower serum testosterone level in the TIA group as previous research has demonstrated [39]. Perhaps multiple comorbidities are required to greatly reduce testosterone, if lifestyle habits influence testosterone and that is why we did not observe extremely low concentrations. More than likely, the HPA axis may not have been dysregulated enough with the short temporal status of a TIA compared to a stroke and thus requires a longer period of ischemia for oxidative stress and inflammation to cause greater dysregulation of the HPA axis. Just as probable, the reduced statistical power of our result from a small sample size could be the reason why we did not observe a significant and larger difference in testosterone between the two groups.

There was a potential significant correlation between increasing age and having hypertension with pathologic atherosclerotic changes of the internal carotid artery, which could lead to TIA [1]. These results agree with current literature, as increasing age and hypertension are well documented risk factors for TIA, in both sexes because of declining endogenous sex hormones [32, 40]. Men are usually younger than females when they get their first stroke, while women are seemingly “protected” before menopause against vascular disease and atherosclerosis-related ischemic strokes due to endothelial dysfunction because of higher levels of estradiol and progesterone compared to men [32, 38, 41]. Thus, reduced vascular integrity from reduced steroid hormones has been shown to lead to atherosclerotic changes, increased arterial stiffness and atherosclerosis, all risk factors for TIA and stroke [38, 41]. As seen in Figure 4, the progesterone and estradiol levels in both study groups were not statistically different and although the estradiol levels were on the lower reference range the results of the linear regression and spearman correlation tests revealed no predictive effect on male estradiol levels on DHEA, testosterone, atherosclerotic changes or stenosis greater than 50%.

Interestingly, an inverse relationship existed between TIA patients with stenosis of greater than 75% and their respective progesterone levels. Those TIA patients with lower progesterone levels showed a potentially significant increased correlation with stenosis of the internal carotid artery greater than 75%. Thus, further supporting the claims that elevated serum progesterone protects against cerebrovascular diseases by maintaining vascular integrity and function [41]. Previous research has shown promising results for progesterone in protecting the brain post-stroke, a leading cause of death and disability [42]. While tPA thrombolysis is currently the sole approved treatment for acute stroke and TIA, it is only suitable for 10% of patients presenting with TIA or stroke [42]. Progesterone could potentially provide a new, safe, and effective therapeutic option for more patients by reducing lesion volume and enhancing recovery from brain artery blockage as shown in experimental studies [42]. However, exogenous progesterone treatment has yet been able to reduce patient mortality or morbidity post stroke.

Results of the simple linear regression analyses of the control group revealed that an increase in HDL could significantly increase testosterone levels by a greater extent. However, the results of the confidence interval were too wide for these results to be applicable to the general population. This finding contrasts with prior research indicating an inverse correlation between HDL and testosterone [43, 44].

On the other hand, HDL is well known for its protective role against atherosclerosis and thus cardiovascular diseases such as TIA and stroke [45]. The anti-inflammatory properties of HDL prevent plaque rupture in arteries by stabilizing atherosclerotic plaques and inhibiting the oxidation of LDL, further reducing the progression of atherosclerosis [45].

The steroid hormone, 17OH P in the control group revealed another possibly important linear regression result. An increase in 17OH P could increase testosterone, but by a lesser extent than DHEA. A positive confirmation of this result with a narrow confidence interval was rationally explained by the role of 17OH P as a precursor hormone to testosterone [26]. Although not directly studied for its effect on testosterone and TIA, testosterone replacement therapy has been shown to reduce cardiovascular events such as TIA in androgen-deficient males, perhaps 17OH P replacement therapy could be beneficial for these males too [46].

The final significant result we observed in the control group regression analysis was that an increase in progesterone could increase DHEA. Previous studies indicated that reduced levels of DHEA may elevate the risk of coronary heart disease and ischemic stroke by decreasing testosterone levels [25]. It is yet to be understood if DHEA can provide the same protective effect against TIA and stroke as testosterone. Unfortunately, our analysis provided no significant difference in the mean DHEA levels of the control group compared with TIA group. Perhaps with a larger sample size these results could be comparable with the current literature. If progesterone has been shown to protect the brain post-stroke and improve vascular integrity [41, 42], progesterone may also be able to protect the brain from the oxidative stress and exacerbated inflammation caused by TIA by increasing DHEA and thus testosterone. That could explain why there was a potentially significant correlation between progesterone with DHEA in the control group rather than the TIA group.

Results of the simple linear regression analyses in the TIA group revealed an interesting bidirectional correlation. DHEA significantly predicted testosterone levels and vice versa. Physiologically, DHEA acts as a precursor hormone to testosterone, therefore a positive predictive value of DHEA to testosterone is easily understood [26]. There are a few potential mechanisms that could explain how testosterone could have a predictive effect on DHEA in TIA patients, most probably, naturally occurring bidirectional negative feedback loops [47]. Previous studies have indicated that men and older individuals are at an elevated risk of suffering from an ischemic stroke because of reduced serum sex hormones [30, 32, 33]. It is essential

to prevent the age-related decline of steroid hormones to lower the already elevated risk for TIA and stroke in older age individuals. Reducing comorbidities with healthy lifestyle changes is an appropriate prevention and therapeutic approach.

The heightened risk of TIA in men is associated with differences in sex hormone levels between genders, prior to female menopause, as well as variations in these levels during aging [32, 33]. After the age of fifty, men undergo a noticeable decrease in testosterone levels, which can also result from sudden illnesses such as an acute stroke [5, 30]. This decline has been linked to larger infarct size and less favorable outcomes following an acute ischemic stroke among elderly males [30]. Therefore, if DHEA and testosterone are potential risk factors for TIA and stroke, they could be screened and monitored in the future. More investigation is necessary to establish whether these hormones and biomarkers could be involved in a direct cause-and-effect relationship with TIA and or stroke.

Potential limiting factors in this study were the relatively small sample size, which could constrain the extent to which the findings can be applied to a broader population of TIA patients. Hormone levels can vary, therefore obtaining multiple measurements over time could offer a more comprehensive insight into their correlation with TIA [5]. Although we stratified our sample size for age and gender, other potential variables that could have impacted the results, included medication usage, concurrent conditions, lifestyle elements, etc.

And finally, the control group was comprised of neurological patients without TIA symptoms, and no history of TIA or stroke. Patients in this group included individuals with conditions like multiple sclerosis, amyotrophic lateral sclerosis, lumbar stenosis, neuropathy, and others. Despite age and gender stratification, variations in other factors might be present and could have impacted the outcomes.

In order to find potential predictors for TIA, this study examined the variations in testosterone levels and other biomarkers between TIA patients and a control group in KBC Firule hospital, Split. Our research showed a unique, significant drop in serum LH levels in the TIA group, a finding rarely reported in current literature.

This implies that after an acute stressful event such as a TIA, there may be dysregulation of the HPG and HPA axes, presumably as a result of elevated cortisol release. Which can lead to oxidative stress, an impaired immune response, and exacerbated inflammation in the brain.

Furthermore, this study demonstrated an inverse relationship between serum progesterone and severe stenosis of the internal carotid arteries, supporting the protective role of progesterone in maintaining vascular integrity. According to these results, controlling and observing hormone levels, especially those of testosterone, progesterone, and LH may be essential to the prevention and treatment of TIA and stroke. Future studies should examine possible treatment approaches that target these hormone pathways by gaining a better understanding of the neuroendocrine reactions of TIA and stroke. This study adds to the growing body of evidence on the importance of monitoring and potentially controlling hormones to minimize and prevent the effects of TIA and stroke.

6. CONCLUSION

The results of our study found a significantly lowered median value of LH in the TIA group compared to the control group, which could be due to fluctuating hormone levels from the acute stress and neurologic dysfunction caused by TIA.

The acute stress of TIA has been noted in the literature to cause lowered testosterone and DHEA values, and although our study could not replicate these results, DHEA and testosterone were shown to bidirectionally influence each other in the TIA group and not the control group. This leads us to believe that a TIA insult had caused a significant change in some hormones more so than others. And with a larger sample size our study could have potentially shown significantly lowered mean testosterone and DHEA values in the TIA group compared to the control group.

The findings that were consistent with the existing literature were as follows: older age and hypertension were significantly correlated with pathologic atherosclerotic changes and progesterone was shown to negatively influence severe stenosis (>75%) of the internal carotid artery in the TIA group. Further supporting the potential protective role progesterone exhibits on the brain.

Perhaps LH, DHEA, and testosterone levels can be used for potential screening in the future for predicting TIA or stroke, or to better monitor the severity and recovery after a TIA or stroke.

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8. SUMMARY

Background: Recent studies have highlighted the potential role of hormonal imbalances in various health conditions, including cardiovascular events. This study investigates the relationship between serum hormone levels such as testosterone, in patients diagnosed with TIA. By analyzing hormones and other known biomarkers, this research aims to identify potential endocrine factors associated with TIA occurrence, contributing to a better understanding of TIA pathogenesis and potentially paving the way for future preventative strategies.

Methods: Fifty patients admitted to the Department of Neurology at the University Hospital Split, Firule in Croatia between 2001 and 2002 were retrospectively included in this study after being stratified for age (older than fifty years) and gender (male). Twenty-five patients who were admitted for TIA were assigned to the TIA group and twenty-five patients who were admitted for a non-TIA/stroke neurological condition were assigned to the control group. The results of their blood samples and ultrasound of both internal carotid arteries were statistically analyzed and compared.

Results: When comparing both patient groups, the median LH levels in the TIA group were significantly lower compared to the control group ($U = 159, P = 0.003$). A potential inverse correlation was found between progesterone levels and the severity of internal carotid artery stenosis in TIA patients ($p(25) = -0.437, P = 0.029$). A Spearman correlation performed in the control group between age and hypertension status with atherosclerotic changes was statistically significant, ($p(25) = 0.795, P < 0.001$) and ($p(25) = 0.480, P = 0.015$), respectively. Linear regression analyses revealed, in the control group, HDL showed a positive correlation with testosterone levels ($b = 12.52, t(23) = 2.50, P = 0.020$). 17OH P, a precursor to testosterone, positively predicted testosterone levels ($b = 2.17, t(23) = 3.73, P = 0.001$), and progesterone levels were positively associated with DHEA levels ($b = 1.76, t(23) = 2.91, P = 0.008$). In the TIA group, a bidirectional correlation was found between DHEA and testosterone. DHEA explained 35% of testosterone variation ($R^2 = 0.350$), and the regression coefficient was significant ($b = 1.25, t(23) = 3.52, P = 0.002$). Conversely, testosterone explained 35% of DHEA variation ($R^2 = 0.350$), and the regression coefficient was significant ($b = 0.281, t(23) = 3.52, P = 0.002$).

Conclusion: This study found a significantly lower median LH level in the TIA group compared to the control group, potentially due to acute stress and neurological dysfunction. While the study couldn't confirm previous findings of lowered testosterone and DHEA levels post-TIA, it did reveal a bidirectional influence between these hormones in the TIA group, suggesting a significant hormonal shift. The study also confirmed existing literature linking older age and hypertension to atherosclerotic changes and demonstrated progesterone's negative influence on severe stenosis of the internal carotid artery in the TIA group, supporting its potential neuroprotective role. Further research is needed to explore the use of LH, DHEA, and testosterone levels in TIA and stroke screening, monitoring, and recovery.

9. SUMMARY IN CROATIAN

Naslov: Razine spolnih hormona u pacijenata s prolaznim ishemijskim udarom.

Cili istraživanja: Nedavne studije istaknule su potencijalnu ulogu hormonske neravnoteže u različitim zdravstvenim stanjima, uključujući kardiovaskularne događaje. Ova studija istražuje odnos između razine hormona u serumu, poput testosterona, u bolesnika s dijagnozom tranzitorne ishemijske atake. Analizom hormona i drugih poznatih biomarkera, ovo istraživanje ima za cilj identificirati potencijalne endokrine čimbenike povezane s pojavom TIA, doprinoseći boljem razumijevanju patogeneze TIA i potencijalno otvarajući put budućim preventivnim strategijama.

Ispitanici i metode: U ovu retrospektivnu studiju uključeno je pedeset pacijenata starijih od pedeset godina, primljenih u Kliniku za neurologiju KBC-a Split, bolnica Firule, između 2001. i 2002. godine. U skupinu s TIA uključeno je dvadeset i pet pacijenata primljenih zbog TIA, a u kontrolnu skupinu dvadeset i pet pacijenata primljenih zbog neuroloških stanja koja nisu TIA/moždani udar. Statistički su analizirani i uspoređeni rezultati njihovih uzoraka krvi i ultrazvuka obje karotidne arterije.

Rezultati: Usporedbom obje skupine pacijenata, medijan razine LH u skupini s TIA bio je značajno niži u usporedbi s kontrolnom skupinom ($U = 159$, $P = 0,003$). Pronađena je potencijalna inverzna korelacija između razine progesterona i težine stenoze karotidne arterije u bolesnika s TIA ($P = -0,437$, $P = 0,029$). Spearmanova korelacija provedena u kontrolnoj skupini između dobi i hipertenzije s aterosklerotskim promjenama bila je statistički značajna, ($P = 0,795$, $P < 0,001$) i ($P = 0,480$, $P = 0,015$). Linearna regresijska analiza u kontrolnoj skupini pokazala je pozitivnu korelaciju između HDL-a i razine testosterona ($b = 12,52$, $t = 2,50$, $P = 0,020$). 17OH progesteron, prekursor testosterona, pozitivno je predvidio razinu testosterona ($b = 2,17$, $t = 3,73$, $P = 0,001$), a razina progesterona bila je pozitivno povezana s razinama DHEA ($b = 1,76$, $t = 2,91$, $P = 0,008$). U skupini s TIA pronađena je dvosmjerna korelacija između DHEA i testosterona. DHEA je objasnio 35% varijacije testosterona ($R^2 = 0,350$), a koeficijent regresije bio je značajan ($b = 1,25$, $t = 3,52$, $P = 0,002$). Obrnuto, testosteron je objasnio 35% varijacije DHEA ($R^2 = 0,350$), a koeficijent regresije bio je značajan ($b = 0,281$, $t = 3,52$, $P = 0,002$).

Zaključci: Ova je studija otkrila značajno nižu medijan razinu LH u skupini s TIA u usporedbi s kontrolnom skupinom, što bi moglo biti posljedica akutnog stresa i neurološke disfunkcije. Iako studija nije mogla potvrditi prethodne nalaze o sniženim razinama testosterona i DHEA nakon TIA, otkrila je dvosmjerni utjecaj između ovih hormona u skupini s TIA, što upućuje na značajnu hormonsku promjenu. Studija je također potvrdila postojeću literaturu koja povezuje stariju dob i hipertenziju s aterosklerotskim promjenama i pokazala negativan utjecaj progesterona na tešku stenozu karotidne arterije u skupini s TIA, što podupire njegovu potencijalnu neuroprotektivnu ulogu. Potrebna su daljnja istraživanja kako bi se istražila upotreba razine LH, DHEA i testosterona u probiru, praćenju i oporavku od TIA i moždanog udara.

