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Original Reserach Article

HSP90 Levels as Novel Protective Signal for the Initial Discovery of Dementia in Patients with Insomnia

Noor Ali Gebur[1*], Makarim Ali Enad[1]
[1]Department of Chemistry, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq

***Corresponding Author:** Noor Ali Gebur
Department of Chemistry, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq

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Abstract: The term insomnia refers to the sleep defect or inability to sleep. A patient's disturbance of sleep to a considerable degree that interferes with day functioning is a main element to take into account of insomnia diagnosis. Cognitive dysfunction and dementia occurs because hypertension. HSP90 is bioactive marker for molecular helper and is related with a number of diseases like hypertension, diabetes and cancers. In this study, we focused to assess the Serum HSP90 in patients with insomnia, and to analyze any correlation with biochemical parameters studied. A case-referent study design included 120 Iraqi individuals, 60 of them suffered from insomnia (36 males and 24 females) against a group of 60 healthy individuals (36 males and 24 females) whose ages were similar to those of the patients aged (20-75) years. The serum HSP90 levels and metabolic parameters including BMI, WHR, SBP, DBP, MDA, AOPPs, 8-OHdG, Vitamin D, Iron, Serotonin, Dopamine, Melatonin and Cortisol were assessed in all individuals. The results were analyzed statistically to examine the differences between the groups and identify the relationship between the studied parameters. According to the statistical analysis, SBP was significantly elevated in insomnia group as compared to healthy referent group (140.50±14.0 versus 109.0±16.2, P=0.02), respectively. DBP was significantly elevated in insomnia group as compared to healthy referent group (90.5±10.62 versus 71.5±12.8, P=0.04), respectively. Serum HSP90 was significantly elevated in insomnia group as compared to healthy referent group (110±10 versus 60±5, P=0.04), respectively. Serum MDA was significantly elevated in insomnia group as compared to healthy referent group (5±0.93 versus 2±0.62, P= 0.01), respectively. Serum AOPPs was significantly elevated in insomnia group as compared to healthy referent group (221±45.1 versus 97.5±31.9, P=0.03), respectively. Serum 8-OHdG was significantly elevated in insomnia group as compared to healthy referent group (3.5±0.6 versus 1.25±0.28, P=0.02), respectively. No strong significant association was observed between HSP90 and other biochemical parameters studied, except SBP and DBP, while weak significant association was found with MDA, AOPPs and 8-OHdG. The current investigation found that insomnia patients had considerably higher levels of HSP90 than the control group. SBP and DBP have a strong positive association with HSP90 levels. These results suggest that HSP90 level in insomnia patients may act as a novel protective signal for the initial discovery ofdementia in patients with insomnia.

Keywords: Insomnia, Hypertention, Dementia, Heat shock proteins 90 (HSP90), Malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPPs), 8-hydroxy-2-deoxyguanosine (8-OHdG), Vitamin D, Iron, Serotonin, Dopamine, Melatonin, Cortisol.

INTRODUCTION

The term insomnia refers to the sleep defect or inability to sleep. A patient's disturbance of sleep to a considerable degree that interferes with day functioning is a main element to take into account of insomnia diagnosis [1]. Insomnia known as defect of sleep with environmental, physical and psychological causes. The sleep disturbance declines of cells functions causing healthy problems [2]. Additionally, hypertension, myocardial infarction, stroke, diabetes, oxidative stress and ischemic attack are related to insomnia [3, 4]. There has been limited research on the connection between the frequency of neurological disorders and insomnia. The

countries are suffer from social and economic problems because increasing of cognitive disturbance like dementia [5]. Cognitive dysfunction and dementia occurs because hypertension [6]. While, these relations are not fully explained. Heat shock proteins (HSP) are molecular helper factors that control protein playing an important role in maintenance activity of cells by wrapping and break down incorrect folding or accumulated proteins [7]. Additionally, cells harmful impacts of many chemical and physical stresses like chronic diseases are shielded by heat shock protein (HSP) [8, 9]. Additionally, infection factors associated with manifestation of HSP [10]. Chronic diseases are able to resisted by mRNA production of HSP [11]. HSP are classified based on their molecular weight (i.e. HSP90, HSP70, HSP60, HSP27 and HSP40), of these, HSP90 is bioactive marker for molecular helper and is related with a number of diseases like hypertension, diabetes and cancers [12]. In this study, we aimed to evaluate the Serum HSP90 in patients with insomnia, and to identify any correlation with biochemical parameters studied.

EXPERIMENTAL

Individuals and Study Design

The clearance for all research before they could begin was given by university of Al-Qadisiyah / Faculty of Science. Informed permission papers were received from each participant before the research started. Accompanied by 120 participants in two groups of 60 insomnia patients (36 male and 24 females), ranging in age from 20 to 75 years, this research was designed to be a case referent study. From October 2024 to December 2024, patients were recorded in the "Diwaniya Teaching Hospital" in Al-Qadisiyah, Iraq. As a referent group, sixty in good health individuals (36 males and 24 females) were added in order to compare the results, whose ages were similar to those of the patients aged (20-75) years.

Exclusion Criteria

This research excluded participants with severe psychiatric diseases, neurological disorders, heart failure, liver diseases, renal disease, use of medications or alcohol, pregnancy and breastfeeding.

Collection of Samples

After an 8–12 hour fast, five milliliters of venous blood were drawn using antecubital venipuncture with G 23 needles from insomnia individuals and a referent healthy group between 8:30 and 10:00 a.m. Five milliliters of blood were left to coagulate at room temperature in a test tube. Following a 15-minute separation by centrifugal force at 3000 X g, the serum was isolated into five tubes then stored.

Demographic Evaluation

By dividing weight in kilograms by length of individual in square meter, the Body Mass Index was estimated as following: BMI = (weight in kg) / (height in meters2), the waist to hip ratio was calculated as the ratio of waist in (cm) to hip in (cm) [13].

Biochemical Evaluation

Using an ELISA micro plate washer and reader, the levels of serum 8-hydroxy-2-deoxyguanosine (8-OHdG) was determined by using a commercial kit from CORTEZ (USA) based on the principles of the Enzyme Linked Immunosorbent Assay (ELISA). Using a commercial kit from Monobind Inc. (USA), the spectrophotometric assay (TYPE 3) was used to measure the levels of Advanced Oxidation Protein Products (AOPPs). Malondialdehyde (MDA) was determined by using a commercial kit from LTA (Italia) based on the principles of the Thiobarbituric Acid Reactive Substances assay (TBARS). Serum HSP90 was evaluated using enzyme-linked immunosorbent test kits (MELSIN, China). Using a commercial kit from LTA (Italia), the serum iron content was determined at 578 nm using the recommendations of a Spectrophotometer. Using a commercial kit from LiNEAR (SPAIN), Enzyme Linked Immunosorbent Assay (ELISA) was used to assess the concentrations of Vitamin D, Serotonin, Dopamine, Melatonin and Cortisol.

Bio-Statistical Analysis

The statistical analysis was conducted by using Microsoft Excel 2010 and SPSS-24 (statistical package for social science-version 24) software. The data were submitted to statistical analysis to examine the differences between the analyzed groups. Pearson's correlation coefficient was applied to evaluate the correlation between parameters.

RESULTS AND DISCUSSION

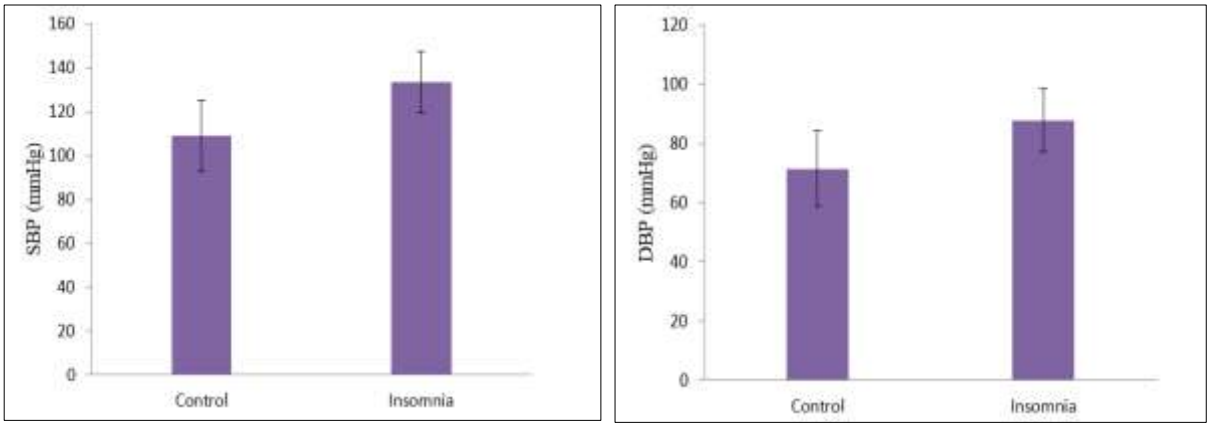
In table-1, the mean values of age, BMI, W/H are shown and demonstrated no significant variation between the patients group and the control group. Nevertheless, the mean values of SBP and DBP show a significant increase in the insomnia group compared to the healthy group, as shown in figure-1 a, b.

Table 1: Demographic data for the insomnia and the control groups

Parameters	Groups		P-value
	Control (n=60) n(%) Mean	Insomnia (n=60) n(%) Mean	
Age (year)	48.6±0.9	48.6±0.9	1.00
Gender Female / Male	24(40%)/36(60%)	24(40%)/36(60%)	1.00

SBP (mmHg)	109.0±16.2	140.50±14.0	0.02
DBP (mmHg)	71.5 ±12.8	90.5 ±10.62	0.04
BMI (Kg/m2)	24.3±2.0	24.8±2.5	0.07
W/H	0.82±0.19	0.84±0.22	0.06
Mood disorders Have/Does not have	3(5%)/57(95%)	12(20%)/48(80%)	0.08
Physical health Excellent/Poor	56(93%)/4(7%)	46(77%)/14(23%)	0.06
Mental health Excellent/Poor	57(95%)/3(5%)	49(81%)/11(19%)	0.09
Anxiety disorders Have/Does not have	3(5%)/57(95%)	7(11%)/(53(89%)	0.08
Took medication to help sleep Have/Does not have	—/60(100%)	10(15%)/50(85%)	0.07
Refreshing sleep Yes/No	60(100%)/—	10(15%)/50(85%)	0.02
Alcohol drinking Have/Does not have	5(8%)/55(92%)	6(10%)/54(90%)	0.53
Life stress Have/Does not have	34(56%)/26(44%)	41(68%)/19(32%)	0.06
Household type Living With parents/Not	40(66%)/20(34%)	27(45%)/33(55%)	0.07
Study state Students and graduate/Not	46(77%)/14(23%)	48(80%)/12(20%)	0.08

Data represented as Mean ±SD, p-value of ≤0.5 was considered significant, SD: Stander deviation, BMI: Body mass index, n: Number of subjects, W/H: The waist to hip ratio, DBP: Diastolic blood pressure, SBP: Systolic blood pressure.



a **b**

Figure 1: Comparison of SBP and DBP between the insomnia and the control groups

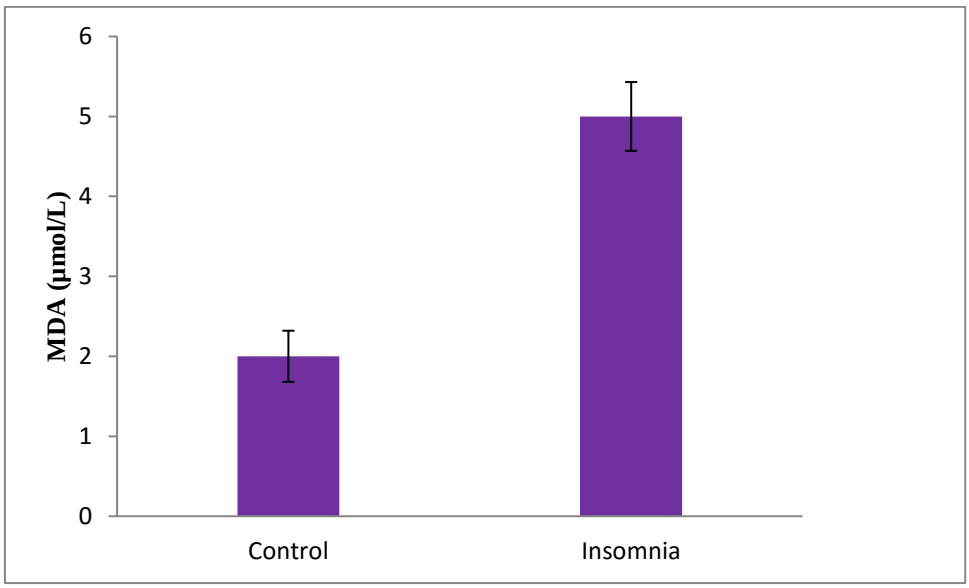
The means of Vitamin D, Iron, Serotonin, Dopamine, Melatonin, Cortisol shown in table-2 demonstrated no significant variations between the insomnia group and the control group. Nevertheless, the means of MDA, AOPPs, 8-OHdG and HSP90 levels showed a significant increase in the insomnia group compared with the control group, as shown in figure-2 a, b, c, d.

Table 2: Biochemical data for the insomnia and the control groups

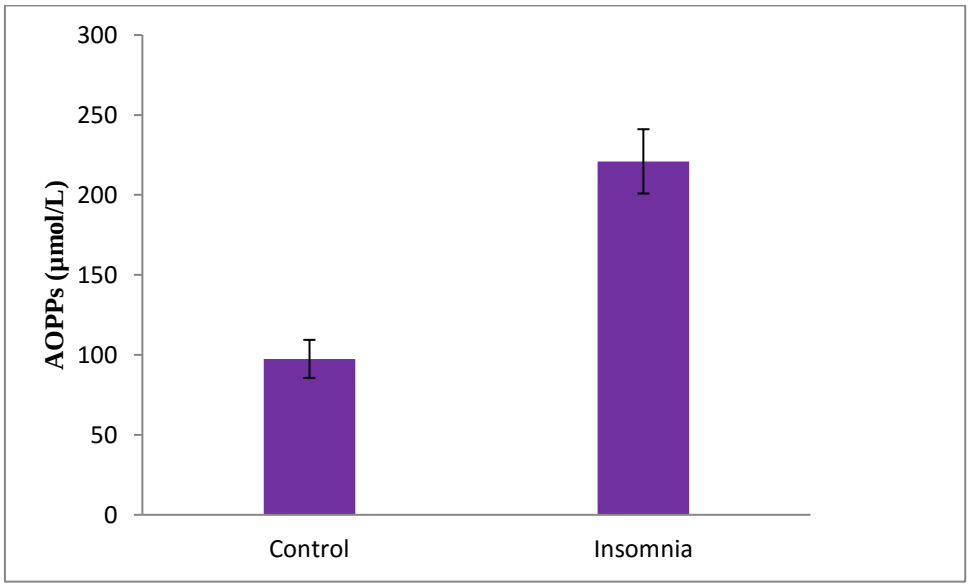
Parameters	**Groups**		**P-value**
	Control Mean ±SD (n=60)	**Insomnia Mean ±SD (n=60)**	
MDA (µmol/L)	2±0.62	5±0.93	0.01
AOPPs (µmol/L)	97.5±31.9	221±45.1	0.03
8-OHdG (ng/mL)	1.25±0.28	3.5±0.6	0.02
Vitamin D (ng/mL)	60±12.3	40.6±17.5	0.07
Iron (µg/dL)	110±10.7	90±9.8	0.10

Serotonin (ng/mL)	180±8.92	110±10.8	0.14
Dopamine (pg/mL)	15±4.94	9±2.32	0.25
Melatonin (pg/mL)	40±12.5	20±5.8	0.06
Cortisol (µg/dL)	10±3.55	7.5±1.5	0.08
HSP90 (ng/mL)	60±5	110±10	0.04

Data represented as Mean ±SD, p-value of ≤0.5 was considered significant, SD: Stander deviation, HSP90: Heat shock protein 90, n: Number of subjects, MDA: Malondialdehyde, AOPPs: Advanced Oxidation Protein Products, 8-OHdG: 8-hydroxy-2-deoxyguanosine.



a



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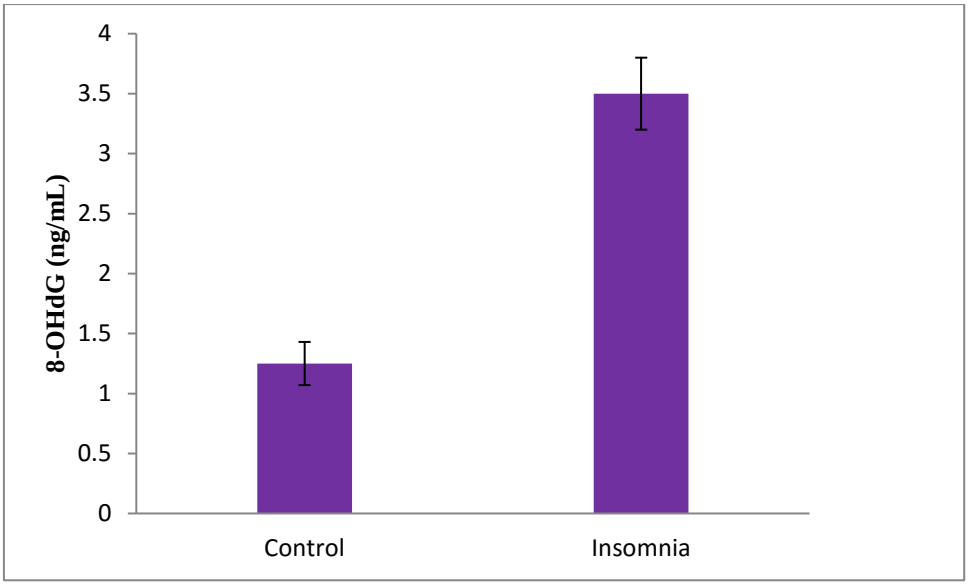
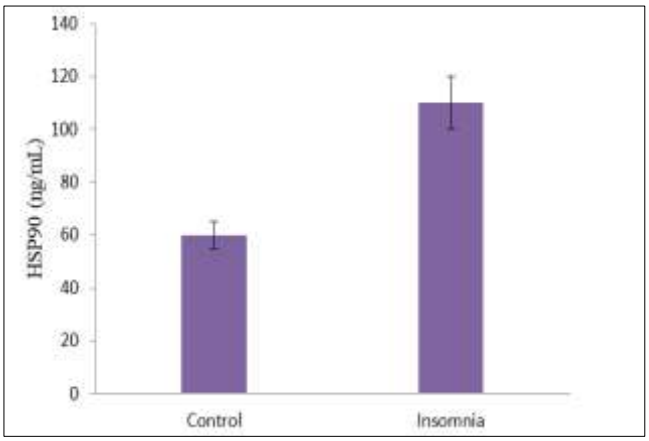


c



d

Figure 2: Comparison of serum a: MDA, b: AOPPs, c: 8-OHdG and d: HSP90 levels between the insomnia and control groups

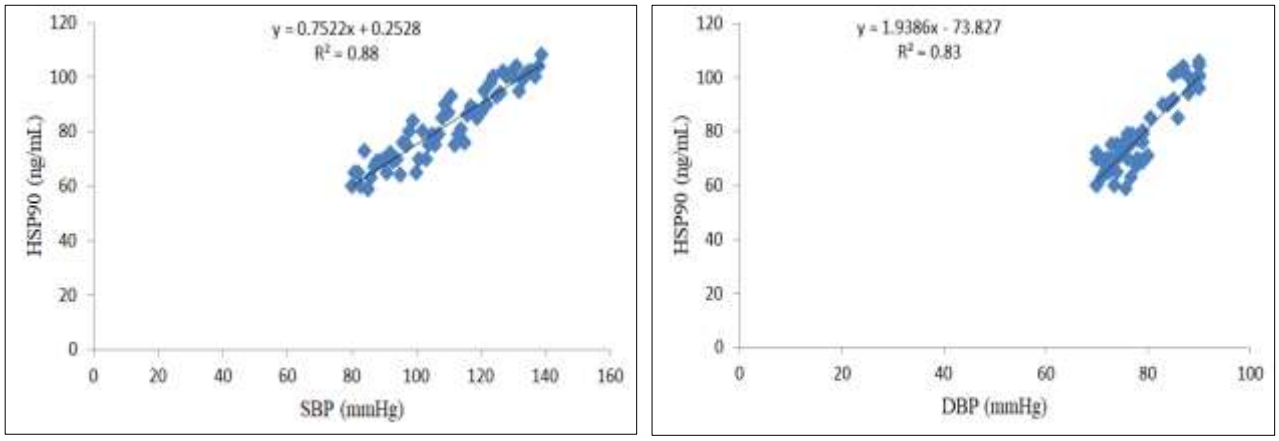
The linear regression analysis shown in table-3 demonstrated the correlation between serum HSP90 and other demographic data in patients with insomnia. No strong significant correlation was observed between HSP90 and other studied biochemical parameters, except that SBP and DBP showed a strong significant positive correlation with HSP90 level, as shown in figure-3 a, b.

Table 3: Correlation between serum HSP90 levels and demographic data in the insomnia group

Parameters	HSP90 (ng/mL)	
Age (year)	r P-value	0.1 0.5
Gender	r P-value	0.2 0.8
SBP (mmHg)	r P-value	0.88 0.02
DBP (mmHg)	r P-value	0.83 0.04
BMI (kg/m2)	r P-value	0.01 0.99
W/H	r P-value	0.07 0.68
Mood disorders	r P-value	0.07 0.70

Physical health	r P-value	0.12 0.51
Mental health	r P-value	0.04 0.98
Anxiety disorders	r P-value	0.23 0.36
Took medication to help sleep	r P-value	0.02 0.88
Refreshing sleep	r P-value	0. 05 0.61
Alcohol drinking	r P-value	0.12 0.50
Life stress	r P-value	0.20 0.47
Household type	r P-value	-0.07 0.65
Study state	r P-value	0.09 0.63

r: Person s correlation, P-value of ≤0.5 was considered significant, W/H: The waist to hip ratio, BMI: Body mass index, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, HSP90: Heat shock protein 90.



a **b**

Figure 3: Correlation between serum HSP90 levels and a: SBP and b: DBP in the insomnia group

The linear regression analysis shown in table-4 demonstrated the correlation between serum HSP90 levels and other biochemical parameters studied in insomnia patients. No strong significant correlation was observed between HSP90 and biochemical parameters. However, a weak but significant correlation was observed between HSP90 and MDA, AOPPs and 8-OHdG.

Table 4: Correlation between serum HSP90 levels and others biochemical parameters in the insomnia group

Parameters	**HSP90 (ng/mL)**	
MDA (µmol/L)	r P-value	0. 72 0.03
AOPPs (µmol/L)	r P-value	0.63 0.04
8-OHdG (ng/mL)	r P-value	0.68 0.01
Vitamin D (ng/mL)	r P-value	-0.23 0.36
Iron (µg/dL)	r P-value	-0.02 0.88
Serotonin (ng/mL)	r P-value	-0. 05 0.61
Dopamine (pg/mL)	r P-value	-0.12 0.50

Melatonin (pg/mL)	r P-value	-0.11 0.55
Cortisol (µg/dL)	r P-value	-0.20 0.47

r: Person s correlation, P-value of ≤0.5 was considered significant, MDA: Malondialdehyde, AOPPs: Advanced Oxidation Protein Products, 8-OHdG: 8-hydroxy-2-deoxyguanosine, HSP90: Heat shock protein 90.

As shown in table-5, the receiver operating characteristic (ROC) curve for HSP90 was illustrated. This study revealed that the cut-off value for HSP90 in the insomnia group was 65%. Furthermore, the area under the curve (AUC) for HSP90 was 0.816 in the insomnia group. The sensitivity of HSP90 was 95%, while its specificity was 70%, as shown in figure-4.

Table 5: Receiver operating characteristic (ROC) and area under the curve (AUC) analysis for HSP90 diagnosis using gauged biomarker

Variable	Group	Cut-off concentration%	Sensitivity%	Specificity%	AUC	Std. Error	95% CI of AUC	P-value
HSP90	Insomnia	65	95	70	0.816	0.038	0.742-0.890	0.000

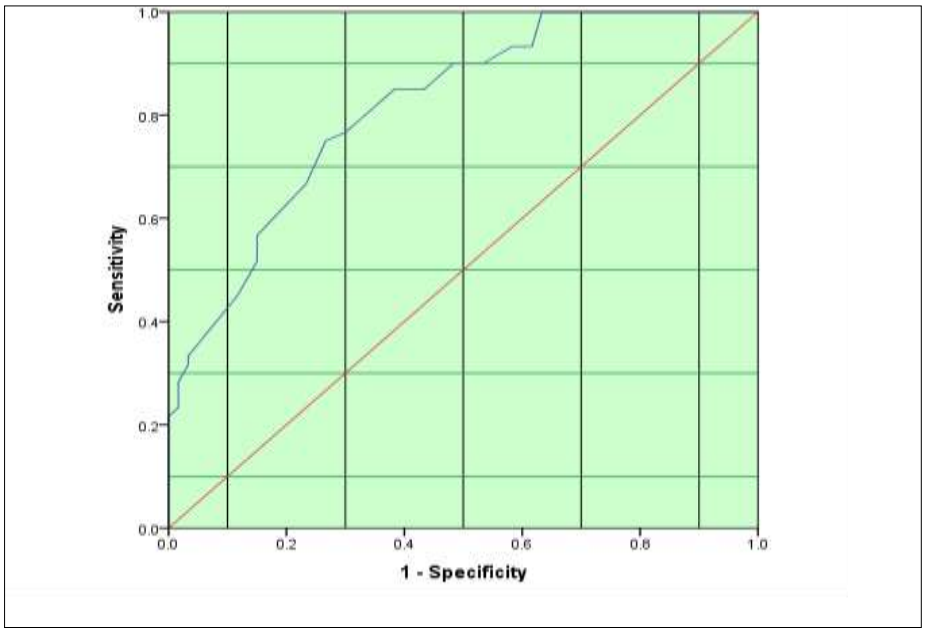


Figure 4: Receiver operating characteristic (ROC) curve analysis for HSP90 in insomnia patients

The current study showed a significant increase in serum HSP90 levels, blood pressure levels and oxidative stress biomarkers levels like (MDA, AOPPs, 8-OHdG) among patients with insomnia, and indicates a strong positive correlation between serum HSP90 and blood pressure in those patients. This can be explained by the fact that chronic insomnia activates the sympathetic nervous system and increases the secretion of stress hormones such as cortisol, which contribute to elevated blood pressure. Chronic hypertension is known to damage cerebral microvasculature, reduce neuronal perfusion, and lead to cognitive decline over time. On the other hand, elevated HSP90 levels may reflect a physiological stress response related to persistent insomnia and hypertension to stabilize other proteins and protect cells from damage. Therefore, these findings suggest that HSP90 could serve as an early biomarker for the potential risk of developing dementia in patients with insomnia. These our findings are consistent with the results of a study about rats suffering from hypertension reported that HSP90 level was elevated with disturbance nitric oxide, the investigation was in hypertension occurs offsetting of defect in role nitric oxide by elevated of HSP90 level [14, 15]. The results of present study are in agreement with the a study concerning obesity demonstrated a significance elevated levels of Hsp90 as compared with control group. The incendiary reply of obesity by increased HSP90 [16, 17]. Similar observations supporting the current findings regarding with hypertensive rats that observed protective response of heart failure by increased levels of heat shock proteins HSP90 with hyperthermia [18, 19]. Our findings align with research related to hypertension by renal failure patients that showed increased levels of Hsp90, hypertension causes increased levels of HSP90 [20, 21]. The current results are in agreement with previous study of hypertensive demonstrated that increase HSP90 as

vasorelaxation role in rats [22]. Similar outcomes was conducted on chickens hearts with hypertension that reported increased Hsp90 as substitutive role against disease [23]. According to other previous researches on hypertension with vascular smooth cell injury that activated AngII by increased HSP90 levels [24, 25]. The results of study about hyperthermia with hypertension found increased levels of mitochondria HSP90 in mice [26]. There were similarities between our results and study on pulmonary arterial hypertension (PAH) that found increased of mtHSP90 as response to stress and regulator of homeostasis of mitochondria [27, 28]. Our findings are agreement with other study on Hypertension that showed increased HSP90 with endothelial disease is regarded with dysfunction of nitric oxide (NO) by the combination between nitric oxide synthase with its proteins [25], the folding will be normal by combination of HSP90 to eNOS endothelial [26]. Nitric oxide is important in homeostasis of blood pressure [29, 30].

CONCLUSION

According to the current study, patients with insomnia have significantly greater levels of HSP90 than the control group. There is a strong positive correlation between HSP90 and blood pressure level. Insomniac patients have high risk factors of dementia, like high blood pressure. These findings imply that HSP90 plays a protective role in the early dysfunction of insomnia that leads to the onset of dementia. Further research is recommended to monitor Tau protein levels in insomnia patients showing elevated HSP90 and blood pressure, as Tau is a key biomarker of early neuronal injury and cognitive decline, and to investigate the relationship between HSP90 and Tau protein to better understand the cellular stress mechanisms linking sleep disturbances to neurodegeneration.

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