PERIÓDICO TCHÊ QUÍMICA

ARTIGO ORIGINAL

A ATIVIDADE DA ENZIMA CONVERSORA DE ANGIOTENSINA 2 E OUTROS COMPONENTES DO SISTEMA RENINA-ANGIOTENSINA (RAAS) DURANTE O ENVELHECIMENTO PROGRESSIVO EM HOMENS HIPERTENSOS.

THE ACTIVITY OF ANGIOTENSIN-CONVERTING ENZYME 2 AND OTHER COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM (RAAS) DURING PROGRESSIVE AGE IN HYPERTENSIVE MEN

فعالية الانزيم المحول للانجيوتنسين 2 والمكونات الأخرى لنظام الرنين انجيوتنسين خلال التقدم بالعمر في الرجال المصابين بارتفاع ضغط الدم

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RESUMO

Introdução: A enzima conversora da angiotensina 2 (ECA2) e o sistema renina-angiotensina-aldosterona (RAAS) são sistemas fisiológicos complexos que contribuem para o controle da pressão arterial, enquanto a atividade e a responsividade do ECA2e RAAS podem ser alteradas devido à idade avançada e hipertensão. **Objetivos**: Este estudo tem como objetivo investigar os níveis de ECA2, Renina, Ang I, Ang II e aldosterona em vários grupos de homens hipertensos de diferentes idades, a fim de fornecer informações sobre o efeito do envelhecimento e da pressão arterial elevada em alguns órgãos do corpo. **Métodos**: Foram comparados os níveis de ECA2, Renina, Ang I, Ang II e aldosterona em 80 homens hipertensos com idades entre 30 e 69 anos. Os indivíduos foram divididos em quatro grupos (20 homens/grupo) de acordo com suas idades. O primeiro grupo tinha entre 30 e 39 anos, o segundo grupo entre 40 e 49 anos, o terceiro grupo entre 50 e 59 anos e o quarto grupo entre 60 e 69 anos. Foi utilizada uma análise de variância unidirecional (ANOVA), seguida do teste de Duncan para os grupos. **Resultados**: Foi observado que a concentração de ECA2diminuiu significativamente nos diferentes grupos com o avanço da idade ($p \leq 0,01$), exceto no quarto grupo amostral em relação ao terceiro grupo. A renina foi reduzida consideravelmente nos outros grupos com o envelhecimento ($p \leq 0,01$), exceto no segundo grupo amostral em relação ao primeiro grupo. Os hormônios peptídicos Angiotensina I (Ang I) e Angiotensina II (Ang II) apresentaram direções opostas de crescimento, diminuindo e aumentando, respectivamente, sendo estatisticamente significativos ($p \leq 0,01$). Além disso, a aldosterona aumentou significativamente nos diferentes grupos com o avanço da idade ($p \leq 0,01$), exceto no quarto grupo em relação ao terceiro grupo. **Discussões**: O impacto fisiológico desses resultados é discutido de acordo com os efeitos da hipertensão e da idade avançada em todos os parâmetros estudados, particularmente a deficiência de ECA2 e os altos níveis de Ang II, apontando para uma aparente disfunção na regulação da pressão arterial durante a idade avançada. **Conclusões**: A deficiência de ECA2 pode ser mediada por uma série de eventos disfuncionais causados pelas alterações presentes no RAAS e em outros parâmetros em resposta à hipertensão e à idade avançada. Baixos níveis de renina e outras alterações circulatórias podem causar um desequilíbrio das proporções de Ang I e Ang II. Além disso, altos níveis de Ang II podem refletir os aspectos desconfortáveis e negativos que contribuíram para a disrupção de muitos órgãos e sistemas durante a hipertensão e a idade avançada.

Palavras-chave: *ECA2, Renina, Angiotensina I, Angiotensina II, Aldosterona.*

ABSTRACT

Background: ACE2 and renin-angiotensin-aldosterone system (RAAS) are complex physiological systems that

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contribute to blood pressure control, whereas the activity and responsiveness of the ACE2 and RAAS be changed due to advanced age and hypertension. **Aim:** This study is to investigate the ACE2, Renin, Ang I, Ang II, and aldosterone levels in various ages of hypertensive men in order to provide insights into the effect of aging and high blood pressure on some body organs. **Methods**: It was compared ACE2, Renin, Ang I, Ang II, and aldosterone levels across 80 hypertensive men with ages between 30 years - 69 years. The persons were divided into four groups (20 men/group) according to their ages. The first group was 30-39 years, the second group was 40-49 years, the third group was 50-59 years, and the fourth group was 60-69. It used a one-way Analysis of Variance (ANOVA), followed by Duncan's test for the groups. **Results**: It was observed that the concentration of ACE2 decreased significantly in different groups with advanced age ($p \leq 0.01$), except in the fourth sample group vs. the third group. The renin was reduced considerably in other groups with aging ($p \leq 0.01$), except in the second sample group vs. the first group. The peptides Angiotensin I (Ang I) and Angiotensin II (Ang II) had opposite growth directions, decreasing and increasing, respectively, it was statistically significant ($p \leq 0.01$). Also, aldosterone increased significantly in different groups with advanced age ($p \leq 0.01$), not including the fourth group vs. the third group). **Discussion**: The physiological impact of these results is discussed according to the effects of hypertension and advanced age on all the studied parameters, particularly ACE2 deficiency and high levels of Ang II, pointed out an apparent dysfunction in blood pressure regulation during advanced age. **Conclusions**: ACE2 deficiency may be mediated by a series of dysfunctional events caused by the present changes in RAAS and other parameters in response to hypertension and advanced ages. Low renin levels and other following changes might cause an imbalance of Ang I and Ang II ratios. In addition, high levels of Ang II might reflect the unfavorable and negative changes that contributed to the disruption of many organs and systems during hypertension and advanced age.

Keywords: *ACE2, Renin, Angiotensin I, Angiotensin II, Aldosterone.*

الملخص

الخلفية: ACE2 ونظام رينين أنجيوتنسين - الألدوستيرون (RAAS) هما نظامان فسيولوجيان معقدان يساهمان في التحكم في ضغط الدم ، في حين تتغير فعالية واستجابة ACE2 و RAAS بسبب تقدم العمر وارتفاع ضغط الدم. **الهدف**: تهدف هذه الدراسة إلى التحقق من مستويات ACE2 و Renin و Ang I و Ang II والألدوستيرون في مختلف الأعمار للرجال المصابين بارتفاع ضغط الدم من أجل توفير نظرة ثاقبة حول تأثيرالتقدم بالعمر والشيخوخة وارتفاع ضغط الدم على بعض أعضاء الجسم. **الطرق:** تمت مقارنة مستويات ACE2 ، و Renin ، و Ang I ، و Ang II و aldosterone لدى 80 رجلاً يعانون من ارتفاع ضغط الدم تتراوح أعمارهم بين 30 عامًا - 69 عامًا, قسم الأشخاص إلى أربع مجاميع (20 رجل/ مجموعة) حسب أعمارهم. المجموعة الأولى تراوحت بين 30-39 سنة ، المجموعة الثانية 40-49 سنة ، المجموعة الثالثة 50-59 سنة ، المجموعة الرابعة 60-69 سنة. استخدم تحليل التباين أحادي الاتجاه (ANOVA) ، متبوعًا باختبار Duncan للمجاميع. **النتائج**: لوحظ أن تركيز ACE2 انخفض بشكل احصائي في المجاميع المختلفة مع تقدم العمر ($p \leq 0.01$) عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة. انخفض الرينين بشكل احصائي في المجاميع الأخرى مع تقدم العمر ($p \leq 0.01$) ، باستثناء المجموعة الثانية مقارنة بالمجموعة الأولى. كان لببتيدي Angiotensin I (Ang I) و Angiotensin II (Ang II) اتجاهات نمو متعاكسة ، تنخفض و ترتفع احصائيا ($p \leq 0.01$) على التوالي. أيضا ، ارتفع aldosterone بشكل احصائي في المجاميع المختلفة مع تقدم العمر ($p \leq 0.01$) عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة. **المناقشة**: تم مناقشة التأثير الفسيولوجي لهذه النتائج وفقًا لتأثيرات ارتفاع ضغط الدم والتقدم بالعمر على جميع المعلمات المدروسة وخاصة نقص ACE2 والمستويات المرتفعة من Ang II ، مما يشير إلى وجود خلل واضح في تنظيم ضغط الدم خلال التقدم بالعمر. **الاستنتاجات**: ان نقص ACE2 ربما توسط سلسلة من الأضطرابات المتسببة من التغيرات الحالية في RAAS وبقية المعلمات الناتجة من جراء ارتفاع ضغط الدم والتقدم بالعمر, وقد يؤدي انخفاض مستويات الرينين وما يتبعة من تغيرات متتابعة إلى عدم توازن نسب Ang I و Ang II اضافة إلى ذلك ، فقد تعكس المستويات العالية من Ang II التغيرات غير المرغوبة والسلبية التي ساهمت في اضطراب العديد من الاعضاء والأنظمة الجسمية أثناء ارتفاع ضغط الدم والتقدم بالعمر.

الكلمات المفتاحية: الانزيم المحول للانجيوتنسين 2, الرنين, انجيوتنسين1, انجيوتنسين2, الدوستيرون.

1. INTRODUCTION:

Hypertension is a severe medical condition diagnosed by systolic blood pressure readings (≥140 mmHg and diastolic blood pressure readings ≥ 90) mmHg. Hypertension is the leading cause of heart, brain, and kidney dysfunctions and other diseases. Moreover, hypertension is considered a significant cause of premature death worldwide and the risk of cardiovascular illnesses (WHO, 2021). It is also a main risk factor for the development of a dissecting aortic aneurysm, angina pectoris, left ventricular hypertrophy (LVH), thoracic and abdominal aortic aneurysms, chronic kidney disease (CKD), atrial fibrillation, diabetes mellitus (DM), vascular dementia and ophthalmologic disease (Goit and Yang 2019). Furthermore, ACE2 is an enzyme located in the cellular membrane of different human body organs, contributing to other factors in regulating blood pressure. It is considered as a main player in this field due to its detrimental low levels during advanced age (South *et al.,* 2020; Zou *et al.,* 2020).

In elderly people, Ang II could contribute to hypertension in either multiple systems atrophy or

pure autonomic failure, as this peptide increases sympathetic and vascular tone (Arnold *et al.,* 2013).

Furthermore, the Ang II-AT1 receptor axis increases with aging, providing a positive feedback loop in vascular inflammation via the recruitment of inflammatory cells in the injured artery, continuing to produce Ang II and vascular inflammation (Ferrario and Strawn, 2006; Conti *et al.,* 2012).

Aldosterone, the primary mineralocorticoid, is synthesized in the zone of the adrenal cortex zona glomerulosa (ZG), tightly regulated by Ang II and potassium levels (Hattangady *et al.,* 2012).

Aldosterone plays a crucial physiological role in regulating the intravascular volume and blood pressure by promoting sodium retention in the kidney. However, excessive aldosterone levels can lead to hypertension and increase the risk of cardiovascular complications (Savard *et al.,* 2013).

Ang II caused aldosterone production by the increased serum sodium and total fluid content (Sparks *et al.,* 2014).

Vasopressin is often released with aldosterone to facilitate water reabsorption into the extracellular fluid by activating aquaporin channels on the apical membrane of principal cells in the collecting tubule (Hall *et al.,* 2019). In addition, aldosterone secretion is triggered by Ang II type 1 receptors (AT1R) binding in the adrenocortical cells, and its response to Ang II is similar to that of vasopressin (Pang *et al.,* 2019).

In view of these controversies, this study attempts to shed some light on the role of ACE2 and RAAS components in hypertensive men during a progressive age

This study aims to investigate the effects of hypertension and progressive age on ACE2, Renin, Ang I, Ang II, and Aldosterone values.

2. MATERIALS AND METHODS:

2.1 Materials

The ACE2, Renin, Ang I, Ang II, and Aldosterone were assayed using Elisa uno human GmbH SN 2950-5702 (Germany) by SUNLONG kit (china). The current study was carried out from October 2021 to February 2022. The sample included eighty hypertensive men divided into four groups (20 men /group) as follows: 1st group, 30-39 years; 2nd group, 40-49 years; 3rd group, 50-59 years; and 4th group, 60-69 years.

Sample individuals have been diagnosed with hypertension and checked medically by a specialist physician according to WHO criteria (hypertension is diagnosed if, when it is measured on two different days, the systolic blood pressure readings on both days are ≥140 mmHg and the diastolic blood pressure readings on both days is ≥90 mmHg). Some of these individuals were excluded due to their attack by diabetes, thyroid disease, heart, and kidney failure, tumors, and those taking hormonal or antihypertensive drugs and smoking.

Table 1: Materials provided with ACE2, Renin, Ang I, Ang II, and ALD ELISA Kit components.

	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Sealed bags	1
4	Microelisa stripplate	96 well plate
5	Standard: 270pg/ml for ACE2 and Ang I 540pg/ml for Renin, Ang II and Aldosterone	0.5ml×1 bottle
6	Standard diluent	1.5ml×1 bottle
7	HRP-Conjugate reagent	6ml×1 bottle
8	Sample diluent	6ml×1 bottle
9	Chromogen Solution A	6ml×1 bottle
10	Chromogen Solution B (Hydroxylysine (Hyl) is an amino acid which arises from a post-translational hydroxy modification of lysine)	6ml×1 bottle
11	Stop Solution	6ml×1 bottle
12	wash solution	20ml(30X) ×1 bottle

2.2. Methods

2.2.1 Blood Samples Collection:

Venous blood samples (8-10 mL) were drawn at 9 - 11 a.m. the blood samples were left for 20 minutes to clot at room temperature to get the serum which was separated by centrifugation at 3000 rpm for 15 min, to assay all the parameters for the current study, serum was transferred into labeled plain tube and stored at -20 C˚until used for evaluation of the current study parameters.

2.2.2 The Procedure of preparation steps for ACE2, Renin, Ang I, Ang II, and Aldosterone.

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).

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

2.2.3 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 for the groups described in tables 6, 7, 8, 9, and 10 (Steel *et al.,* 1997).

3. RESULTS AND DISCUSSION:

3.1 RESULTS

3.1.1. ACE2

ACE2 decreased significantly ($P \leq 0.01$) in the second (7.200 ±0.695 pg/ml), third (6.050 ±0.944 pg/ml), and fourth groups (5.950 ±0.887 pg/ml) in comparison with the first group (12.450 ±0.887 pg/ml). ACE2 decreased significantly in both the third and fourth groups in comparison with the second group and decreased not significantly in the fourth group in comparison with the third group. (Figure 2).

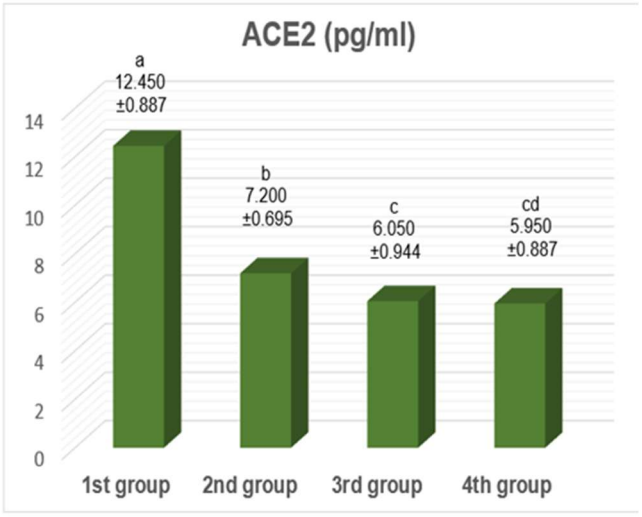

Figure 2: The levels of ACE2 enzyme during different groups.

- The values represent mean ± SD.
- Different small letters represent significant differences ($p \leq 0.01$) between groups.
- Similar small letters represent no significant differences between groups.

3.1.2. RENIN

The renin levels showed a significant decrease ($p \leq 0.01$) in the third (13.000 ±0.858pg/ml) and fourth groups (11.800 ±0.951pg/ml) and a non-significant decrease in the second group (16.850 ±0.933pg/ml) compared to the first group (17.100 ± 0.911pg/ml). Moreover, renin levels decreased significantly in both the third and fourth groups compared to the second group. There was a significant decrease in the fourth group compared to the third group, as in Figure 3.

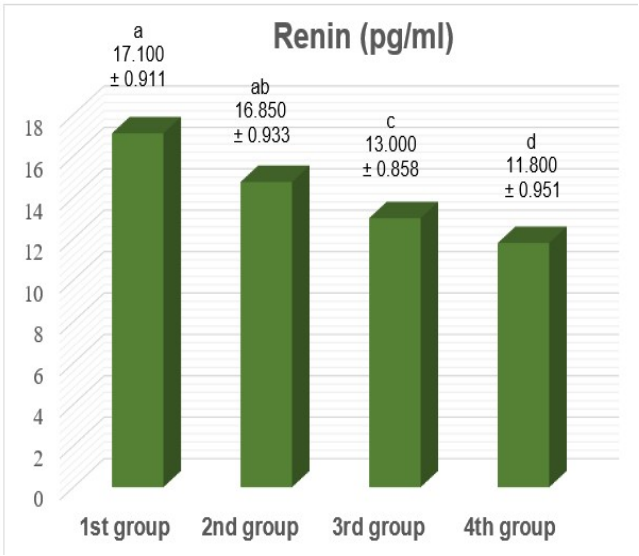

Figure 3: The levels of Renin enzyme during different groups.

- The values represent mean ± SD.
- Different small letters represent significant differences ($p \leq 0.01$) between groups.
- Similar small letters represent no significant differences between groups.

3.1.3. ANG I

Ang I levels decreased significantly ($p \leq 0.01$) in the second (23.800 ±0.833pg/ml), third (19.950 ±0.887pg/ml), and fourth groups (18.800 ±0.894pg/ml) compared to the first group (24.850 ±0.812pg/ml). Furthermore, Ang I levels decreased significantly in both the third and fourth groups compared to the second group. Additionally, a significant decrease in Ang I was observed in the fourth group when compared to the third group, according to Figure 4.

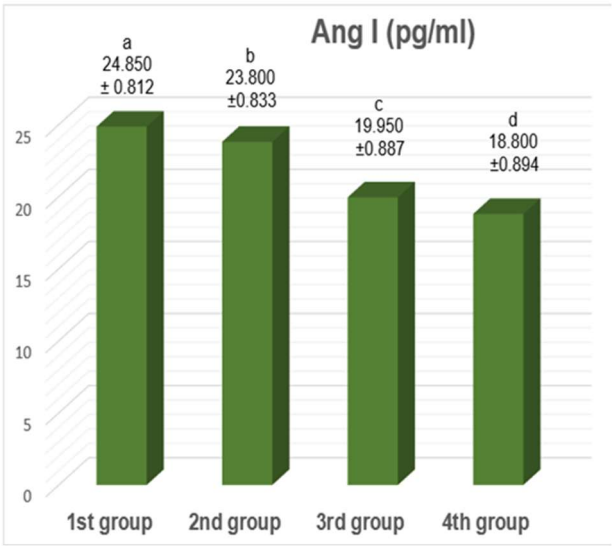

Figure 4: The levels of Ang I during different groups.

- The values represent mean ± SD.
- Different small letters represent significant differences ($p \leq 0.01$) between groups.

3.1.3. ANG II

The concentration of Ang II showed significant increases ($p \leq 0.01$) in the second (21.300 ± 0.923 pg/ml), third (27.850 ± 0.933 pg/ml), and fourth groups (31.500 ± 0.945 pg/ml) compared to the first group (17.800 ± 0.894 pg/ml). Furthermore, the third and fourth groups exhibited significantly higher Ang II levels than the second group. Notably, the fourth group demonstrated a significant increase in Ang II concentration compared to the third group, as in Figure 5.

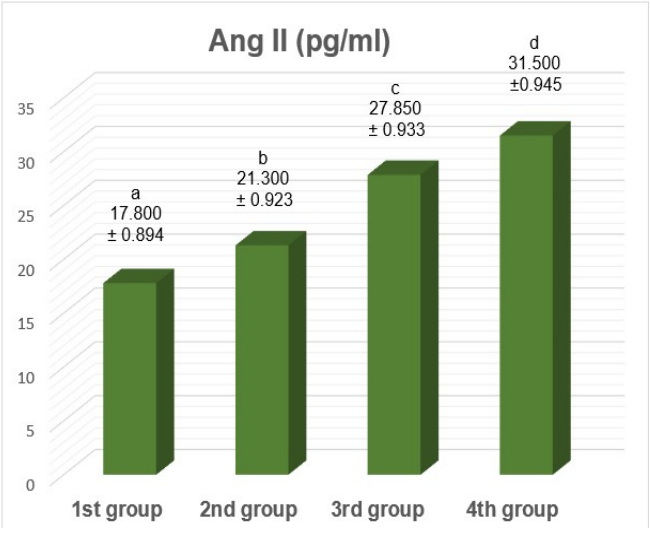

Figure 5: The levels of Ang II during different groups.

- The values represent mean ± SD.

- Different small letters represent significant differences ($p \leq 0.01$) between groups.

3.1.4. ALDOSTERONE

Aldosterone levels increase significantly ($p \leq 0.01$) in the second (18.250 ± 0.910 pg/ml), third (37.700 ± 0.864 pg/ml), and fourth (37.900 ±0.911 pg/ml) groups, compared to the first group (16.200 ± 0.894 pg/ml). Significant increases were observed in the third and fourth groups compared to the second group. However, there was no significant increase in the fourth group compared to the third group, presented in Figure 6.

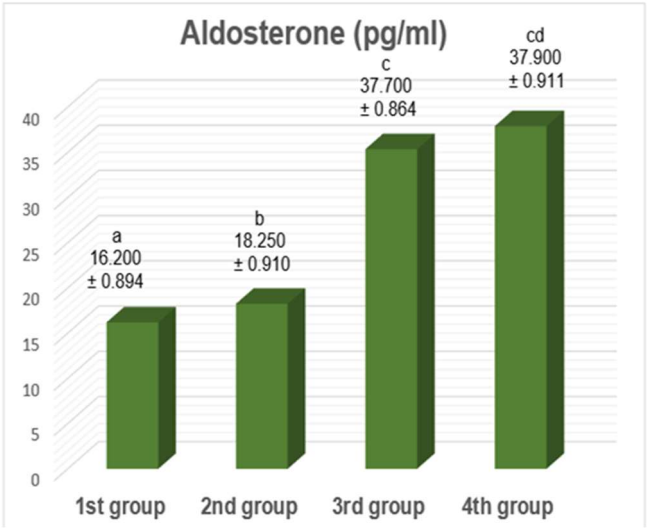

Figure 6: The levels of Aldosterone hormone during different groups.

- The values represent mean ± SD.
- Different small letters represent significant differences ($p \leq 0.01$) between groups.
- Similar small letters represent no significant differences between groups

33.2. DISCUSSION

The present results revealed that ACE2 decreased significantly ($p \leq 0.01$) in different groups (Figure 2). The deficiency of the present enzyme can be attributed to the impacts of oxidative stress production and hypertension, which may occur or worsen due to advanced age and aging. This oxidative stress production and endothelial dysfunction are further exacerbated by the high levels of Ang II present during progressive aging (Figure 4) and by the ACE2 deficiency, knowing that this ACE2 enzyme may be a protective agent against the effects of Ang II and its detrimental changes by converting Ang II to Ang (1–7). In addition, the current deficiency of ACE2 during advanced age may be caused by the Ang II action via its triggered cleavage of ACE2.

The current findings concerning the impact of oxidative stress, hypertension following ACE2 deficiency, and high levels of Ang II are in consistent with the study of Liguori *et al.* (2018), Luo *et al. (2020),* Tain and Hsu (2022), who considered that oxidative stress is an essential factor in endothelial damage, vascular dysfunction, cardiovascular remodeling, and the pathophysiology of hypertension. Oxidative stress leads to impaired hypertension-coordinated regulatory systems. Similarly, Wang *et al.,* 2021; Gu *et al.,* 2021 mentioned that ACE2 levels reduced significantly during advanced age and aging by creating a proinflammatory change in renin-angiotensin signaling caused by the high levels of Ang II that be detrimental to vascular and endothelial factors. In the same line as the present results, Basu *et al*. (2017) and Bártová *et al.* (2020) showed that patients with hypertension and ACE2 deficiency were associated with the upregulation of Ang II levels and downregulation of Ang 1-7.

The present reduction in renin levels, as in Figure 3, may be explained via excessive oxidative stress, hypertension, and advanced age, enhanced by the current high levels of Ang II (Figure 5), thereby stimulating aldosterone secretion, which enhances electrolyte reabsorption, particularly sodium, and promotes water retention. Oxidative stress may mainly contribute to RAAS functions, specifically in renin decline and increased Ang II.

Also, Jang *et al.,* 2018 found that renin levels decreased and Ang II increased due to oxidative stress production and impaired tissue function in elderly hypertensive individuals. Moreover, Peti-Peterdi and Harris (2010); Quadri *et al.* (2016) agree with the present results about the role of Ang II in suppressing renin synthesis and releases via a negative feedback mechanism.

On the other hand, the present findings revealed that Ang I decreased significantly ($p \leq 0.01$) in different groups (Figure 4). This deficiency may be explained by the present significant decline of renin levels during progressive age, as in Figure 3. Secondly, the possible influence of progressive age, hypertension, oxidative stress, endothelial dysfunction, and inflammation on ACE increment

activity. Thirdly, the negative feedback mechanism by the present Ang II (Figure 5).

Studies are along the same lines and ideas about the role of hypertension, advancing age, and oxidative stress and their impact on both renin and Ang I deficiency, such as the study by Amraei and Rahimi (2020) that renin converts angiotensinogen to Ang I, therefore, the deficiency in renin levels may also lead to Ang I reduction. Zhu *et al.* (2020) showed that aging, hypertension, oxidative stress, endothelial dysfunction, and inflammation indirectly influence ACE activity positively. Therefore, AngI is converted to Ang II by ACE.

On the other hand, it is worth mentioning that the current high levels of Ang II are a sine qua non to explain and discuss the findings of this study. Ang II (Figure 5) results from aging, hypertension, oxidative stress, and inflammation. It has a detrimental impact on some current findings, such as ACE2, renin, and Ang I (Figure s 2, 3, 4). Many others concluded the same results, Chen *et al.,* 2020; Cook and Ausiello, 2021 found that Ang II levels increased with advanced age and hypertension due to ACE2 deficiency, whereas this enzyme was responsible for the conversion of Ang II to Ang 1-7, which led to a decrease in Ang 1-7. Also, the Ang 1-7 age-related deficiency is associated with a concomitant enhanced Ang II production, moreover, Ang II increased in aging and hypertensive mice related to oxidative stress, endothelial dysfunction, and inflammation (Jia *et al.,* 2019; Birk *et al.,* 2021).

On the other hand, the present elevation of aldosterone may be attributed to the current increases in the Ang II (Figure s 5) and decreases in renin (Figure 3) associated with advanced age, hypertension, and inflammation in the study sample. These findings agree with the studies of Barrett *et al.* (2019); Rieder *et al.* (2021), who showed that the elevation of aldosterone in hypertensive patients might be caused due to the high levels of Ang II that positively correlated with the synthesis and secretion of this hormone. Moreover, Rajamohan *et al.* (2012) found that high aldosterone levels are associated with oxidative stress caused by the increment of Ang II that can facilitate or attenuate reactive oxygen species (ROS) production or degradation, respectively, in hypertensive men and rats.

4. CONCLUSIONS:

ACE2 deficiency may be considered a key player in the detrimental changes in RAAS in response to hypertension and progressive age. The renin-angiotensin-aldosterone system changes unfavorable, including low renin levels and high levels of both Ang II and aldosterone, simultaneously reflected these negative changes via their abilities to reabsorption of water and blood vessels contractions, which might be contributed to the disruption of many organs and systems during hypertension and progressive 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. Rashid Rahim Hateet and Dr. Asad Yahea for their help and support.

5.3. Funding source

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5.4. Competing Interests

There is no potential conflict of interest in this publication.

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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 was obtained to publish this study.

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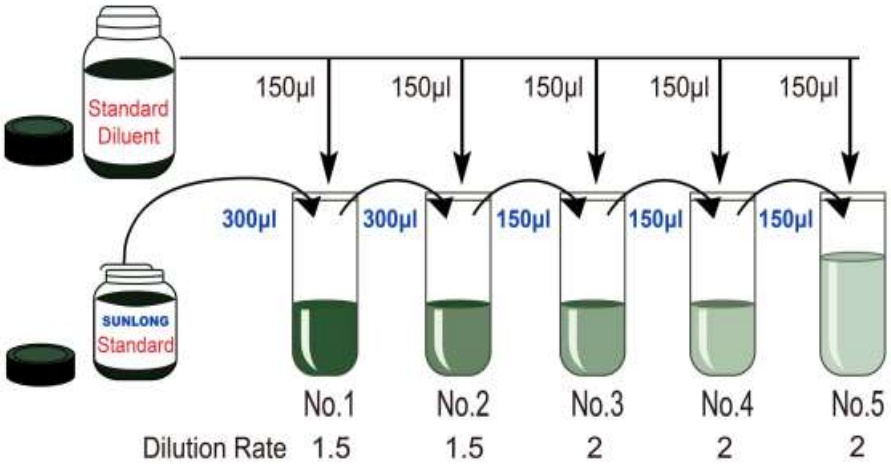
7. REFERENCES:

1. Amraei, R., & Rahimi, N. (2020). COVID-19, renin-angiotensin system and endothelial dysfunction. Cells, 9(7), 1652. DOI: 10.3390/cells9071652.
2. Arnold, A. C., Okamoto, L. E., Gamboa, A., Shibao, C., Raj, S. R., Robertson, D., & Biaggioni, I. (2013). Angiotensin II, independent of plasma renin activity, contributes to the hypertension of autonomic failure. Hypertension, 61(3), 701-706. DOI:10.1161/HYPERTENSIONAHA.111.00377.
3. Barrett, K. M., Barman, S. M.,Brooks, H. L. and Yuan, J. X. J. (2019).Ganong's Review of Medical Physiology. A lange medical book. 26 edt, New York, Chicago, San Francisco, Athens, London, Madrid, Mexico City, Milan, New Delhi, Singapore, Sydney and Toronto: by McGraw-Hill Education. pp 1606-1614.
4. Bártová, E., Legartová, S., Krejčí, J., & Arcidiacono, O. A. (2020). Cell differentiation and aging accompanied by depletion of the ACE2 protein. Aging (Albany NY), 12(22), 22495. DOI: 10.18632/aging.202221.
5. Basu, R., Poglitsch, M., Yogasundaram, H., Thomas, J., Rowe, B. H., & Oudit, G. Y. (2017). Roles of angiotensin peptides and recombinant human ACE2 in heart failure. Journal of the American College of Cardiology, 69(7), 805-819. DOI: 10.1016/j.jacc.2016.11.064.
6. Birk, M., Baum, E., Zadeh, J. K., Manicam, C., Pfeiffer, N., Patzak, A., ... & Gericke, A. (2021). Angiotensin II induces oxidative stress and endothelial dysfunction in mouse ophthalmic arteries via involvement of AT1 receptors and NOX2. Antioxidants, 10(8), 1238. https://doi.org/10.3390/antiox10081238 .
7. Chen, J., Jiang, Q., Xia, X., Liu, K., Yu, Z., Tao, W., ... & Han, J. D. J. (2020). Individual variation of the SARS-CoV-2 receptor ACE2 gene expression and regulation. Aging cell, 19(7), e13168. DOI: 10.1111/acel.13168.
8. Conti, S., Cassis, P., & Benigni, A. (2012). Aging and the renin-angiotensin system. Hypertension, 60(4),878-883. DOI:10.1161/HYPERTENSIONAHA.110.155895.
9. Cook, J. R., & Ausiello, J. (2022). Functional ACE2 deficiency leading to angiotensin imbalance in the pathophysiology of COVID-19. Reviews in Endocrine and Metabolic Disorders, 23(2), 151-170. https://doi.org/10.1007/s11154-021-09663-z .
10. Fang, L., Karakiulakis, G., & Roth, M. (2020). Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? The lancet respiratory medicine, 8(4), e21. https://doi.org/10.1016.
11. Ferrario, C. M., & Strawn, W. B. (2006). Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. The American journal of cardiology, 98(1), 121-128. DOI: 10.1016/j.amjcard.2006.01.059.
12. Goit, L. N. and Yang, S. (2019). Treatment of Hypertension: A Review. Yangtze Medicine, 3(2): 101-123.
13. Gu, J., Yin, J., Zhang, M., Li, J., Wu, Y., Chen, J., & Miao, H. (2021). Study on the clinical significance of ACE2 and its age-related expression. Journal of Inflammation Research, 14, 2873. https://doi.org/10.2147.
14. Guignabert, C., de Man, F., & Lombès, M. (2018). ACE2 as therapy for pulmonary arterial hypertension: the good outweighs the bad. European Respiratory Journal, 51(6). DOI: 10.1183/13993003.00848-2018.
15. Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E. (2019). Obesity, kidney dysfunction, and hypertension: mechanistic links. Nature reviews nephrology, 15(6), 367-385. DOI: 10.1038/s41581-019-0145-4.
16. Hattangady, N. G., Olala, L. O., Bollag, W. B., & Rainey, W. E. (2012). Acute and chronic regulation of aldosterone production. Molecular and cellular endocrinology, 350(2), 151-162. DOI: 10.1016/j.mce.2011.07.034.
17. Hurr, C., & Young, C. N. (2016). Neural control of non-vasomotor organs in hypertension. Current hypertension reports, 18, 1-11. DOI:10.1007/s11906-016-0635-8.
18. Hurr, C., & Young, C. N. (2016). Neural control of non-vasomotor organs in hypertension. Current hypertension reports, 18, 1-11. DOI:10.1007/s11906-016-0635-8.
19. Jang, I. A., Kim, E. N., Lim, J. H., Kim, M. Y., Ban, T. H., Yoon, H. E., Park, C. W., Chang, Y. S., & Choi, B. S. (2018). Effects of Resveratrol on the Renin-Angiotensin System in the Aging Kidney. Nutrients, 10(11), 1741. https://doi.org/10.3390/nu10111741.
20. Kuriakose, J., Montezano, A. C., & Touyz, R.

M. (2021). ACE2/Ang-(1-7)/Mas1 axis and the vascular system: vasoprotection to COVID-19-associated vascular disease. Clinical Science, 135(2), 387-407. https://doi.org/10.1042/CS20200480 .
21. Laghlam, D., Jozwiak, M., & Nguyen, L. S. (2021). Renin–angiotensin–aldosterone system and immunomodulation: A state-of-the-art review. Cells, 10(7), 1767. DOI: 10.3390/cells10071767.
22. Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., ... & Abete, P. (2018). Oxidative stress, aging, and diseases. Clinical interventions in aging, 757-772. https://doi.org/10.2147/CIA.S158513.
23. Luo, J., Mills, K., le Cessie, S., Noordam, R., & van Heemst, D. (2020). Ageing, age-related diseases and oxidative stress: what to do next? Ageing research reviews, 57, 100982. DOI: 10.1016/j.arr.2019.100982.
24. Ma, L., Song, Y., Mei, M., He, W., Hu, J., Cheng, Q., ... & Yang, S. (2018). Age-related cutoffs of plasma aldosterone/renin concentration for primary aldosteronism screening. International Journal of Endocrinology, 2018. https://doi.org/10.1155/2018/8647026.
25. Pang, H. W., Linares, A., Couling, L., Santollo, J., Ancheta, L., Daniels, D., & Speth, R. C. (2019). Novel high molecular weight albumin-conjugated angiotensin II activates β-arrestin and G-protein pathways. Endocrine, 66, 349-359. https://doi.org/10.1155/2018/8647026.
26. Peti-Peterdi, J., & Harris, R. C. (2010). Macula densa sensing and signaling mechanisms of renin release. Journal of the American Society of Nephrology, 21(7), 1093-1096. DOI:10.1681/ASN.2009070759.
27. Quadri, S. S., Culver, S. A., Li, C., & Siragy, H. M. (2016). Interaction of the renin-angiotensin and cox systems in the kidney. Frontiers in bioscience (Scholar edition), 8, 215.
28. Rajamohan, S. B., Raghuraman, G., Prabhakar, N. R., & Kumar, G. K. (2012). NADPH oxidase-derived H2O2 contributes to angiotensin II-induced aldosterone synthesis in human and rat adrenal cortical cells. Antioxidants & redox signaling, 17(3), 445-459. DOI: 10.1089/ars.2011.4176.
29. Rieder, M., Wirth, L., Pollmeier, L., Jeserich, M., Goller, I., Baldus, N., ... & Lother, A. (2021). Serum ACE2, angiotensin II, and aldosterone levels are unchanged in patients with COVID-19. American journal of hypertension, 34(3), 278-281. DOI: 10.1093/ajh/hpaa169.
30. Savard, S., Amar, L., Plouin, P. F., & Steichen, O. (2013). Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. Hypertension, 62(2),331-336. DOI:10.1161/HYPERTENSIONAHA.113.01060.
31. South, A. M., Brady, T. M., & Flynn, J. T. (2020). ACE2 (angiotensin-converting enzyme 2), COVID-19, and ACE inhibitor and Ang II (angiotensin II) receptor blocker use during the pandemic: the pediatric perspective. Hypertension, 76(1), 16-22. DOI: 10.1161/HYPERTENSIONAHA.120.15291.
32. Sparks, M. A., Crowley, S. D., Gurley, S. B., Mirotsou, M., & Coffman, T. M. (2014). Classical renin-angiotensin system in kidney physiology. Comprehensive Physiology, 4(3), 1201. DOI: 10.1002/cphy.c130040.
33. Spence, J. D. (2018). The Spectrum of Subclinical Primary Aldosteronism and Incident Hypertension. Annals of Internal Medicine, 168(10): 753-754.
34. Tain, Y. L., & Hsu, C. N. (2022). Oxidative stress-induced hypertension of developmental origins: preventive aspects of antioxidant therapy. Antioxidants, 11(3), 511. https://doi.org/10.3390/antiox11030511.
35. Wang, A., Tian, X., Zuo, Y., Wang, X., Xu, Q., Meng, X., ... & Wang, Y. (2021). High lactate dehydrogenase was associated with adverse outcomes in patients with acute ischemic stroke or transient ischemic attack. Ann Palliat Med, 10(10), 10185-95.95 | https://dx.doi.org/10.21037.
36. Wang, G., Jing, J., Li, J., Pan, Y., Yan, H., Meng, X., ... & Wang, Y. (2021). Association of elevated hs-CRP and multiple infarctions with outcomes of minor stroke or TIA: subgroup analysis of CHANCE randomised clinical trial. Stroke and Vascular Neurology, 6(1). DOI: 10.1136/svn-2020-000369.
37. World Health Organization (2021) Hypertension https://wwwwhoint/news-room/fact-sheets/detail/hypertension.
38. Zhu, Z., Cai, T., Fan, L., Lou, K., Hua, X., Huang, Z., & Gao, G. (2020). The potential role of serum angiotensin-converting enzyme in coronavirus disease 2019. BMC Infectious Diseases, 20, 1-8. https://doi.org/10.1186/s12879-020-05619-x.
39. Zou, X., Chen, K., Zou, J., Han, P., Hao, J., & Han, Z. (2020). Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Frontiers of medicine, 14, 185-192. DOI: 10.1007/s11684-020-0754-0.



(Figure 1). *Dilution steps for ACE2, Renin, Ang I, Ang II and ALD*

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

Table 2 :For ACE2 and Ang I

Standards Density	Standards Numbers	Dilution Stepes
180pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
120pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
60pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
30pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
15pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

Table 3: For Renin, Ang II, and ALD

Standards Density	Standards Numbers	Dilution Stepes
360pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
240pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
120pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
60pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
30pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

Table 4: The mean and standard deviation of ACE2, Renin, Ang I, Ang II, and Aldosterone.

		N	Mean	Std. Deviation	Std. Error
ACE2	1.00	20	12.4500	.88704	.19835
	2.00	20	7.2000	.69585	.15560
	3.00	20	6.0500	.94451	.21120
	4.00	20	5.9500	.88704	.19835
	Total	80	7.9125	2.81134	.31432
Ang I	1.00	20	24.8500	.81273	.18173
	2.00	20	23.8000	.83351	.18638
	3.00	20	19.9500	.88704	.19835
	4.00	20	18.8000	.89443	.20000
	Total	80	21.8500	2.68658	.30037
Ang II	1.00	20	17.8000	.89443	.20000
	2.00	20	21.3000	.92338	.20647
	3.00	20	27.8500	.93330	.20869
	4.00	20	31.5000	.94591	.21151
	Total	80	24.6125	5.47837	.61250
Renin	1.00	20	17.1000	.91191	.20391
	2.00	20	16.8500	.93330	.20869
	3.00	20	13.0000	.85840	.19194
	4.00	20	11.8000	.95145	.21275
	Total	80	14.6875	2.50869	.28048
ALD	1.00	20	16.2000	.89443	.20000
	2.00	20	18.2500	.91047	.20359
	3.00	20	37.7000	.86450	.19331
	4.00	20	37.9000	.91191	.20391
	Total	80	27.5125	10.41541	1.16448

N : The number of repetitions in each group

Table 5: (ANOVA) of ACE2, Renin, Ang I, Ang II and ALD

Parameters		Sum of Squares	freedom Degrees	Mean Square	F
ACE2	Between Groups	568.337	3	189.446	256.876
	Within Groups	56.050	76	.737	
	Total	624.387	79		
AngI	Between Groups	514.300	3	171.433	233.076
	Within Groups	55.900	76	.736	
	Total	570.200	79		
AngII	Between Groups	2306.038	3	768.679	899.455
	Within Groups	64.950	76	.855	
	Total	2370.987	79		
Renin	Between Groups	433.638	3	144.546	172.864
	Within Groups	63.550	76	.836	
	Total	497.188	79		
ALD	Between Groups	8509.038	3	2836.346	3536.707
	Within Groups	60.950	76	.802	
	Total	8569.988	79		

Table 6: Duncan for ACE2

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
4	20	5.9500		
3	20	6.0500		
2	20		7.2000	
1	20			12.4500
Sig.		.714	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.

Table 7: Duncan for Renin

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
4	20	11.8000		
3	20		13.0000	
2	20			16.8500
1	20			17.1000
Sig.		1.000	1.000	.390

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 for Ang I

No. of groups	N	Subset for alpha = 0.01			
		1	2	3	4
4	20	18.8000			
3	20		19.9500		
2	20			23.8000	
1	20				24.8500
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.

Table 9: Duncan for Ang II

No. of groups	N	Subset for alpha = 0.01			
		1	2	3	4
1	20	17.8000			
2	20		21.3000		
3	20			27.8500	
4	20				31.5000
Sig.		1.000	1.000	1.000	1.000

N: The total number of patients in each group (Sample Size).

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Table 10: Duncan for ALD

No. of groups	N	Subset for alpha = 0.01		
		1	2	3
1	20	16.2000		
2	20		18.2500	
3	20			37.7000
4	20			37.9000
Sig.		1.000	1.000	.482

N: The total number of patients in each group

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.