PERIÓDICO TCHÊ QUÍMICA

ARTIGO ORIGINAL

ESTUDO DO PAPEL DOS HORMÔNIOS VASOPRESSINA, ENDOTELINA-1 E ALBUMINA SERICA DURANTE O ENVELHECIMENTO PROGRESSIVO EM HOMENS HIPERTENSOS

STUDYING THE ROLE OF VASOPRESSIN HORMONE, ENDOTHELIN-1, AND SERUM ALBUMIN DURING PROGRESSIVE AGE IN HYPERTENSIVE MEN

دراسة دور هورمون الفازوبريسين , الاندوثيلين وألالبومين خلال التقدم بالعمر في الرجال المصابين بارتفاع ضغط الدم

Hasan, Methaq Mohamed*
Department of Biology, College of Science, University of Misan, Maysan, Iraq.

Khalifa, Ahmed Aboud
Department of Biology, College of Science, University of Misan, Maysan, Iraq.

** Corresponding author*
e-mail: ali_abid78@yahoo.com

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RESUMO

Introdução: Tanto a hipertensão arterial descontrolada quanto a idade avançada levam a má qualidade de vida, patogênese renal e doença cardiovascular por meio de distorções em marcadores neuro-hormonais e inflamatórios, como arginina vasopressina (AVP), albumina e endotelina-1, que contribuíram para o regulação da pressão arterial na idade avançada. **Objetivos**: O presente estudo investigou o papel do hormônio arginina vasopressina (AVP), albumina sérica, proteínas totais e endotelina-1 (ET-1) em homens hipertensos com idade avançada. **Métodos**: Foram comparados os níveis de AVP, ET-1, proteína total e albumina sérica em 80 homens hipertensos com o avanço da idade entre divididos com idades de 30 anos a 69 anos, divididos em quatro grupos (20 homens/grupo) de acordo com as suas idades. O o primeiro grupo foi de 30-39 anos, o segundo grupo de 40-49 anos, o terceiro grupo de 50-59 anos e o quarto grupo de 60-69 anos, utilizando a Análise de Variância de um fator (ANOVA), seguida do teste de Duncan para os grupos. **Resultados**: A arginina vasopressina aumentou significativamente em diferentes grupos ($p \leq 0,01$), salvo no quarto grupo vs. o terceiro grupo. O endotelina-1 aumentou significativamente em diferentes grupos ($p \leq 0,01$), exceto no terceiro grupo contra o segundo grupo. A albumina sérica diminuiu significativamente em diferentes grupos ($p \leq 0,01$), exceto para o terceiro grupo contra o segundo grupo. As proteínas totais diminuíram significativamente ($p \leq 0,01$), com exceção do terceiro grupo contra o segundo grupo. **Discussão**: O impacto fisiológico desses resultados foi discutido de acordo com os efeitos da hipertensão e da idade avançada sobre os parâmetros estudados; particularmente, o incremento da arginina vasopressina e níveis elevados de endotelina-1 que indicaram uma evidente disfunção na regulação da pressão arterial durante a idade avançada. **Conclusões**: Níveis elevados do hormônio AVP e sua capacidade de constrição dos vasos sanguíneos e reabsorção de sódio e água podem ser indicados como hipertensão contínua e de manutenção durante a idade avançada. Altos níveis de ET-1 podem ser detectados durante hipertensão e idade avançada, e ambos são considerados inflamação de baixo grau.

Palavras-chave: *AVP, ET-1, Albumina Sérica, Proteínas totais.*

ABSTRACT

Background: Vasopressin (AVP) hormone and total serum protein (including albumin) change during the progressive age in hypertensive men and are associated with cytokines release (endothelin-1 (ET-1)), leading to many renal and cardiovascular disorders**. Aim:** This study is to investigate AVP, ET-1, total protein, and serum albumin levels in various ages of hypertensive men to provide insights into the effects of aging and high blood pressure. **Methods:** The levels of AVP, ET-1, total protein, and serum albumin were compared in 80 hypertensive

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men as they aged, divided into four groups (20 men/group) based on their ages ranging from 30 to 69 years. The first group consisted of men aged 30-39 years, the second group included men aged 40-49 years, the third group had men aged 50-59 years, and the fourth group had men aged 60-69. One-way Analysis of Variance (ANOVA) was used to analyze the data, followed by Duncan's test for the groups. **Results:** AVP increased significantly ($p \leq 0.01$) in different groups, except the fourth group against the third group. High levels of ET-1 were released significantly ($p \leq 0.01$) in different groups except in the third group vs. the second group. Both serum albumin and total protein reduced significantly ($p \leq 0.01$) in different groups (with some exceptions). **Discussion:** The physiological impact of these results was discussed according to the effects of hypertension and advanced age on the studied parameters; particularly, AVP increment and high levels of ET-1 indicated an evident dysfunction in blood pressure regulation during advanced age. **Conclusions:** High levels of AVP hormone and its ability to constrictions of blood vessels and reabsorption of Na and water might be indicated as continuous and maintenance hypertension during advanced age. High levels of ET-1 detection might be pointed to a low-grade inflammation during hypertension and advanced age.

Keywords: *AVP, ET-1, Serum Albumin, Total protein.*

الملخص

تتغير مستويات كل من هورمون الفاسوبريسين والبروتين الكلي (من ضمنها ألبومين المصل) خلال التقدم بالعمر في الرجال المصابين بارتفاع ضغط الدم ويترافق هذا التغير مع انطلاق مجموعة من السايتوكينات (من ضمنها الاندوثيلين -1) الامر الذي يؤدي الى العديد من الاضطرابات القلبية والكلوية. **الهدف**: تهدف هذه الدراسة إلى التحقق من مستويات الفاسوبريسين , الاندوثيلين والبروتين الكلي (ألبومين المصل) عند تقدم العمر في الرجال المصابين بارتفاع ضغط الدم وذلك لزيادة الفهم حول تأثيرات الشيخوخة وارتفاع ضغط الدم . **طريقة العمل**: لمقارنة هورمون الفاسوبريسين, البروتين الكلي ,ألبومين المصل والاندوثيلين-1 تم اخذ ثمانين رجلاً مصابًا بارتفاع ضغط الدم تتراوح أعمارهم بين (30 - 69 سنة) قسموا إلى أربع مجاميع (20 رجل / مجموعة) وفقًا لأعمارهم ، المجموعة الأولى 30-39 سنة ، المجموعة الثانية 40-49 سنة ، المجموعة الثالثة 50-59 سنة ، المجموعة الرابعة 60-69 سنة و باستخدام تحليل التباين أحادي الاتجاه (ANOVA) المتبوع باختبار Duncan. **النتائج**: أظهر الفاسوبريسين زيادة معنوية ($p \leq 0.01$) (عدا المجموعة الرابعة بالمقارنة مع المجموعة الثالثة) في المجاميع المختلفة. زاد مستوى الاندوثيلين -1 معنويا ($p \leq 0.01$) (باستثناء المجموعة الثالثة بالمقارنة مع المجموعة الثانية) في المجاميع المختلفة. انخفض ألبومين المصل معنويا ($p \leq 0.01$) (عدا المجموعة الثالثة بالمقارنة مع المجموعة الثانية). انخفضت البروتينات الكلية معنويا ($p \leq 0.01$) (عدا المجموعة الثالثة بالمقارنة مع المجموعة الثانية) في المجاميع المختلفة. **المناقشة**: تمت مناقشة البعد الفسيولوجي لهذه النتائج وفقًا لتأثيرات ارتفاع ضغط الدم والتقدم بالعمر على المقاييس المدروسة وعلى وجه الخصوص فقد أشرت زيادة كل من الفاسوبريسين و الاندوثيلين وجود خلل واضح في تنظيم ضغط الدم خلال التقدم بالعمر. **الاستنتاجات**: المستويات المرتفعة من هرمون الفاسوبريسين وقابليته على قبض الأوعية الدموية وإعادة امتصاص الصوديوم والماء يمكن ان يؤشر استمرار وادامة ارتفاع ضغط الدم أثناء التقدم بالعمر كما ان الكشف عن المستويات العالية من الاندوثيلين هو الاخر قد يؤشر التهابًا منخفض الدرجة أثناء ارتفاع ضغط الدم.

الكلمات المفتاحية: الفاسوبريسين، اندوثيلين-1، ألبومين المصل، البروتين الكلي.

1. INTRODUCTION:

Hypertension is an established risk factor for cardiovascular diseases, and its prevalence of hypertension among older adults is high (Shukuri *et al.,* 2019). Old people are more susceptible to several non-communicable diseases, such as cancer, diabetes, arthritis, hypertension, and several others (Munday *et al.,* 2019). Several factors contribute to regulating blood pressure, including vasoconstrictor and vasodilator agents, such as the vasopressin hormone (Patel and Ali, 2017). AVP is a potent neurohormone involved in the regulation of arterial blood pressure. The main stimuli that trigger AVP release include hyperosmolality, hypovolemia, hypotension, hypoxia, hypoglycemia, and strenuous exercise (Proczka *et al.,* 2021). AVP is secreted from the posterior pituitary gland in response to various triggers, such as high serum osmolarity, which acts on osmoreceptors in the hypothalamus, angiotensin II, low blood volume that causes a decreased stretch in the low-pressure baroreceptors, leading to the production of AVP, and low blood pressure causes a decreased stretch in the high-pressure baroreceptors, also leading to the production of AVP (Shahoud *et al.,* 2021).

Aging was accompanied by AVP elevation, which represents dysfunction of the vasopressin pathway and total peripheral resistance. It promotes the reabsorption of water through the vasopressin receptor (V2), besides the vasoconstriction enhancement via the vasopressin receptor (V1) (Tamma *et al.,* 2015). Furthermore, the major hormone is responsible for maintaining bodily water through the antidiuretic effects of the kidney, as noted by Gonzalez *et al*. (2020). On the other hand, aging and hypertension are considered low-grade inflammation and are

associated with some cytokines released, including interleukins and ET-1 (Bukowska *et al.,* 2022). ET-1 is a 21-amino acid peptide with broad biological activity linked to various disorders (Fagan *et al.,* 2001). ET-1 can raise blood pressure by disturbing some regulatory mechanisms and maintaining intravascular fluid volume (Speed *et al.,* 2015). In addition, ET-1 plays a vital role in maintaining intravascular volume by regulating the tubular reabsorption of water and electrolytes in the kidneys (Kohan *et al.,* 2011). It also interacts with other hormones like aldosterone (Andreis *et al.,* 2002), AVP, and natriuretic peptides to regulate fluid and electrolyte balance (Rossi, 2004).

The ET-1 is a powerful inflammatory mediator that may be crucial in inflammatory pulmonary disorders (Datta *et al.,* 2011). In several pathological processes, overstimulation of ET-1/ETA signaling may upset the balance in regulating blood pressure and may subsequently lead to hypertension (Kohan *et al.,* 2011). The ET-1 released associated with hypertension has downregulated angiotensin-converting enzyme (ACE2) expression and decreased its activity (Zhang *et al.,* 2013). Furthermore, ET-1 associated with progressive age (Yanes *et al.,* 2005; Yanes and Reckelhoff, 2011) mediated vasoconstriction tone that contributed to the pathogenesis of hypertension (Stauffer *et al.,* 2008).

On the other hand, albumin is the most prevalent protein circulating in human plasma (3.5-5 g/dL), synthesized by hepatocytes, and quickly released into the bloodstream at a rate of 10-15 gm/day. Although only a small amount is stored in the liver, albumin is crucial in regulating plasma osmotic pressure. It is a vital transporter of both endogenous and exogenous ligands, such as drugs (Chang and Holcomb, 2016). Høstmark *et al.,* 2005 noted that the decrease in serum albumin levels related to the possibility of injury with cardiovascular diseases during advanced age accompanied by blood pressure increment. Devi and Kumar 2012 mentioned a reverse correlation between advanced age and total serum proteins and their fractions (albumin and globulin) due to the low rate of hepatic protein synthesis.

This study aims to investigate the effects of hypertensive and progressive age on vasopressin, endothelin-1, serum albumin, and total protein values.

2. MATERIALS AND METHODS:

2.1 Materials

The current study was carried out from October 2021 to February 2022. The sample comprised eighty hypertensives men, divided into four groups of 20 men each, based on their ages: the 1st group (30-39 years), 2nd group (40-49 years), 3rd group (50-59 years), and 4th group (60-69 years). The individuals in the sample have been diagnosed with hypertension by a specialist physician following WHO criteria. However, individuals who were also diagnosed with diabetes, thyroid disease, heart and kidney failure, and tumors and those taking hormonal, antihypertensive drugs or smoking were excluded from the study. Serum AVP and serum ET-1 were assayed by using Elisa uno human GmbH SN 2950-5702 (Germany) by SUNLONG kit (china), serum albumin and total serum proteins assayed by BioSystems S.A. A15 automated. Costa Brava 30, 08030 Barcelona SN 831055272 (Spain).

Table 1: Materials provided with AVP and ET-1 kits

	User manual	**1**
	Closure plate membrane	**2**
	Microelisa stripplate	**96 well plate**
	Sealed bags	**1**
	Standard diluent	**1.5ml×1 bottle**
	Standard : 22.5pg/ml for AVP and 180pg/ml for ET-1	**0.5ml×1 bottle**
	Sample diluent	**6ml×1 bottle**
	HRP-Conjugate reagent	**6ml×1 bottle**
	wash solution	**20ml(30X) ×1 bottle**
	Stop solution	**6ml×1 bottle**
	Chromogen solution A	**6ml×1 bottle**
	Chromogen solution B	**6ml×1 bottle**

2.1.1. Total protein biosystems kit components

A. Reagent 10×50mL
B. Copper (II) acetate 6mmoL/L,
C. potassium iodide 12 mmoL/L,

D. sodium hydroxide 1.15 mol/L, and
E. detergent are the ingredients in the reagent.

2.1.2. Serum albumin (ALB) biosystems kit components

A. Reagent Bromocresol reagent. Succinate buffer 75 mmol/L pH 4.2, BCG 0.12 mmol/L, tensioactive 2 g/L (w/v) 5×50 Ml.
B. Acetate buffer 100 mmol/L
C. bromocresol green 0.27 mmol/L
E. detergent, pH 4.1.

2.2. Methods

Venous blood samples (8-10 mL) were drawn during the interval of 9 to 11 a.m. and left to clot for 20 minutes at room temperature to obtain serum, which was separated by centrifugation at 3000 rpm for 15 min to assay all parameters for the procedure of AVP and ET-1.

2.2. The Procedure of preparation steps for AVP and ET-1.

1. Dilution of the standard

   The standard was diluted by small tubes first, and then a 50 µl volume was pipetted from each tube to the microplate well. Each tube was used for two wells, comprising ten wells, as summarized in (tables 2 and 3). Figure1.

2. Microelisa Stripplate: An empty well was left as blank control in the microelisa stripplate. The samples were loaded onto the bottom without touching the well wall and mixed well with gentle shaking. In the sample wells, 40µl of the sample dilution buffer and 10µl sample were added (dilution factor is 5).

3. Incubation: After being sealed with a closure plate membrane, the plate was incubated for 30 minutes at 37℃.

4. Dilution: The concentrated washing buffer was diluted with distilled water (30 times for 96T and 20 times for 48T).

5. Washing: The closure plate membrane was peeled off, and the well was aspirated and refilled with a washing solution. The washing solution was discarded after resting for 30 seconds, and the washing procedure was repeated five times.

6. HRP-Conjugate Reagent: 50 µl HRP-Conjugate reagent was added to each well except the blank control well.

7. Incubation: The plate was incubated as described in Step 3.

8. Washing: The washing procedure was repeated as described in Step 5.

9. Coloring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mixed with gentle shaking, and incubated at 37℃ for 15 minutes. The plate was kept away from light during coloring.

10. Termination: 50 µl stop solution was added to each well to terminate the reaction. The stop solution was 0.16M sulfuric acid. The color of the well changed from blue to yellow.

11. Reading: The absorbance OD was read at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. The assay was carried out within 15 minutes after adding the stop solution.

2.2.2 Total Protein

Reagent preparation

The reagent and standard ware provided ready to use.

1. Pipette into labeled test tubes; the reagents are described in Table 2.

Table 2: The total protein reagents description

	Blank	Standard	Sample
Distilled water	20	----	----
Protein Standard (S)	---	20	----
Sample	---	----	20
Reagent (A)	1.0 mL	1.0 Ml	1.0 Ml

2. Mix thoroughly and let stand the tubes for 10 minutes at room temperature.

3. 3. Read the absorbance (A) of the Standard and the Sample at 545 nm against the Blank. The color is stable for at least 2 hours.

2.2.3. Serum albumin

1. Serum albumin was pipetted into labeled test tubes, and the reagents used are described in Table 3

Table 3: The Serum albumin reagents description

	Blank	Standard	Sample
Cc	**-----**	**10 µL**	**-----**
Albumin Standard (S)	**-----** **10Ml**	**1.0Ml**	**10 µL** **1.0Ml**
Sample Reagent (A)	**10ml**		

2. The reagents were mixed thoroughly and left stood the tubes for about 1 minute at room temperature.
3. Finally, the absorbance (A) of the standard and the sample was read at 630 nm against the Blank. The color was stable for 30 minutes.

2.2.4 Statistical Analysis

The statistical analysis was performed by one-way Analysis of Variance (ANOVA), as illustrated in Tables 4 and 5, followed by Duncan's test by using SPSS Statistics for the groups described in Tables 6, 7, 8, and 9 (Steel *et al.,* 1997).

3. RESULTS AND DISCUSSION:

3.1. RESULTS:

3.1.1. AVP

The AVP showed a significant increase in the second group (P ≤ 0.01, and 4.050 ±0.998 pg/ml), third (5.800 ±0.894pg/ml), and fourth groups (6.000 ±0.917pg/ml) compared to the first group (2.950 ±0.759pg/ml). Additionally, there was a significant increase in AVP levels in the third and fourth groups compared to the second group. Although there was a slight increase in the fourth group compared to the third group, it was not statistically significant, see Figure 2.

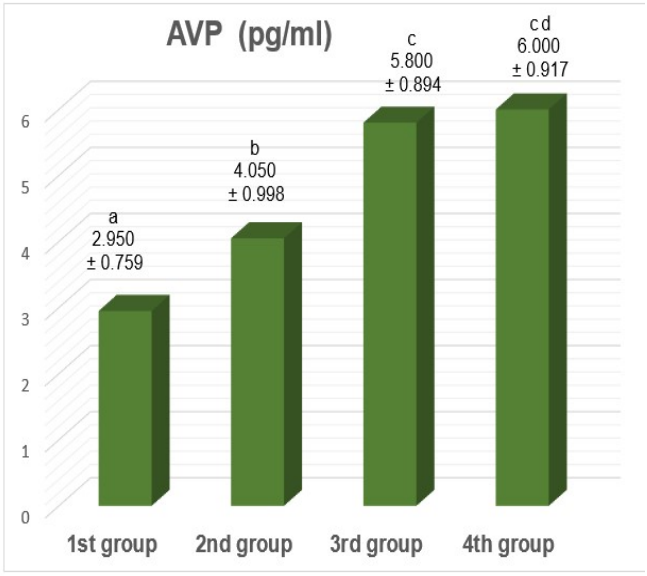

Figure 2: The levels of AVP hormone during different groups.

3.1.2. ET-1

There was a significant increase (p ≤ 0.01) in ET-1 levels in the second (26.250 ±0.966pg/ml), third (26.800 ±0.951pg/ml), and fourth groups (29.800 ±0.833pg/ml) compared to the first group (22.000 ±0.858pg/ml). Additionally, there was a non-significant increase in ET-1 levels in the third group compared to the second group. Moreover, there was a significant increase in ET-1 levels in the fourth group compared to both the second and third groups (Figure 3).

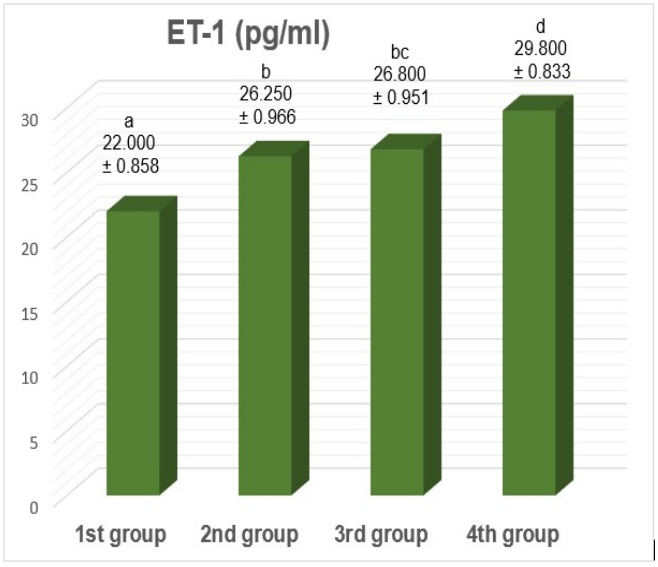

Figure 3: The levels of ET-1 during different groups.

3.1.2. Serum albumin (ALB)

The serum albumin decreased significantly ($p \leq 0.01$) in the second (43.650 ± 0.875 g/L), third (43.550 ± 0.998 g/L), and fourth groups (40.000 ± 0.858 g/L) in comparison with the first group (47.450 ± 0.998 g/L) and decreased not significantly in third group in comparison with the second group, in addition, decreased significantly in the fourth group in comparison with the second and third group (Figure 4).

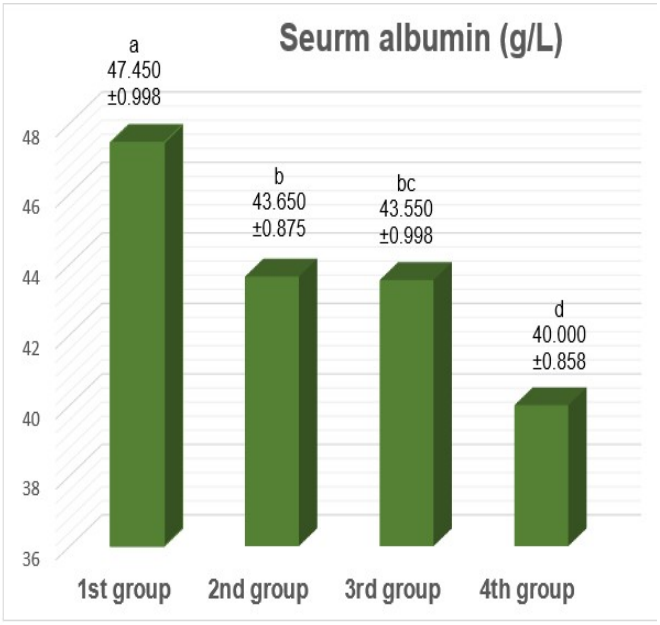

Figure 4: The levels of serum albumin during different groups.

3.1.2. Total Protein (T. P.)

Total protein decreased significantly ($p \leq 0.01$) in the second (70.850 ±0.988 g/L), third (67.000±0.917 g/L), and fourth groups (64.200 ±0.695 g/L) in comparison with the first group (73.450 ± 0.825 g/L) and decreased significantly in both third and fourth groups in comparison with the second group, in addition, decreased in the fourth group in contrast with the third group, as in Figure 5.

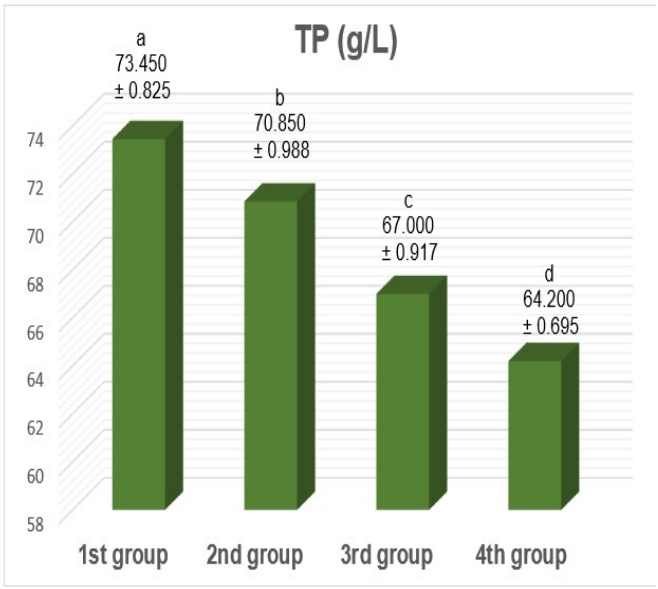

Figure 5: The levels of Total protein during different groups.

3.2. DISCUSSION

The present results revealed that AVP levels increased significantly ($p \leq 0.01$) in different groups (Figure 2), and high secretion of the ET-1 (Figure 3) probably caused these high levels of AVP.

These findings are in consent with the study of Yamamoto *et al.* (1991), Rossi (2004), Bukowska *et al.* (2022), which mentioned that the high levels of ET-1 caused a significant increase in AVP levels in hypertensive rats. Furthermore, the present high levels of ET-1 enhanced the release and inhibition of aldosterone and renin, thereby increasing a high level of AVP and blood pressure. Moreover, renin deficiency associated with hypertension and advanced age is considered the main cause of high levels of AVP production (Birder *et al.,* 2019; Yilmaz *et al.,* 2019; Kostov, 2021).

The present results revealed that ET-1 increased significantly ($p \leq 0.01$) in different groups, as described in Figure 3. AVP concentrations were higher in aged rats than in young adults, as reported by *Sauvant et al.,* 2014. Additionally, in hypertensive patients, AVP levels increased significantly due to dilutional hyponatremia caused by water retention without increased serum osmolality (Cowen *et al.,* 2013; Tamma *et al.,* 2015; Aleksandrowicz *et al.,* 2020).

Aging and hypertension are characterized by low-grade inflammation, oxidative stress, and endothelial dysfunction, which lead to increased

levels of inflammatory blood markers and cytokine release; this grade of inflammation may explain the high levels of ET-1 observed in Figure 3. Furthermore, the elevated levels of AVP are responsible for the ET-1 increment.

The present results agree with the findings of many authors who mentioned that aging and hypertension are considered low-grade inflammation; therefore, more inflammatory markers and cytokines released, such as ET-1 (Yanes *et al*. 2005; Puzianowska-Kuźnicka *et al*. 2016; Subhi *et al.,* 2019; Jankowich and Choudhary, 2020; Mesquita *et al.,* 2021). In addition, Avolio *et al.,* 2011 concluded that aging and inflammation might be the main causes of endothelial dysfunction and oxidative stress associated with arterial inflammation and age-related arterial remodeling.

Faramarzi and his coworkers 2012 mentioned that ET-1 levels increased significantly in elderly hypertensive persons due to the lack of proper functioning of endothelial cells, which led to endothelial dysfunction and alterations in ET-1 signaling pathways, releasing more ET-1. Spontaneously hypertensive rats have been shown to significantly increase ET-1 levels associated with endothelial dysfunction, inflammation, and oxidative stress (Bukowska *et al.,* 2022).

The results revealed that serum albumin and total protein levels decreased significantly in different groups ($p \leq 0.01$) (Figures 4 and 5). Both progressive age and hypertension, related to oxidative stress, endothelial dysfunctions, and inflammation, have been the major cause of these current changes. Besides that, the high levels of current ET-1 might be caused a decline in kidney and liver functions, thereby reducing serum albumin and total protein.

The present results agree with the studies of Komers and Plotkin 2016; Zou *et al.*, 2020, who mentioned that high levels of ET-1 have been shown to enhance glomerular albumin permeability, leading to proteinuria and reduced albumin levels both *in vitro* and *in vivo* in hypertensive patients and mice with the progressive age). Moreover, hypoalbuminemia (caused by synthesis reduction, high catabolism, vascular permeability, and renal and enteral losses of albumin concentrations) is associated with progressive age and hypertension (Arques and Ambrosi, 2011; Filippatos *et al.,* 2011; Arques, 2018; Cai *et al.,* 2021).

Furthermore, albumin deficiency played a pathological role in kidney performance and negatively changed the glomerular filtration rate (GFR), thereby causing high levels of aldosterone and hypertension occurrence (Pontremoli *et al.,* 2006; Catena *et al.,* 2017; Mirfakhraee *et al.,* 2021).

On the other hand, aging is associated with low serum albumin levels (hypoalbuminemia) in individuals with vascular endothelial dysfunction (Choi *et al.,*2021). Deficiency of albumin may act due to its osmotic effects as a risk factor for many cardiovascular diseases, such as hypertension in elderly people (Arques, 2018; Manolis *et al.,* 2022; Choi and Fernadndez, 2021).

Total protein and serum albumin decreased significantly in elderly hypertensive individuals due to high levels of Ang II, which promote ROS production and inflammation. In addition, the increased glomerular pressure caused hypertrophy and renal fibrosis, resulting in a vascular permeability increase, thus, an increase in urinary albumin excretion (Al-Najdi and Khalifa, 2021; Choi *et al.,* 2021; Myette *et al.,* 2021; Li *et al.,* 2022).

4. CONCLUSIONS:

Endothelin-1 represents an inflammatory biomarker for low-grade inflammation and other harmful effects, including age-related arterial remodeling and endothelial damage in hypertensive and elderly individuals. The lowest serum albumin and total protein levels might reflect renal and liver dysfunctions, proximal tubular reabsorption impairments, and increased glomerular permeability to albumin. High levels of aldosterone and AVP hormones, along with their abilities to reabsorb sodium and water, and constrict blood vessels, might indicate sustained and ongoing hypertension in those patients with progressive hypertension age.

5. DECLARATIONS

5.1. Study Limitations

This study is limited to the sample size and the analysis performed under experimental conditions.

5.2. Acknowledgements

I extend my thanks to Dr. Asad Yahea and Dr. Rashid Rahim Hateet for their help and support.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

There is no potential conflict of interest in this publication.

5.5. Open Access

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

Permission was issued to conduct this study by all health institutions in Maysan province and in the institute where this study was conducted. The Reference Number is: 325 / Dated in 22-11-2021.

6.2. Informed Consent

The consent of the participants to participate and publish this study was obtained.

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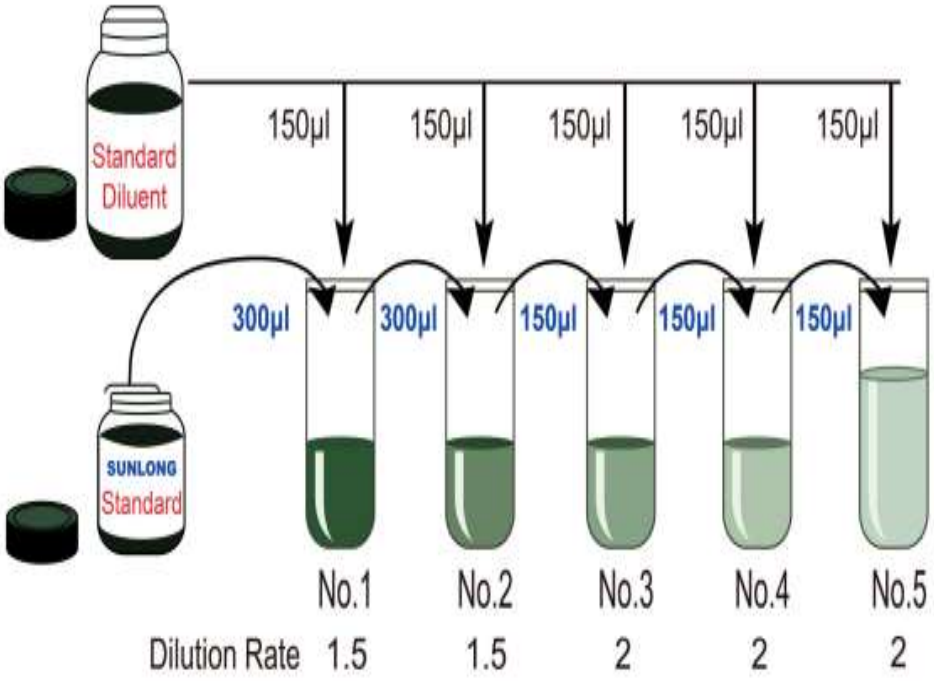

Figure 1. *Dilution steps for AVP and ET-1*

Source of the Figure 1: Human antidiuretic hormone (ADH) or Vasopressin (AVP) and ET-1 human ELISA Kit (SUNLONG)

Table 2: *The standard dilution for AVP*

Dilute the standard	**Serial of Standards**	**Dilution procedures**
15pg/ml	Standard No.1	300µl Original Standard +150µl Standard diluents
10pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
5pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
2.5pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
1.25pg/ml	Standard No.5	150µl Standard No.4+ 150µl Standard diluent

Table 3: *The standard dilution for ET-1*

Dilute the standard	**Serial of Standards**	**Dilution procedures**
120pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
80pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
40pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
20pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
10pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

Table 4: *The mean and standard deviation of AVP, ET1, ALB and T P.*

Parameters/groups		N	Mean	Std. Deviation
AVP	Group1	20	2.9500	.75915
	Group2	20	4.0500	.99868
	Group3	20	5.8000	.89443
	Group4	20	6.0000	.91766
	Total	80	4.7000	1.54592
ET1	Group1	20	22.0000	.85840
	Group2	20	26.2500	.96655
	Group3	20	26.8000	.95145
	Group4	20	29.8000	.83351
	Total	80	26.2125	2.93686
ALB	Group1	20	47.4500	.99868
	Group2	20	43.6500	.87509
	Group3	20	43.5500	.99868
	Group4	20	40.0000	.85840
	Total	80	43.6625	2.80571
T P	Group1	20	73.4500	.82558
	Group2	20	70.8500	.98809
	Group3	20	67.0000	.91766
	Group4	20	64.2000	.69585
	Total	80	68.8750	3.66432

N : The total number of patients in each group

Table 5: *(ANOVA) of AVP, ET-1, ALB, and T. P.*

Parameters		Sum of Squares	freedom degrees	Mean Square	Calculated F
AVP	Between Groups	127.700	3	42.567	52.947
	Within Groups	61.100	76	.804	
	Total	188.800	79		
ET1	Between Groups	619.238	3	206.413	252.411
	Within Groups	62.150	76	.818	
	Total	681.388	79		
ALB	Between Groups	555.438	3	185.146	211.754
	Within Groups	66.450	76	.874	
	Total	621.888	79		
T. P	Between Groups	1004.050	3	334.683	448.606
	Within Groups	56.700	76	.746	
	Total	1060.750	79		

Table 6: *Duncan of AVP*

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
1	20	2.9500		
2	20		4.0500	
3	20			5.8000
4	20			6.0000
Sig.		1.000	1.000	.483

N: The total number of patients in each group.

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.

Table 7: Duncan of ET-1

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
1	20	22.0000		
2	20		26.2500	
3	20		26.8000	
4	20			29.8000
Sig.		1.000	.058	1.000

N: The total number of patients in each group.

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.

Table 8 : Duncan of ALB

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
4	20	40.0000		
3	20		43.5500	
2	20		43.6500	
1	20			47.4500
Sig.		1.000	.736	1.000

N: The total number of patients in each group.

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.

Table 9: *Duncan of T. P.*

No. of groups	N	Subset for alpha = 0.01			
		1	2	3	4
4	20	64.2000			
3	20		67.0000		
2	20			70.8500	
1	20				73.4500
Sig.		1.000	1.000	1.000	1.000

N: The total number of patients in each group.

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.