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Activity of RAS and some Biochemical and Hormonal Levels in Hypertensive Men at Different Age Groups

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{ واللَّهُ أَخْرَجَكُمْ مِّنْ بُطُونِ أُمَّهَاتِكُمْ لَا تَعْلَمُونَ شَيْئاً وَجَعَلَ لَكُمُ السَّمْعَ وَالأَبْصارَ والافْئِدَةَ لَعَلَّكُمْ تَشْكُرُونَ}

صدق الله العلي العظيم

سورة النحل، الآية (٧٨)

I certify that this thesis entitled "Activity of RAS and some Biochemical and Hormonal Levels in Hypertensive Men at Different Age Groups "has been prepared under our supervision at the college of science, university of misan; as a partial fulfillment of the requirements for the degree of master of biology.

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Recommendation of Head of Biology Department

In view the available recommendations; I forward this thesis debate by examining committee.

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Dedication

I would like to dedicate my work:

To my parents

To my husband, who has always been supporting and encouraging me

To my lovely children: Sajjad, Yousif, Hussein and Iham

To my brothers and sisters

To science, pioneers and students

I dedicate my modest effort

Acknowledgment

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Methaq

Summary

The present study aimed to investigate the role of ACE2, RAAS system and some biochemical and hormonal parameters in hypertensive men with increying age in Maysan province. The sample included eighty hypertensive men aged (30-69) years who visited Al-Sadder Teaching Hospital and some clinical centers, divided into four groups (20 men / group) according to their ages as the following:

- First group: 30-39 years.
- Second group: 40-49 years.
- Third group: 50-59 years.
- Fourth group: 60-69 years.

The present results revealed that:

1- Angiotensin converting enzyme 2 decreased significantly ($P \leq 0.01$) (except fourth group vs third group) in different groups with progressive age.
2- Renin decreased significantly ($P \leq 0.01$) (except second group vs first group) in different groups with progressive age.
3- Angiotensin I decreased significantly ($P \leq 0.01$) in different groups with progressive age.
4- Angiotensin II increased significantly ($P \leq 0.01$) in different groups with progressive age.
5- Aldosterone increased significantly ($P \leq 0.01$) (except fourth group vs third group) in different groups with progressive age.
6- Vasopressin increased significantly ($P \leq 0.01$) (except fourth group vs third group) in different groups with progressive age.

7- Endothelin -1 increased significantly ($P \leq 0.01$) (except third group vs second group) in different groups with progressive age.

8- Total protein decreased significantly ($P \leq 0.01$) in different groups with progressive age.

9- Serum albumin decreased significantly ($P \leq 0.01$) (except third group vs second group) in different groups with progressive age.

10- Lactate dehydrogenase increased significantly ($P \leq 0.01$) in different groups with progressive age.

11- Alanine transaminase increased significantly ($P \leq 0.01$) (except second group vs first group) in different groups with progressive age.

12- Aspartate transaminase increased significantly ($P \leq 0.01$) (except second group vs first group) in different groups with progressive age.

13- Alkaline phosphatase increased significantly ($P \leq 0.01$) (except fourth group vs third group) in different groups with progressive age.

The physiological impact of these results be discussed according to the effects of hypertension and progressive age on all the studied parameters, particularly, ACE2 deficiency and high levels of Ang II which pointed out a clear dysfunction in blood pressure regulation during progressive age.

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List of abbreviations

Abbreviations	Equivalences
ACE	Angiotensin converting enzyme
ACE I	ACE inhibitor
ACE2	Angiotensin converting enzyme 2
ACTH	Adrenocorticotropic hormone
ADH (AVP)	Antidiuretic hormone (Vasopressin)
AGT	Angiotensinogen
ALD	Aldosterone
ALP	Alkaline phosphatase
ALT (SGPT)	Alanine transaminase (Serum glutamic pyruvic transaminase)
Ang I (ATI)	Angiotensin I
Ang II (ATII)	Angiotensin II
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ANS	Autonomic nervous system
APCC	Aldosterone - producing cell cluster
APCC	Aldosterone - producing cell cluster
ARB	Angiotensin type 1receptor blocker
ARR	Aldosterone renin ratio
AST (SGOT)	Aspartate transaminase (Serum glutamic oxaloacetic transaminase)
AT1R	Angiotensin 1 receptor
BK	Bradykinin
BP	Blood pressure
Ca^{+2}	Calcium ions
CCC	Cardiovascular control centers
CKD	Chronic kidney disease

COVID-19	Coronavirus disease-19
CVS	Cardio vascular system
DM	Diabetes mellitus
DNA	Deoxy ribonucleic acid
ECE	Endothelin converting enzyme
ECF	Extracellular fluid
ELISA	Enzyme – linked immunosorbent assays
ET-1	Endothelin -1
ETA, ETB	Endothelin-1 receptors
GFR	Glomerular filtration rate
HRP	Horseradish peroxidase
IBM	Index body mass
ICF	Intracellular fluid
IL-6	Interleukin-6
IL-8	Interleukin-8
JGC	Juxtaglomerular cells
K^+	Potassium ions
LDH	Lactate dehydrogenase
LVH	Left ventricular hypertrophy
M R	Mas receptor
Na^+	Sodium ions Na^+
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
OD	Optical density
PA	Primary aldosteronism
ppET-1	Pepro-endothelin-1
RAAS	Renin angiotensin aldosterone system
RAS	Renin angiotensin system

RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
SARS	Severe acute respiratory syndrome
SCD	Serious cardio disease
SD	Standard deviation
SPSS	Statistical package for the social sciences
TGF1	Transforming growth factor 1
TP	Total protein
V1A, V1B	Vasopressin receptors
WHO	World Health Organization
ZG	Zona glomerulosa

Chapter One

Introduction

Introduction

Systemic circulation provides oxygenated blood to all organs in the body, which is essential to be maintained in all times via the power of blood pressure (Lowry, 2016). Blood pressure (BP), is the circulating blood force against the walls of blood vessels (WHO, 2021).

Blood pressure controlled by both neural and humoral control, neural control including autonomic nervous system (ANS), cardiovascular control centers (CCC) and vasomotor tone secondly humoral control including as, catecholamines, nitric oxide (NO), renin angiotensin-aldosterone system (RAAS) and atrial natriuretic peptide (ANP) (Patel and Ali, 2017).

Renin angiotensin system (RAS) or renin angiotensin aldosterone system (RAAS) is a peptide cascade a highly known for playing a crucial part in controlling arterial blood pressure regulation of arterial blood pressure and sodium homeostasis, in addition to cardiovascular regulation and remodeling (Van Thiel *et al.*, 2015). The regulation of blood pressure is an important issue that may be controlled by the RAS and its components such as, renin, angiotensin I (Ang I) and angiotensin II (Ang II) (Arendse *et al*., 2019).

The elevation of blood pressure (hypertension), is a serious medical condition, diagnosed by the systolic blood pressure readings (≥140 mmHg and/or the diastolic blood pressure readings ≥ 90) mmHg, hypertension considered as a main cause of heart, brain, kidney dysfunctions and other diseases, moreover, hypertension considered as a major reason for an early death worldwide and the risk for cardiovascular diseases (WHO, 2021), also it is a main risk factor for development of a serious cardio disease (SCD), 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).

Hypertension influenced by several factors such as the progressive age and aging which they were primary risk factors and incidents for cardiovascular diseases, the vasculature undergoes structural and functional changes with progressive age characterized by endothelial dysfunction, wall thickening, reduced distensibility and arterial stiffening, in addition to activation of proinflammatory and profibrotic signaling pathways, induced by some vasoactive agents, like angiotensin II, endothelin-1 (ET-1) and aldosterone (Harvey *et al.*, 2016).

Age was associated with higher systolic and diastolic blood pressure, that probably reflected blood vessels stiffening and reduced arterial compliance, thereby the pulse pressure increased with aging (Burt and Harris, 1994; Chrysant and Chrysant, 2014).

On the other hand, Ismail and his colleagues 2020 mentioned that aldosterone increased markedly in elderly people, RAS and its components activated with progressive age.

As long as, renal structural and functional detrimental changes occurred and renal mass declined with progressive age, some dysfunctional aspect in RAS and its components appeared including renin and angiotensin II secretion (Zhou *et al.*, 2008; Yoon and Choi, 2014).

Furthermore, angiotensin converting enzyme 2 (ACE2) an enzyme located on the cellular membrane of different human body organs, contributed with other factors in regulation of blood pressure, thereby, considered as a main player in this field due to their detrimental effects when their levels decreased during progressive age (South *et al.*, 2020 ; Zou *et al.*, 2020 ; Alghatrif *et al.*, 2021 ; Ambrocio-Ortiz *et al.*, 2021 ; Gu *et al.*, 2021).

On the other hand, many studies demonstrate both of hypertension and progressive age have a negative effect on different body organs particularly, liver, heart, brain and kidneys …etc., which reflected in the efficiency of these organs and fluctuations of their secretions. Liver enzymes Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) (Arques, 2018) and lactate dehydrogenase (LDH) enzyme (Ribeiro and Uehara, 2022), aldosterone hormone (Funder *et al.*, 2016) and arginine vasopressin (AVP) (antidiuretic hormone (ADH)) (Gonzalez *et al.*, 2020) increased significantly during both hypertension and progressive age.

Moreover, renin, total proteins and serum albumin levels decreased remarkedly in hypertensive men with progressive age (Inoue *et al.*, 2020; Myette *et al.*, 2021). On the other hand, both aging and hypertension considered as a low-grade inflammation and associated with some cytokines released including interleukins and (ET-1) (Bukowska *et al.*, 2022).

In view of these controversy, this study is an attempt to shed some light about the role of ACE2, RAS and some biochemical and hormonal parameters in hypertensive men during progressive age in maysan province.

Aim of the study:

Investigate the following parameters:

1- Angiotensin converting enzyme (ACE 2).

2- Renin.

3- Angiotensin I (Ang I).

4- Angiotensin II (Ang II).

5- Aldosterone.

6- Vasopressin (AVP).

7- Endothelin -1 (ET-1).

8- Total protein (T.P).

9- Serum albumin.

10- Lactate dehydrogenase (LDH).

11- Alanine transaminase (ALT).

12- Aspartate transaminase (AST).

13 - Alkaline phosphatase (ALP).

Chapter Two

Literatures Review

2.1 Renin angiotensin system

Renin was the first component measured by Tiegerstedt and Bergman in 1898 when they injected kidney extracts into rabbits and induced hypertension in their model (Basso and Terragno, 2001). Constriction of the renal artery leading to hypertension thus, driving the discovery of Angiotonin, later termed Angiotensin in his experiment in dogs (Goldblatt *et al.*,1934; Page and Helmer, 1940).

Angiotensin was subsequently purified and two forms were resolved: Angiotensin I and Angiotensin II, therefore, the existence of Angiotensin converting enzyme (ACE) was predicted and subsequently isolated and characterized (Skeggs *et al.*, 1956).

In Buenos Aires, the discovery of the RAS began with Dr Juan C. Fasciolo arrival, a young physician, at the institute chaired by Dr Houssay, he was interested in performing his doctoral thesis in physiology, under the direction of prof Houssay, the subject, selected by Houssay, was to repeat the experiment described by Goldblatt in the dogs to find out the nature of the mechanisms that lead to the emergence of high blood pressure (Basso and Terragno, 2001).

Laragh and his colleagues 1960 showed that the sodium-retaining hormone, aldosterone, appears to participate in the blood pressure regulation of normal subjects, and in excess can produce hypertensive vascular disease in human and in animals.

Aldosterone considered as additional components of RAS, hence, it called renin angiotensin aldosterone system (RAAS) (Reid, 1998) plays a crucial part in controlling blood pressure (Watkins *et al.*,1976), the counter regulatory axis of RAS was then described, pioneered with the discovery of ACE2 by two independent research groups (Donoghue *et al.*, 2000 ; Tipnis *et al.*, 2000).

Moreover, Gheblawi and his colleagues 2020 illustrated in the following schematic the historical timeline discovery of the major RAS components, Fig (2.1).

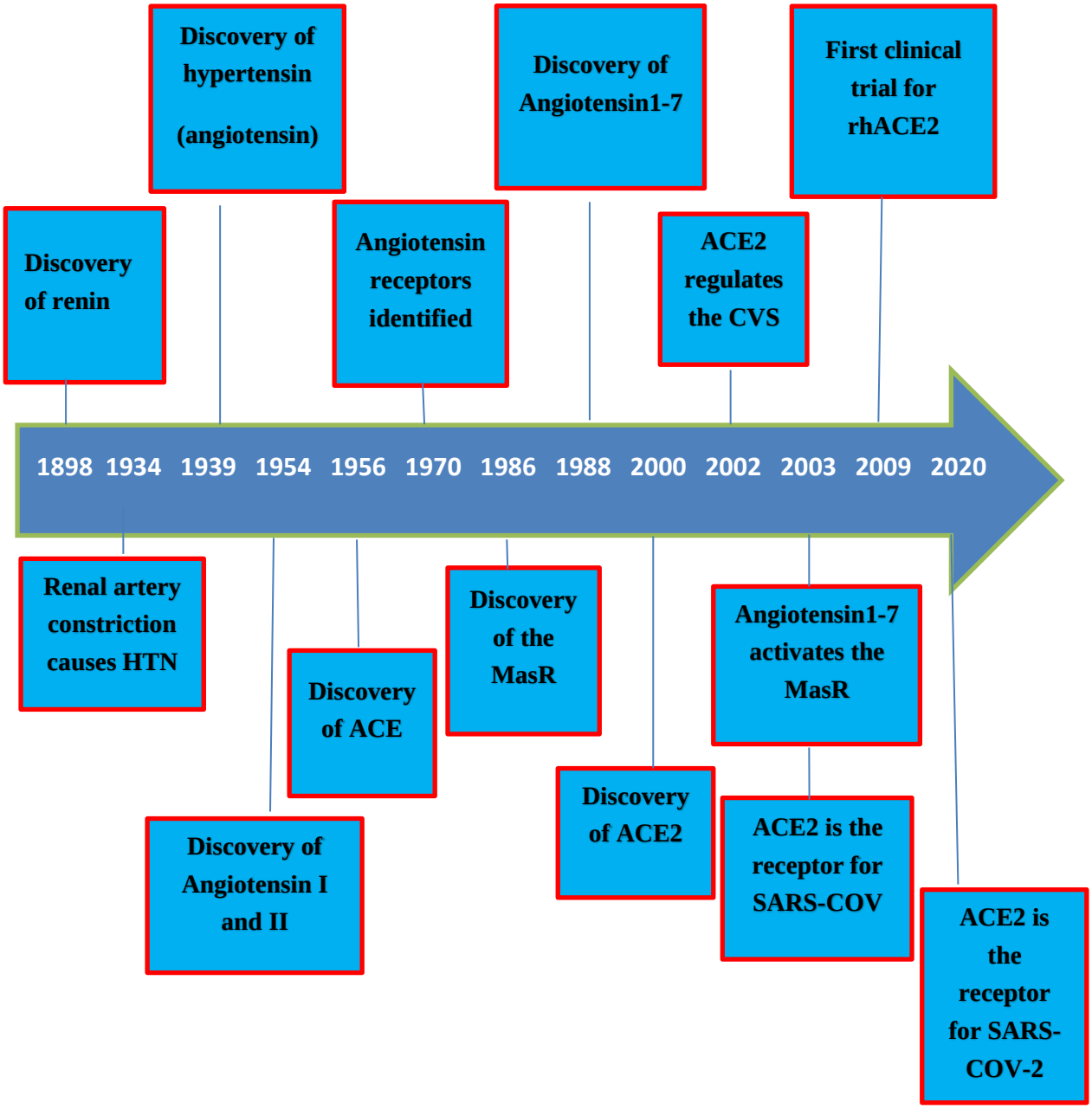


Figure (2.1) Historical timeline of discovery of the major renin-angiotensin system (RAS) components, including ACE2 (Gheblawi *et al.*, 2020).

Renin angiotensin system composed of renin and angiotensin, defined as signaling pathway which regulated homeostatic vascular function, in reaction to the renal blood pressure, both components increased arterial pressure, reduction salt delivery to the distal convoluted tubule (Crowley *et al.*, 2005; Drummond *et al.*, 2019).

Moreover, Renin angiotensin system is a crucial hormonal system in the physiological control of blood pressure; indeed, RAS dysregulation is believed to play a significant role in the emergence of cardiovascular disorders (Chappell, 2016), it's an important system which regulated salt levels, the equilibrium of extracellular fluids (ECF) and blood pressure (Kinsman *et al.*, 2017).

Survival of mammals live on land depend on adaptations to life-threatening circumstances such as deprivation of salt and water and marked sweating, vomiting, diarrhea, or tissue injury with hemorrhage, one of these adaptations is the activation of the renin angiotensin aldosterone system (Weber, 2001).

Ryu and his team 2007 noted that classical view of RAS pathway begins with renin cleaving its substrate Angiotensinogen (AGT) to produced inactive peptide Ang I which converted by ACE to Ang II, this conversion occurred most extensively in the lung, Fig (2.2).

Renin angiotensin aldosterone system, has the aldosterone beside the other components of RAS is responsible for more chronic alterations via the baroreceptors reflex that responds in a short-term manner to arterial reduction thereby, blood pressure elevated with prolonged manner (Hall *et al.,* 2019; Liu *et al.*,2019).

Renin was an aspartyl protease which released by the kidneys specifically from juxtaglomerular cells (JG cells) in response to perceived low both renal perfusion and blood pressure, renin played a vital role in the rate-limiting step of the RAAS, that responsible for the homeostasis of blood volume and mean arterial blood pressure, in addition renin binding to pro-renin receptors, causing Angiotensinogen to Ang I conversion (Nguyen *et al.,* 2002).

Furthermore, renin is a highly specific enzyme that catalyzes the first step in the RAS chemical cascade by producing Ang I which can be converted to Ang II by ACE or Ang1-7 by ACE 2 (Arendse *et al.,* 2019).

Renin was stored in form of prorenin, and the cleavage of prorenin facilitates its conversion to renin by intracellular microsomes, juxtaglomerular cells are responsible for the exocytosis of renin and have similar structures to smooth muscle that reflects in their location within the tunica media of glomerular afferent arterioles (Ren *et al.,* 2019).

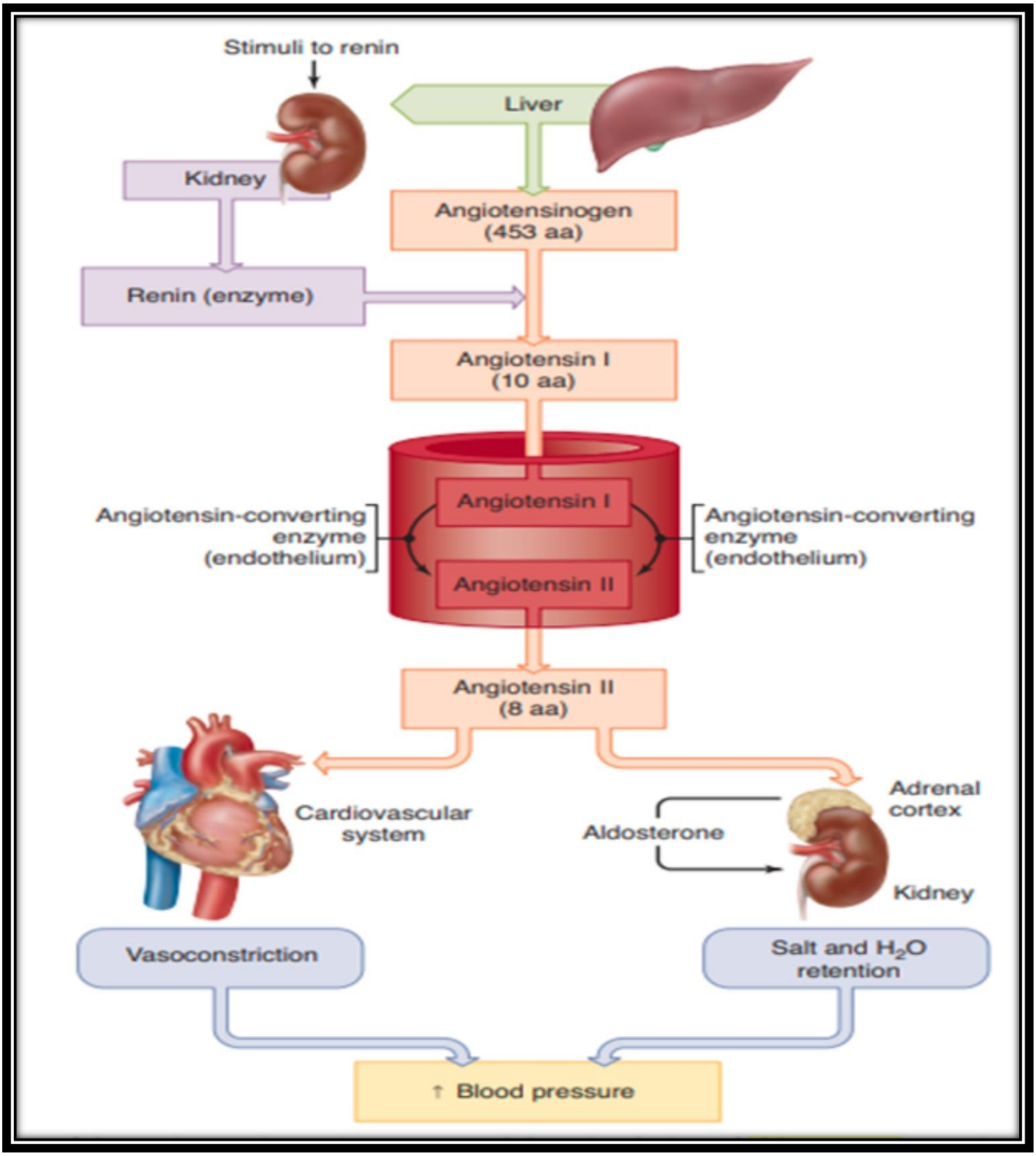


Figure (2.2) RAS and aldosterone's secretion stimulated by Angiotensin II

(Barrett *et al.*, 2019).

Renin that expressed by JG cells in response to reduction in renal blood flow cleaves circulating Angiotensinogen generated by the liver to make the inactive decapeptide Ang I (Ang 1– 10), the bioactive peptide Ang II (Ang 1–8) is then formed when the C-terminal dipeptide of Ang I was cleaved by ACE known as ACE1, a big 200 k Da protein found mostly at the endothelial surface of pulmonary arteries (Dzau, 1988 ; Guignabert *et al.,* 2018).

Renin release by JG considered to be the main rate-limiting step in providing circulating renin, remarkably, the stimuli that inhibit renin secretion e.g. increased arterial pressure or Ang II and increase intracellular free calcium, this elevation of free calcium levels lead to enhanced depletion of secretory granules, thus, this unique feature of renin secretion is commonly referred to as the calcium paradox, the reason of the opposite effect of calcium on renin secretion which can be found in granules (the origin of the renin storage) (Peters *et al.,* 2002).

The cleavage of AGT (renin's substrate) represents the rate-limiting step in the enzyme cascade that makes up the classic circulating RAS, AGT may be also produced in the brain, large arteries, kidneys, adrenal glands and adipose tissues, although the liver is the main source of AGT (Koizumi *et al.,* 2016).

Kobayashi and his colleagues 2020 found that higher renin levels were associated with higher body mass index, previous myocardial infarction, chronic obstructive pulmonary disease and dilated cardiomyopathy.

Low salt or use of an Ang 1 receptor (AT1R) blocker/ACE inhibitor (ACEi) can increase renin secretion (Shao *et al.,* 2013; Momoniat *et al.,* 2019).

Angiotensin II is the result of Ang I cleavages by ACE and its action increased the reabsorption of sodium and water by constriction of afferent glomerular arterioles, moreover, Ang II caused the production of both of AVP and aldosterone whereas, they increased serum sodium levels and total fluid content

(Sparks *et al.,* 2014),therefore, Ang II indirectly regulates blood volume by its action on adrenal cortex (Karnik *et al.*, 2015).

Angiotensin II, the final effector of RAS, causes vasoconstriction, both directly and indirectly, by stimulating Ang II type 1 receptor (AT1 receptor) present on the vasculature, and by increasing sympathetic tone and AVP release (Catarata *et al.*, 2020).

2.2 Angiotensin converting enzyme

Angiotensin converting enzyme is a kininase II subunit. (Alhenc-Gelas *et al.*,2019), ACE a well-known component of the RAS, which controls blood pressure, it is a membrane-bound, zinc-dependent dipeptidase that catalyzes the conversion of the decapeptide Ang I to the potent vasopressor octapeptide Ang II by removing the two C-terminal amino acids (Zhao and Xu, 2008).

The human ACE gene codes for a 180-kDa protein with two homologous domain that is found on chromosome 17. ACE operates as an ectoenzyme at the cell surface, hydrolyzing circulating peptides, and is linked to the plasma membrane by a single carboxyterminal transmembrane domain (Imai *et al.*, 2010).

ACE has a homolog called ACE2, which differs about ACE in that in its a carboxypeptidase that selectively eliminates carboxy-terminal hydrophobic or essential amino acids, this homolog appeared to be crucial for heart function (Riordan, 2003).

ACE2 is an enzyme located in the cellular membrane of different human body organs in varying degrees (Ni *et al.,* 2020), mainly in the epithelium, it has been shown that ACE2 is expressed in the lungs, liver, kidneys, stomach, intestines, arteries, veins, heart, oral mucosa, nasopharynx, colon, thymus gland, bladder, and central nervous system (Zou *et al.,* 2020 ; Ambrocio-Ortiz *et al.*, 2021).

The conversion of Ang I to Ang II and the degradation of bradykinin (BK) are two well-known functions of ACE, both of which are vital in blood pressure management, ACE involved in a variety of processes, including kidney development, male fertility, hematopoiesis, erythropoiesis, myelopoiesis and immunological responses, in addition to blood pressure management (Bernstein *et al.,* 2012).

Moreover, ACE2 expressed predominantly in the renal tubular and vascular endothelial cells (Tipnis *et al.,* 2000; Donoghue *et al.,* 2000), in addition Bártová and his colleagues 2020 found that localization of ACE2 not only in the plasma membrane and cytoplasm but, also in the cell nucleus.

The vital active product of ACE2 was the vascular vasodilator Ang (1-7), that produced in two ways, firstly, ACE2 can hydrolyze Ang II directly to produced Ang (1–7), lowering Ang II concentrations while boosting Ang (1–7) levels, secondly, ACE2 may convert Ang I to Ang (1–9), which was subsequently cleaved by ACE to produce Ang (1–7) (Santos *et al.*, 2018 ; Ni *et al.*, 2020).

Angiotensin converting enzyme is a family member of the RAS and acts as a metallo-carboxypeptidase cleaving Ang II to Ang-(1-7), Ang II acts on the Ang II type I receptor (AT1R), leading to pro-inflammatory and pro-fibrotic signaling, while Ang (1-7) acts on the Mas oncogenic receptor (Mas R), leading to anti-inflammatory and anti-fibrotic signaling, thus, ACE2 can shift the RAS balance by reducing the amount of Ang II and at the same time increasing the amount of Ang (1-7) molecules, therefore, ACE2 not only re-establishes the physiologic balance of the RAS, but it also clearly shifts it toward resolution of inflammation and fibrosis (Kaschina and Unger, 2003 ; Santos *et al.*, 2019 ; Kuriakose *et al.,* 2021).

Flores-Muñoz and his team 2012 noted that the counter-regulatory axis of the RAS peptide Ang 1-7, has been identified as a potential therapeutic target in cardiac remodeling, acting via the Mas R, Fig (2.3).

In addition to its enzymatic function, ACE2 is the receptor for viruses belonging to the severe acute respiratory syndrome (SARS)-coronavirus (CoV) and SARS-Cov-2 subtypes, which cause SARS and coronavirus disease-19 (COVID-19) respectively, endothelial ACE2 is cleaved by proteases, shed into the circulation, and measured as soluble ACE2, plasma ACE2 activity increased in cardiovascular diseases and may have prognostic significance in disease severity (Kuriakose *et al.,* 2021).

Considering the importance of ACE2 in cardiovascular pathophysiology, it has been suggested that the loss of ACE2 catalytic function and dysregulation of the RAS may be responsible for the cardiovascular symptoms reported in COVID-19 patients (Nishiga *et al.*, 2020; Zhang *et al.*, 2020).

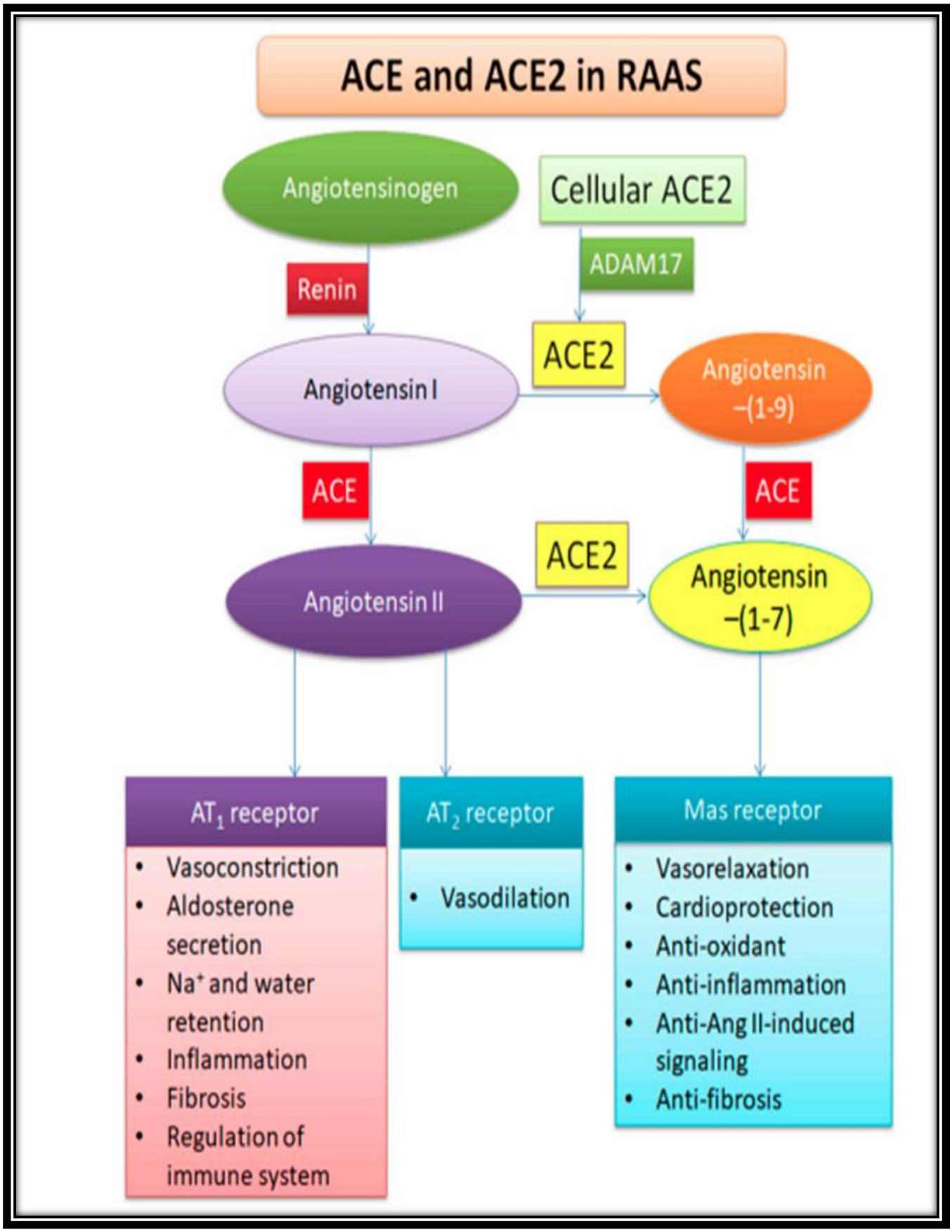


Figure (2.3) The vital action of ACE and ACE2 in RAAS (Xiao *et al*., 2020).

2.3 RAS and hypertension

Hypertension, is a serious medical condition, diagnosed by systolic and / or diastolic blood pressure measurements of at least 140 and 90 mmHg, respectively, hypertension considered as a cause of main heart, brain, kidney dysfunctions and other diseases, moreover, hypertension considered as a major reason for an early death worldwide also its a risk for cardiovascular diseases (WHO, 2021).

Goit and Yang 2019 noted that hypertension is a main risk factor for development of serious cardio disease (SCD), a dissecting aortic aneurysm, angina pectoris, LVH, thoracic and abdominal aortic aneurysms, CKD, atrial fibrillation, DM, vascular dementia and ophthalmologic disease, Fig (2.4).

Moreover, one of the most prevalent disorders in the western world is hypertension, which has been found to respond well to a number of pharmacological classes, hypertension is also one of the leading causes of morbidity and mortality worldwide (Mills *et al.*, 2020).

Hypertension, beside other cardio vascular diseases such as atherosclerosis, myocardial infarction…. etc… are all linked to RAS (Hoogwerf, 2010).

Bader, 2013 concluded that the ACE2 / Ang (1-7) / Mas axis exerts preventive effects in conditions such as hypertension, DM and other cardiovascular illnesses, in addition, ACE2 modulates the local actions of the RAS in cardiovascular tissues.

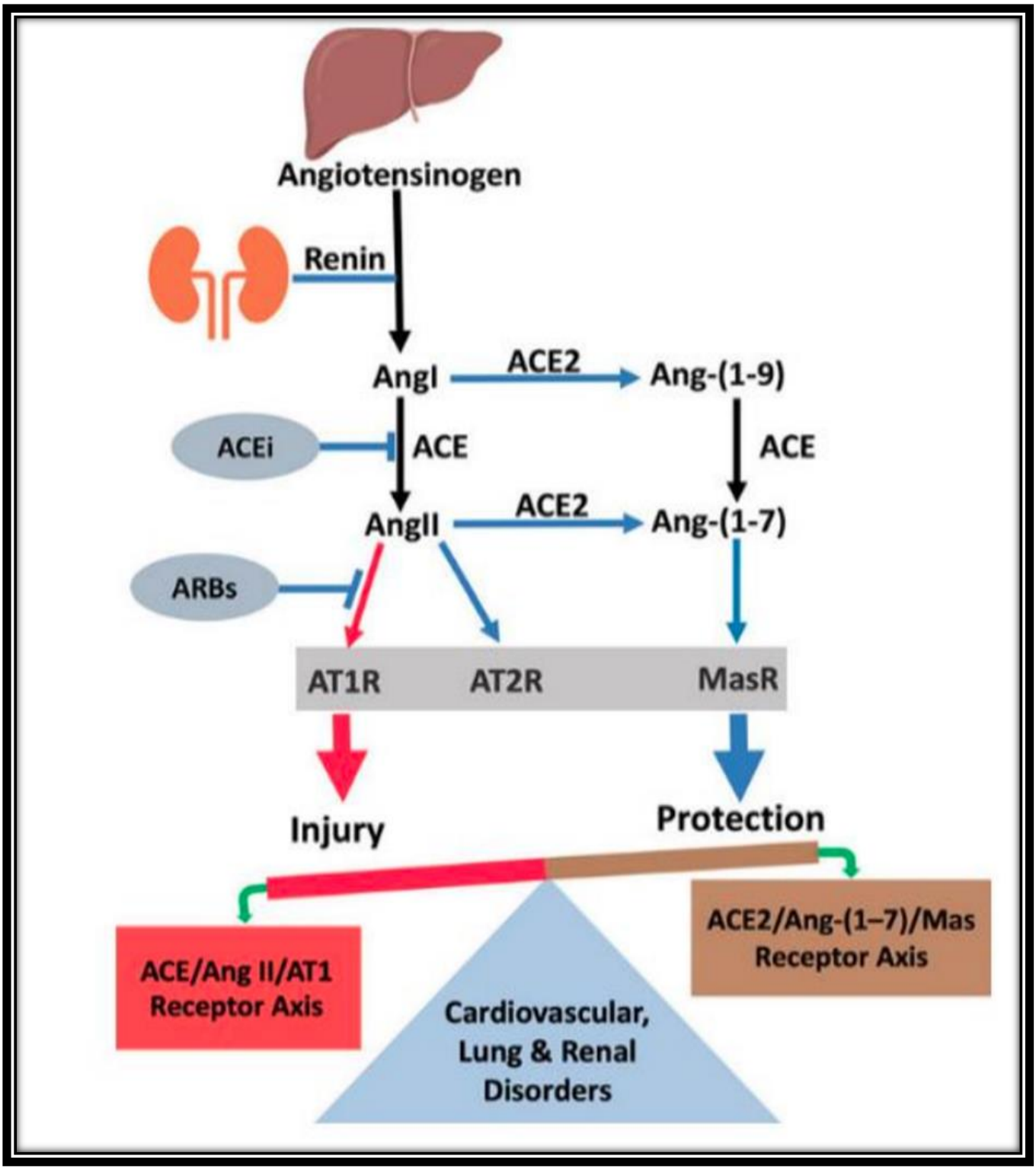


Figure (2.4) RAS with vasodilatations axis (ACE/Angiotensin II/AT1 receptor) and overprotective ACE2/Ang-(1-7)/Mas R axis (Sharma *et al.*, 2021).

Pinheiro and his colleagues 2019 suggested that a systemic arterial hypertension may be caused by the combined impact of both ACE and ACE2 polymorphisms.

The RAAS acts to manage blood volume and arteriolar tone on a long-term basis to regulate the blood pressure, the alterations in blood volume chronically, by RAAS may be lead to the development of hypertension, whereas, (for example) the reduction in blood volume that reached one (or both) kidneys because of renal artery stenosis may be resulting in RAAS activation and in appropriate elevation circulating blood volume and arteriolar tone caused a development of hypertension (Santos *et al.,* 2019).

Lo and his team 2013 found that recombinant human ACE2 reduces Ang II-induced hypertension, lowers plasma Ang II and increases plasma Ang (1–7) levels.

Furthermore, blood pressure changes are greater in Ang II-treated rats, overexpression of ACE2 results in increased the expression of anti-hypertensive components of RAAS (Ang 1–7, Mas R and AT2 R) reduced the elevated blood pressure (Diez-Freire *et al.,* 2006; Rentzsch *et al.,* 2008).

Ang(1–7) has shown blood pressure-lowering effects in hypertensive animals, the anti-hypertensive effects of ACE2/Ang 1–7 generated interest in potential cardioprotective effects against hypertensive heart diseases, a group of disorders that includes heart failure, ischemic heart disease, hypertensive heart disease, and left ventricular hypertrophy (Riet *et al.,* 2015 ; Patel *et al.,* 2016).

2.4 ACE2 and endothelin

Endothelin-1, is the most common member of the endothelin peptide family, discovered in 1987 by Yanagisawa and his team in porcine aortic endothelial cells as a potent endothelial cell-derived vasoconstrictor peptide (Yanagisawa *et al.*, 1988).

The ET family of peptides (ET-1, ET-2, and ET-3) affect cells from the endothelium, epithelium, muscle, and nervous systems in almost every organ and system, endothelin converting enzymes (ECE) in the ET system convert relatively inactive precursor peptides (big ETs 1 and 2, as well as big-ET 3) into active peptides, these peptides' biological effects are mediated by ETA and ETB receptors, which are primarily found in very high concentrations in endothelial and epithelial cells in the kidneys (Speed *et al.*, 2015, a).

Endothelin -1 is a 21-amino acid peptide with broad biological activity that has been linked in a variety of disorders (Fagan *et al.*, 2001), through its role in producing pro-inflammatory mediators in the lungs (Marshall, 2003).

Moreover, it was recognized as a multifunctional peptide with cytokine-like activity that contributes to almost every aspect of physiology and cell function, over the last three decades, more than 30000 researches publications on endothelin have been published, resulting in the discovery and subsequent regulatory approval of a new class of treatments known as endothelin receptor antagonists (Barton and Yanagisawa, 2019).

ET-1, like the interleukins (IL-6 and IL-8) and granulocyte-macrophage colony-stimulating factor, it’s a powerful inflammatory mediator that may be quite important in inflammatory pulmonary diseases (Datta *et al.*, 2011).

Endothelin -1 implicated with the kidneys' tubular reabsorption of water and electrolytes, which helps to maintain intravascular volume (Kohan *et al.*, 2011), by aldosterone (Andreis *et al.*, 2002), AVP and natriuretic peptides secretions (Rossi, 2004).

Endothelin -1 able to raise blood pressure by upsetting a few of the control mechanisms (involved blood pressure regulation as integrative process of complex interactions for nervous, endocrine, cardiovascular systems and renal balance of fluids) and maintaining intravascular fluid volume (Speed *et al.*, 2015, b).

Through the control of catecholamine secretion and its potent vasoconstrictor impact, ET-1 influenced peripheral vascular resistance (Ohara-Imaizumi and Kumakura, 1991), ET-1 inhibited the releasing of renin but can immediately stimulated the releasing of aldosterone, in addition to its synergistic interactions with Ang II, ET-1 increased the production of Ang II (AT II) via increasing ACE activity, moreover, ATII then increased the production of ET-1 by improving the activity of endothelin converting enzyme (Kostov, 2021), Fig (2.5).

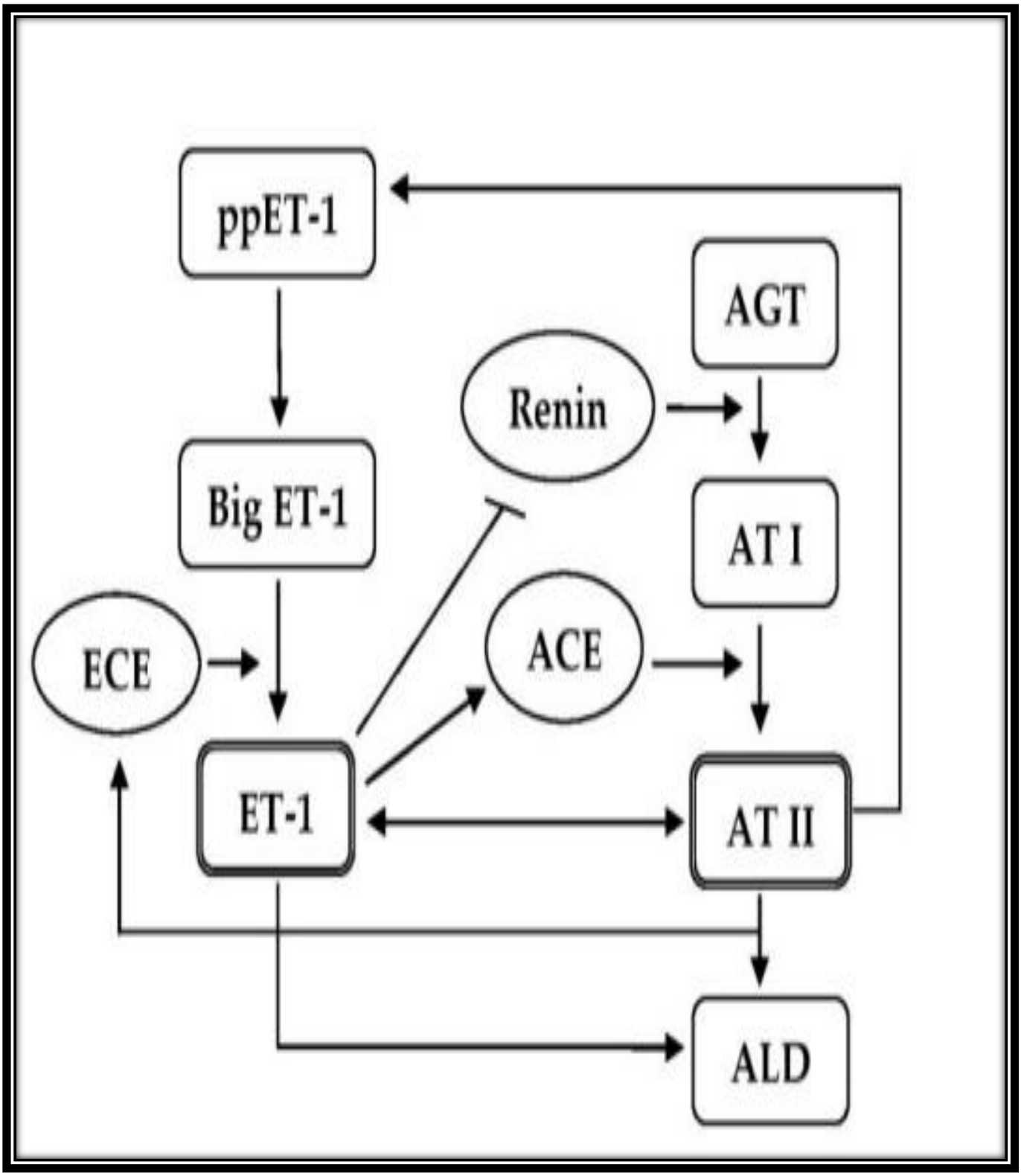


Figure (2.5) ET-1 and Angiotensin II work in concert to promote the development of hypertension (Kostov, 2021).

Overstimulation of ET-1/ETA signaling may disturb the balance in the regulation of blood pressure in a number of pathogenic conditions, which may then result in the development of hypertension (Kohan *et al.*, 2011).

There is interaction between ET-1 and RAS, particularly the ACE-AngII-AT1R axis, played an important task in mediating airway inflammation responses associated with hypertension and released of ET-1 that downregulated the ACE2 expression and decreased its activity (Shrikrishna *et al.*,2012; Zhang *et al.*, 2013).

Chen and his colleagues 2013 found that ET-1 and the RAS involved in the pathogenesis of cardiac dysfunction, the Mas R (the Ang -1-7 receptor) is a functional binding site for Ang-1-7 which is considered a critical component of the RAS and exerts cardioprotective effects, where they demonstrated that ET-1 downregulates Mas R expression at the transcription level in human cardiomyocytes via the ETA receptor and they provide novel insights concerning the function of ET-1 and the Ang-(1-7) / Mas axis in cardiac pathophysiology.

On the other hand, the increment in ET-1 production in the wall of vascular may enhanced low-grade inflammation and oxidative stress with the endothelial dysfunction development and increased vasoconstrictor activity, in addition, the increase in ET-1 levels can assist with atherosclerotic alterations and arterial aging which linked to increased arterial stiffness and the manifestation of isolated hypertension, moreover, ET-1 affected myocardial contractility, water-salt balance, baroreceptor activity and renal hemodynamics, as a part of the intricate regulation of blood pressure via stimulating sympathetic activity (Kostov, 2021).

2.5 Aldosterone and Regulation of Blood Pressure

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

Physiologically, aldosterone plays a key role in the maintenance of intravascular volume and blood pressure through sodium retention in the kidney, excess aldosterone causes hypertension and induces cardiovascular complications (Savard *et al.,* 2013).

Aldosterone, often released simultaneously with AVP in order to support water reabsorption to the extra cellular fluid (ECF) by mobilizing aquaporin channels to the apical (lumen-facing) membrane of principal cells in the collecting tubule (Hall *et al.,* 2019), after verifying Ang II, type1 receptors (AT1R) binding in the adrenocortical cells, aldosterone secretion responses to Ang II in an equivalent manner (Pang *et al.,* 2019).

The autonomous secretion of aldosterone, independent of Ang II and sodium status, is known as primary aldosteronism (PA), PA is the most common cause of endocrine-related hypertension with a prevalence of 5% - 10% in hypertensive population (Calhoun *et al.,* 2002), which considered as a risk factor for cardiovascular complications and must be detected and targeted early (Funder *et al.,* 2016).

Brown and his colleagues (2017) showed that patients with higher aldosterone and renin suppression levels have an increased risk for hypertension through increased mineralocorticoid receptor activation.

On the other hand, Tu and his group (2018) noted that age is a strong determinantal agent for blood pressure when they used observational data collected from normotensive men enrolled in a prospective cohort study, they found aldosterone sensitivity increases as plasma renin activity decreases and this increase may be attributed to the blood pressure increase.

High levels of aldosterone increased blood pressure and suppressed renin secretion for unknown reasons, plasma renin is also low in 10-15% of patients

with essential hypertension and normal circulating aldosterone levels, increased aldosterone secretion causes hypertension (Barrett *et al.*, 2019).

On the other hand, aldosterone exerts its major physiological role via its receptors (MRS R) located in distal portion of the nephron, particularly distal convoluted tubule, the collecting duct's cortical and medullary sections (Kolkhof and Bärfacker, 2017; Laursen *et al.*, 2018).

The reduction of aldosterone be regulated by Ang II, Adrenocorticotropic hormone (ACTH) and potassium ions (K^+) in both acute (during minutes) and chronic (during weeks, months and years) actions, in addition, Klotho protein, natriuretic peptides and circadian clock are factors that regulating aldosterone production too (Tsilosani *et al.*, 2022).

On the other hand, Wang and his team (2020) indicated that aldosterone is an independent regulator of water permeability, aldosterone decreases vasopressin-stimulated water transport and attenuated osmotic water in rats.

2.6 ACE2 and vasopressin

Vasopressin commonly known as antidiuretic hormone (ADH), is the major hormone in charge of maintaining bodily water through the kidney's antidiuretic effects (Gonzalez *et al.*, 2020).

Moreover, AVP is a potent neurohormone involved in the regulation of arterial blood pressure, increased release of AVP into the circulation is associated with hypertension whereas AVP release stimulated by hyperosmolality, hypovolemia, hypotension, hypoxia, hypoglycemia and strenuous exercise (Proczka *et al.*, 2021), there are at least three kinds of vasopressin receptors, V1A, V1B and V2 (Di giglio *et al.*, 2017).

Vasopressin secreted from posterior pituitary gland in response to the number of triggers such as high serum osmolarity (that acts on osmoreceptors

which located in hypothalamus), Ang II , low blood volume (causes a decline stretch in the low-pressure baroreceptors) and blood pressure decline (causes decreased stretch in the high-pressure baroreceptors) (McClure *et al.*, 2009; Shahoud *et al.*, 2021).

Vasopressin, has many functions including increase water reabsorption in collecting duct, increase the plasma volume and arterial pressure (McClure *et al.*, 2009), as soon as AVP released, its cardiovascular effects exerts mainly through V1aR that mediates vasoconstriction and increases the vascular resistance (Japundžić-Žigon *et al.*, 2020).

Moreover, AVP and Ang II acted on AT1R and V1aR, respectively, exert vasoconstriction, increase cardiac contractility, stimulate the sympathoadrenal system and elevate blood pressure, on the other hand, AVP released from vasopressinergic neurons in response to Ang II (Szczepanska-Sadowska *et al.*, 2018).

Vasopressin examined as renin mediator which has been hypothesized as negative regulator of renin secretion and synthesis in vitro whereas it suggested to primarily inhibit the release of renin through a high cytosolic calcium ion (Ca^{+2}) concentration in JG cells (Neder, 2018).

Moreover, AVP increased in hypertensive patients due to the low levels of renin and high levels of ET-1 which enhanced also the release of aldosterone in elderly people (Yilmaz *et al.*, 2019).

Moreover, Spulecki and his group (2016) pointed that in the perioperative setting, continued use of ACE inhibitors within 24 hours before surgery remains controversial and it has been suggested that the morning dose of the ACE inhibitor be held, and those patients experienced shorter duration of decreased mean arterial pressure, no incidence of refractory hypertension from withholding the morning dose of the ACE inhibitor, patients undergoing coronary artery bypass or vascular

surgery where they noted that those patients receiving AVP demonstrated improved hemodynamic stability with small, intermittent doses, without profound ischemic changes, for management (prevention and treatment) of ACE inhibitor–associated hypotension.

2.7 Albumin and Total Proteins

Albumin is the most abundant circulating proteins (3.5 - 5 g/dL) found in human's plasma, synthesized by hepatocytes, secreted rapidly into the bloodstream at the rate of 10 - 15 gm/day, stored as a little amount in the liver, acts as a significant modulator of plasma osmotic pressure and a transporter of endogenous and exogenous (i.e. drugs) ligands (Chang and Holcomb, 2016).

Albumin plays a physiological role in regulating blood pressure by effecting on osmotic pressure whereas, water moves into bloodstream resulting increase the intracellular fluid (ICF) in turn decreased the extracellular fluid volume, leading to increasing the secretion of vasopressin and aldosterone (Barrett *et al.,* 2019).

Moreover, in essential hypertensive patients, increased albumin excretion is associated with endothelial changes within the glomerular capillaries (Stehouwer *et al.,* 2002), Microalbuminuria is thought to currently imply broad vascular damage in addition to glomerular endothelial damage, which represents microvascular injuries (Deckert *et al.,* 1989).

However, an imbalance between albumin filtration and reabsorption in the kidney could lead to an increase in albumin excretion in the urine, and glomerular endothelial dysfunction is known to be a major factor in an excessively elevated albumin glomerular filtration rate (Ballermann, 2007).

Høstmark and his team (2005) noted that decrease in serum albumin levels related to possibility of injury with cardiovascular diseases during progressive age

which accompanied with blood pressure increment, moreover, it is also has a vital metabolic function as an antioxidant and of molecules carriers.

Liu and his colleagues (2009) showed the treatment with bovine serum albumin increased significantly the ACE /ACE2 expression as well Ang II production in human kidney cells, therefore, they concluded a relationship between the albumin protein and some of the RAS components.

Moreover, human serum albumin (HAS) identified as a potent physiological inhibitor of the ACE (responsible of Ang I transformation into Ang II), so, activity of this enzyme suppressed completely by HAS, thus Ang I conversion reduced to a little amount in vivo (Zheng *et al.*, 2019).

Devi and Kumar (2012) mentioned a revers correlation between progressive age and serum total proteins and its fractions (albumin and globulin) due to the low rate of hepatic protein synthesis.

2.8 Lactate dehydrogenase

Lactate dehydrogenase is a terminating enzyme in the metabolic pathway of anaerobic glycolysis with end product of lactate from glucose, lactate can also be formed and utilized by different cell types under fully aerobic conditions this enzyme manifests as a cytoplasmic enzyme, released from injured cells and its elevation indicated to a tissue damage (Liaw *et al.,* 1997; Kumar *et al.,* 2018; Forkasiewicz *et al.*, 2020; Gupta, 2022).

Sarkar and Sogan (2013) showed that high levels of serum LDH in both damage tissues and endothelial vascular dysfunction represented main cause for preeclampsia in pregnant women, in addition, many other elevations in this enzyme be found also during hypertension post oxidative stress, renal and cardiac damage in rats (Oyagbemi *et al.,* 2017).

Jaiswar and his team (2011) found that the high LDH activity had a significant correlation with high blood pressure, similarly, LDH activity elevated significantly in women with preeclampsia/eclampsia and correlate well with the severity of the disease and poor outcomes of these preeclamptic and eclamptic women.

Furthermore, LDH increased significantly in hypertensive men during progressive age, obesity and atherosclerosis, that resulting in vascular endothelial damage, moreover, atherosclerosis might be the LDH importance explanation (Cai *et al.*, 2021), whereas, Yan and his team (2021) consider LDH as a marker of inflammation that associated with hypertension, in addition, Gupta and his colleagues 2018 showed a significant elevation of LDH associated with presence of hypertension's complications.

Serum LDH levels increased in proportion to the clinical severity of idiopathic pulmonary arterial hypertension and its role be assessed as a biomarker and mediator involved in the pathogenesis of idiopathic arterial hypertension (Hu *et al.*, 2015).

Lactate dehydrogenase is an enzyme that is widely detectable in cytoplasm of every type of cells of the human body and is a sensitive indicator of cell injury and/or increment the permeability of cell membrane, whereas, atherosclerosis begins with endothelial cell weakness and increased permeability of the cell membrane, releasing LDH into circulation, it was suggesting that serum LDH progressively increased with the atherosclerosis progression and demonstrating that the pathophysiology of atherosclerosis was connected to LDH (Jing *et al.*, 2004).

2.9. Liver Enzymes and RAS

ALT and AST are the common liver enzymes well-known as a good marker of both liver's health and damage (Li *et al.*, 2016; Vagvala and O'Connor, 2018). These markers can be beneficial in the evaluation and treatment hepatic dysfunction patients (Gaeini *et al.*, 2020), ALT is the most specific marker of liver function that used as an indirect marker of liver inflammation or injury, however, AST found in many tissues beside the liver, thus it's a less specific marker (Lee *et al.*, 2004).

Moreover, the liver enzymes alanine and aspartate aminotransferase (ALT and AST), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP) have been widely used as a good marker of liver health (Hanley *et al.*, 2004).

ALT and AST known as serum glutamic pyruvic transaminase (SGPT or GPT) and serum glutamic oxaloacetic transaminase (SGOT or GOT) respectively, catalyzed the reversible transfer of the amino group from amino acids to α -keto acids (Ndrepepa, 2021). The mean half-life of ALT and AST are 87 hours and 17 hours respectively (Dufour *et al.*, 2000).

Alkaline phosphatases are group of isoenzymes, located on the outer layer of the cell membrane, they catalyze the hydrolysis of organic phosphate esters present in the extracellular space, in the liver, alkaline phosphatase (ALP) is cytosolic and present in the canalicular membrane of the hepatocyte (Green and Sambrook, 2020).

The human alkaline phosphatases (hALP) are found anchored on the cell membrane by glycosylphosphatidylinositol, they are released in the serum by the action of specific phospholipase (Sharma *et al.*, 2014). Liver of the patients which have an elevated ALP considered as the source for this enzyme, ALP half-life is about 7 days and its clearance independent of bile duct patency or functional capacity of the liver, however, the site of degradation of alkaline phosphatase is

not known, serum alkaline phosphatase levels may remain elevated for up to 1 week after the resolution of biliary obstruction (Green and Sambrook, 2020).

AST has multiple metabolic functions, such as maintenance of the nicotinamide adenine dinucleotide/ reduced nicotinamide adenine dinucleotide (NAD+ / NADH) ratio in cells, synthesis of purine and pyrimidine bases, urea synthesis, proteins synthesis and gluconeogenesis (Ndrepepa, 2021).

ALT leaked by damaged hepatocytes into the extracellular space and ultimately plasma, so that ALT activity and/ or amount will be increased in animals with damaged hepatocytes when compared with normal hepatocytes (Aulbach and Amuzie, 2017).

Multiple factors affected the amount of AST and ALT in the blood and some mechanisms have been proposed to explain the rise in AST and ALT levels (McGill, 2016), firstly, the most frequent cause of increased activity of aminotransferases (including AST) in circulation is either by direct damage of tissue (cell necrosis or damage of cell membrane and protein leakage caused by various numerous harmful substances or stressors), or apoptosis (in conditions of physiological cellular renewal or increased apoptotic stimuli), second, the raised levels of AST in the blood may be caused by enhanced AST gene expression, Thirdly, macroenzymes (macro AST) indicated a different mechanism of asymptomatic enzyme increase associated with protracted serum AST clearance ….etc (Ndrepepa, 2021).

Rahman and his coworkers (2020) mentioned that the prevalence of elevated liver enzymes was higher in hypertensive individuals, increased serum ALT and AST activities were positively associated with hypertension and age in Bangladeshi men.

Moreover, Cai and his colleagues (2021) noted that ALT, AST and ALP were higher in hypertensive more than normotensive Chinese human male with

aging, while AST and ALT are more specific for myocardium and liver illnesses respectively. Shimizu and his team 2013 mentioned that the high levels of ALP are positively associated with hypertension.

Furthermore, El-Raziky and his group (2005) found that, ALT, AST and aldosterone were significantly higher in patients with chronic liver diseases and enhanced the potential use of these enzymes as a prognostic marker in chronic liver diseases.

On the other hand, Wu and his group (2018) found that ACE2 deficiency promoted liver inflammation and led to increment in AST, ALT and ALP concentrations, thus, ACE2 may have a capability to protect the liver.

It is noteworthy, that ALT, AST and ALP levels, increased with some cases especially in liver fibrosis because of the high levels of both Ang II and reactive oxygen species (ROS) that indicated an inflammatory response associated with the pathogenesis of liver injury and fibrosis (Colmenero *et al.*, 2009 ; Grace *et al.*, 2012).

Wu and his team (2015) mentioned that Ang II has an ability to promoted liver extracellular matrix hyperplasia, inducing activation, contraction, emigration and propagation of hepatic cells, initiating liver fibrosis, stimulating transforming growth factor 1 (TGF1) secretion and augmenting the pro-fibrogenesis of TGF 1 through its receptor AT1R (Zhu *et al.*, 2009 ; Yang *et al.*, 2009).

Moreover, ACE inhibitors and angiotensin type 1 receptor blockers prominently ameliorate the hepatic fibrosis in several animal models (Wu *et al.*, 2015).

Furthermore, ACE2 exerts an opposite effects Ang II, so, it's might servs as anti-regulatory strategy to ACE, adjusting the liver's fibrosis and antifibrosis balance, moreover, ACE2 can serve as a dual function similarly RAS blockade i.e… ACE inhibitors (ACE i) and angiotensin type I receptor blockers (ARB), so,

the therapeutics targeting ACE2 recovering and rebuilding self-regulation and balance of the RAS may be a promising approach to prevent and / or slow the liver fibrosis thereby reduction AST and ALT levels in rats (Warner *et al.*, 2007 ; Wu *et al.,* 2015).

2.10 Aging and the RAS

Aging including the progressive changes from infancy till eventual death, gerontology, the study of aging, is currently of great interest because our society is expected to see a sharp increase in the population of seniors, a goal of gerontology is to increase health span, or the number of years during which a person can fully utilize all of their body's functions, rather than necessarily life span.

Moreover, aging is a slow process during which the body undergoes changes that eventually bring about death, even if there is no specific disorder or disease, medical science tried to extend the life span of human with healthy and prolong the period in which the body functions normally, moreover , there is many factors which effected on aging (Mader and Windelspecht, 2018).

Aging is associated with the buildup of damaged macromolecules, including as DNA, proteins, and lipids, that have been chemically changed by excessively produced ROS is what causes cellular aging (Conti *et al.*, 2012).

also its accompanied major composition body changes and progressive physiological changes which disturbed gradually the homeostasis of organs and systems, resulted in decline in functions and increased the risk of many illness with advancing age … i.e. cardiovascular diseases (Frith *et al.*, 2009 ; Leblanc *et al.*, 2013 ; Fuloria *et al.,* 2021).

Maloberti and his group (2019) indicate that Structural and functional arterial properties commonly impair with aging process, these effects on vasculature could act at many levels from microcirculation to large vessels.

Furthermore, Leblanc and his colleagues 2009 noted that endothelium dependent dilation declines with age in coronary arterioles for both male and female rats. Older people are more susceptible to several non-communicable diseases, such as cancer, diabetes, arthritis, hypertension and several others (Munday *et al.,* 2019). Moreover, Shukuri and his colleagues 2019 mentioned that the hypertension is a recognized as a risk factor for cardiovascular diseases and the prevalence of hypertension among older adults was high, these older adults are disproportionately affected by hypertension.

On the other hand, aging causes a series of changes in the activity and responsiveness of the RAAS components, the elderly people predisposed to fluid and electrolyte imbalances, as well as acute renal damage and chronic kidney disease due to RAAS alterations, which was key player in the various pathways implicated in renal aging (Yoon and Choi, 2014). Vascular aging associated with an increased vascular smooth muscle tone, RAAS hyperactivity and oxidative stress, contributed to arterial inflammation and age-related arterial remodeling (Lacolley *et al.*, 2015). moreover, chronic RAAS activation played a major role in age-associated arterial proinflammation and arterial remodeling (Xiao *et al.*, 2015).

In addition, Nanba and his group (2018) found that aging associated with increased aldosterone - producing cell cluster (APCC) expression, this histopathologic finding parallels an age-related autonomous aldosteronism and abnormal aldosterone physiology that provides potential explanation for age-related cardiovascular risk.

Furthermore, ACE2 activity decreased in aged rats (Xudong *et al.,* 2006) and in hypertensive elderly mice in comparison with the younger rodents, similarly renin has a same downregulation in elderly rodents (Bártová *et al.,* 2020).

The expression of ACE2 decreased in individuals with progressive age in both sexes, whereas, both COVI–19 and SARS-CoV viruses enter host cell via the same receptor (ACE2), so, that might be the leading cause of SARS -CoV-2 incidence (Zhou *et al.,* 2020; Gu *et al.,* 2021).

Moreover, Lakatta 2018 indicated that Ang II in elderly people mediated the proinflammatory changes leading to increase in RAS signaling throughout the body, exaggerated forms of this proinflammatory profile are also salient pathophysiologic features of hypertension which are highly prevalent at older ages.

Furthermore, 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).

Angiotensin converting enzyme2, may has an important role in the prevention of Ang II-mediated heart failure because the age-dependent cardiomyopathy in ACE2 null mice is associated with increased Ang II-mediated oxidative stress (Oudit *et al.,* 2007), moreover, uncontrolled Ang II dependent ROS generation, takes place as a consequence of age-associated activation of RAS (Conti *et al.,* 2012).

On the other hand, ACE involved in development of inflammation by its associated with increment of Ang II, in turn, Ang II plays a key role in the regulation of the vascular inflammatory response by activating the recruitment of inflammatory cells to the infected arteries, in addition, inflammatory cells can produce Ang II, resulting in a local positive feedback response, thereby perpetuating the inflammatory cycle (Ferrario and Strawn, 2006).

Moreover, Funder and his colleagues (2016) found that aldosterone levels increased in contrast with the decrease of renin levels during progressive age in hypertensive individuals, in addition, renin levels and aldosterone renin ratio

(ARR) correlated negatively and positively with progressive age in hypertensive men, respectively (Alnazer *et al.,* 2021).

Aging was accompanied with AVP elevation which represents a dysfunction of vasopressin pathway, more total peripheral resistance and promoted reabsorption of water through the vasopressin receptor (V2), besides the vasoconstriction enhancement via the vasopressin receptor (V1) (Tamma *et al.,* 2015).

On the other hand, elevation of ET-1 was a associated with progressive age (Yanes *et al.,* 2005 ; Yanes and Reckelhoff, 2011) and mediated vasoconstriction tone which contributed to the pathogenesis of hypertension (Stauffer *et al.,* 2008), moreover, both of inflammation and aging caused the endothelial dysfunction and high amounts of ET-1 that sharing with a progressive arterial stiffening (Avolio *et al.,* 2011).

Moreover, Wang and his colleagues 2021 noted that elderly hypertensive patients have a high levels of LDH, that considers as a clear sign of some organs' damage, including liver, kidneys and blood vessels, moreover, LDH is a sensitive sign of cell damage and / or enhanced membrane permeability, which initiated with endothelial dysfunction and leads to LDH release (Kogawa *et al.,* 2022).

Aging associated with low levels of serum albumin (hypoalbuminemia) in individuals with vascular endothelial dysfunction (Choi *et al.*,2021), deficiency of albumin may be acts 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).

On the other hand, in elderly hypertensive individuals, serum total proteins decreased significantly due to some organ's dysfunctions, particularly liver, heart and kidney which was the main damage indicator for these organs (Memon *et al.,* 2017; Lee *et al.,* 2019; Al najdi and Khalifa, 2021).

Furthermore, Aziz and Kamran (2019) reported that the urine proteins increased significantly with hypertension, that means a significant serum total proteins reduction in those hypertensive elderly patients.

Age advancement associated with liver enzymes that considered as a real marker in some organ's dysfunction, particularly in liver and heart of hypertensive men with advancing age (Hossain *et al.*, 2016; Liu *et al.*, 2018; Wong *et al.*, 2018 Gu *et al.*, 2021).

Chapter Three

Materials and Methods

3.1 - Materials

3.1.1- Subjects of the Study:

The present study was conducted in some hospitals, private health clinic centers and laboratories in Maysan province, during October 2021 till February 2022. The sample of this study including eighty hypertensive men, divided (according to their ages) into four groups (20 men per group) as the following:

- First group: 30-39 years.
- Second group: 40-49 years.
- Third group : 50-59 years.
- Fourth group : 60-69 years.

Sample's individuals have been diagnostic with hypertension and checked medically by specialist physician (in according to WHO criteria), some of these individuals were excluded due to their attack by diabetes, thyroid disease, heart and kidney failure, pituitary tumors, in addition to those whom taking hormonal and or antihypertensive drugs and smoking, Fig (3.1).

A questionnaire has been designed to obtain the actual information about the sample.

3.1.2 Experimental design

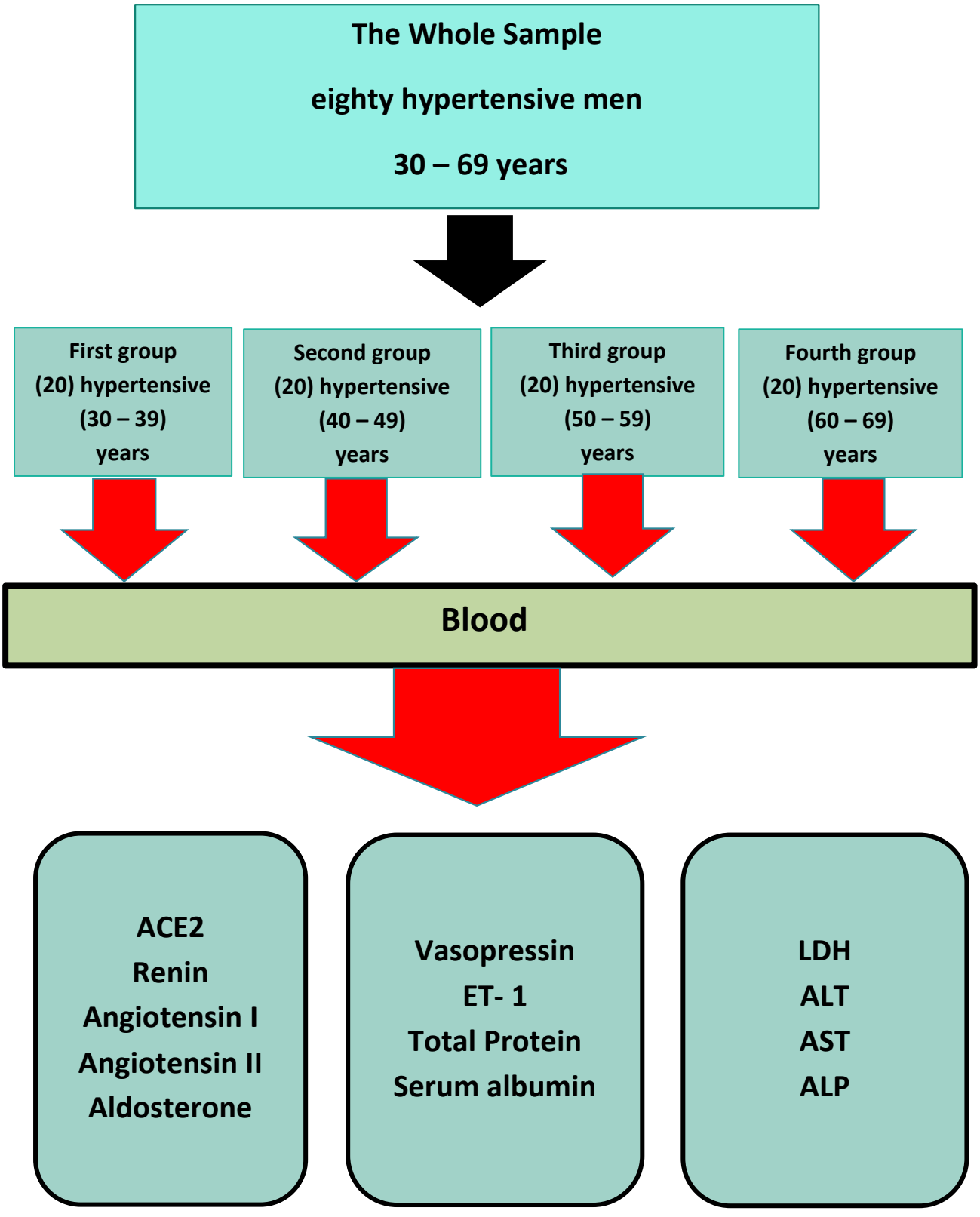


Figure (3.1) Experimental design.

3.1.3 Instruments and Equipment:

The instruments and equipment used in this study and their origin:

Table (3.1) The instruments and equipment used in this study

No.	Instruments and equipment	Origin
1	Alcohol	Iraq
2	Gel and Activator tube	Jordan
3	Cotton	Turkey
4	Enzyme – linked Immunosorbent Assays (ELISA)	Germany
5	spectrophotometer	Spain
6	Eppendorf tubes (1.5 ml)	Germany
7	Frozen deep freeze	Germany
8	Gloves	China
9	Gel tubes	Jordan
10	Micro pipettes	China
11	Power Spin TM Centrifuge	Japan
12	Syringe	Jordin
14	Tips (10ml,20ml, 100ml and 200 ml)	China
15	Plain tubes	China
14	Cold box	China
15	Micro pipettes (10ml,20ml, 100ml and 200 ml)	Germany
16	Staining rackes	China
17	Test tubes for dilution	China
18	Bio Systems A-15	Spain

3.1.4 Laboratory kits:

The laboratory kits were used in this study and shown in table (3. 2)

Table (3. 2) Kits and their supplies

NO	Kits	Origin
1	ACE2	China
2	Renin	China
3	angiotensin I	China
4	angiotensin II	China
5	AVP	China
6	ALD	China
7	ET -1	China
8	Serum albumin	China
9	TP	China
10	LDH	China
11	ALT	China
12	AST	China
13	ALP	China

3.1.5 Diagnostic kits

3.1.5.1 Elisa kit

The contents of Elisa kits shown in the following:

Table (3.3) ACE2 Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：270 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.4) Renin Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	١.5ml×1 bottle
6	Standard：270 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.5) Angiotensin I Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：540 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.6) Angiotensin ii Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：540 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.7) Aldosterone Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：540 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.8) AVP Elisa kit components

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：22.5 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

Table (3.9) Endothelin-1 Elisa kit

No.	Item	Specifications
1	User manual	1
2	Closure plate membrane	2
3	Microelisa stripplate	96 well plate
4	Sealed bags	1
5	Standard diluent	1.5ml×1 bottle
6	Standard：180 pg/ml	0.5ml×1 bottle
7	Sample diluent	6ml×1 bottle
8	HRP-Conjugate reagent	6ml×1 bottle
9	wash solution	20ml(30X) ×1 bottle
10	Stop solution	6ml×1 bottle
11	Chromogen solution A	6ml×1 bottle
12	Chromogen solution B	6ml×1 bottle

3.1.5.2 BioSystems Automated Kits

The contents of BioSystems kits are listed as the following:

a- Total protein BioSystems kit components

A. Reagent 10×50 mL. Copper (II) acetate 6 mmoL/L, potassium iodide 12 mmoL/L, sodium hydroxide 1.15 mol/L, detergent are the ingredients in the reagent.

Additional Equipment

Analyzer, spectrophotometer or photometer able to read at 545 ± 10 nm.

b- Serum albumin BioSystems kit components:

Reagent. 5×50 Ml. Acetate buffer 100 mmol / L, bromocresol green 0.27 mmol / L, detergent, pH 4.1

Additional Equipment

Analyzer, spectrophotometer or photometer able to read at 630 nm (610).

c- LDH BioSystems kit components:

- Reagent: lactate 62.5 mmol/L, pH 9.4, N-Methyl-D- glucamine 0.406 mol/L.
- Reagent: NAD+ 50 mmol/L

Additional Equipment:

Analyzer, photometer or spectrophotometer with a thermosettable cuvette at 30 or 37oC for readings at 340 nm.

Cuvettes with 1.0 cm light path.

d- ALT BioSystems Kit Components:

- Reagent: Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, Ph 7.3.

- Reagent: NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

Supplementary Apparatus:

Analyzer, spectrophotometer, or photometer with a cell holder thermostat that can read at 340 nm and can operate at 30 or 37 °C. 1 cm light path in the cuvettes.

e- AST BioSystems Kits Components:

- Reagent: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, Sodium hydroxide 255 mmol/L, pH 7.8.
- Reagent: NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

Additional Tools:

Include an analyzer, spectrophotometer, or photometer with a cell holder that can read at 340 nm and can be thermostated at 30 or 37 ºC .

f- ALP BioSystems Kit Components:

- Reagent: 0.4 mol/L of 2-amino-2-methyl-1-propanol, 1.2 mmol/L of zinc sulfate, and N-hydroxyethylethylenediaminetriacetic Mg acetate 2.5 mmol / L, pH 10.4, acid 2.5 mmol / L.
- Reagent: 60 mmol/L of 4-Nitrophenylphosphate.

Additional Equipment:

Analyzer, spectrophotometer or photometer with cell holder thermostat able at 25, 30 or 37º C and able to read at 405 nm. Cuvettes with 1 cm light path.

3.2 Methods

3.2.1 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 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 hormones and the other parameters**.**

3.2.2 Determination of RAS System components:

3.2.2.1 Determination of ACE2:

ACE2 was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human ACE2 kit (Chang *et al.*, 2020).

3.2.2.1.1 The principle:

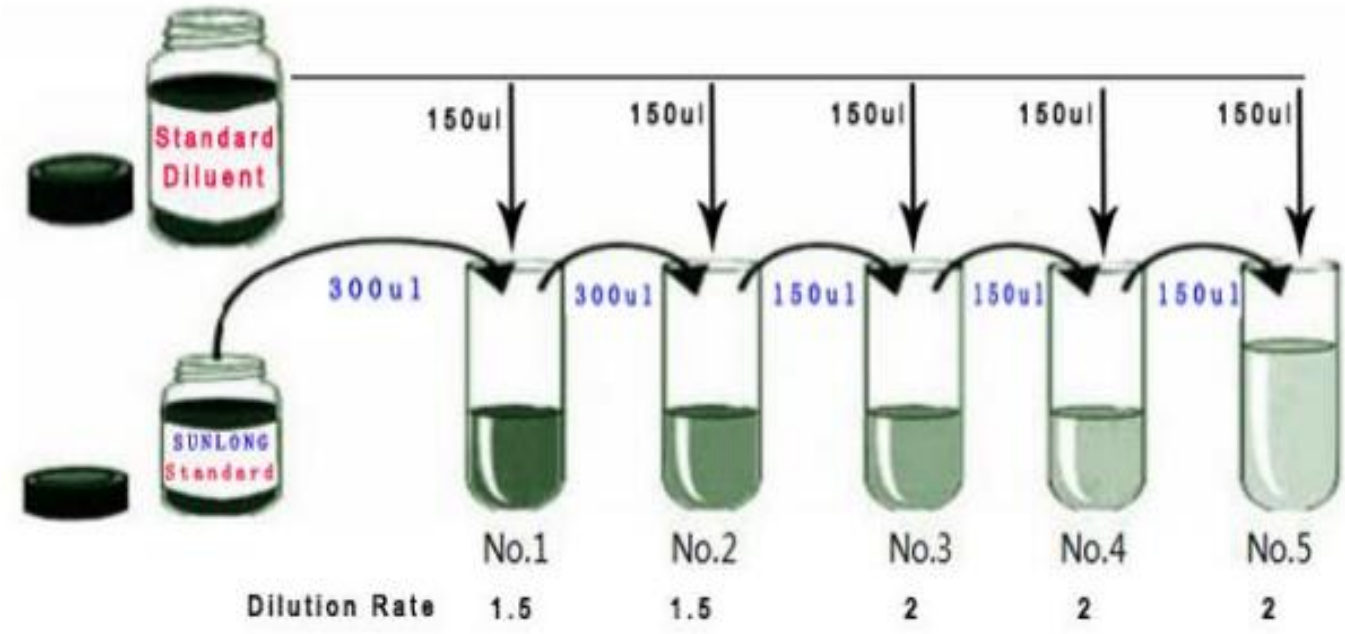
This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to ACE2 Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for ACE2 is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain ACE2 and HRP conjugated ACE2 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of ACE2 You can calculate the concentration of ACE2 in the samples by comparing the OD of the samples to the standard curve.

3.2.2.1.2 The procedure:

1. Dilution of Standards:

Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37°C after sealed with Closure plate membrane.

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

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.

7. Incubation as described in Step 3.

8. Washing as described in Step 5.

9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.

10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.2.2.2 Determination of Renin Enzyme:

Renin was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human renin kit (Derkx *et al.,* 1987; Wu *et al.,* 2019).

3.2.2.2.1 The principle

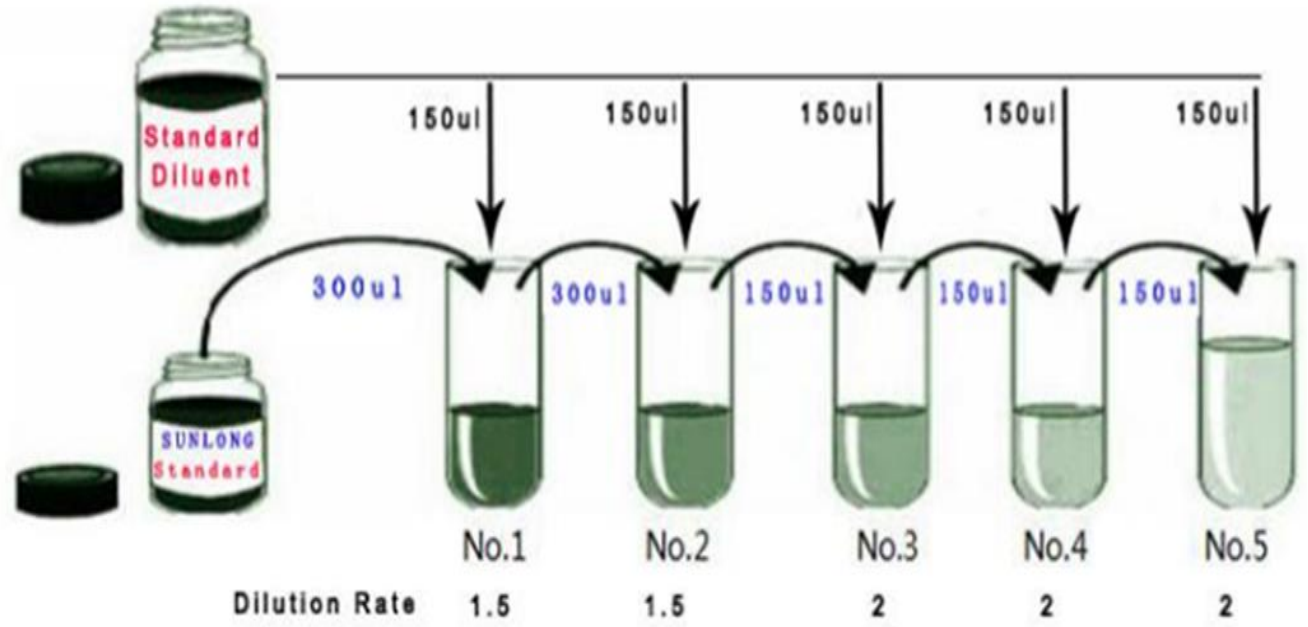
This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to renin Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for renin is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain renin and HRP conjugated renin antibody will appear blue in color and then turn yellow after the addition of the

stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of renin You can calculate the concentration of renin in the samples by comparing the OD of the samples to the standard curve.

3.2.2.2.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. The control well should be left empty on the micro Elisa strip plate, the bottom should be loaded with samples of the sample wells without touching the wall of

the well, then gently shaken to properly blend with the 40 µl of dilution buffer and 10 µl of sample (the factor of dilution is 5) that are added.

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, The OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.2.3 Determination of Angiotensin I:

Angiotensin I was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human angiotensin I kit (Derkx *et al.,* 1987).

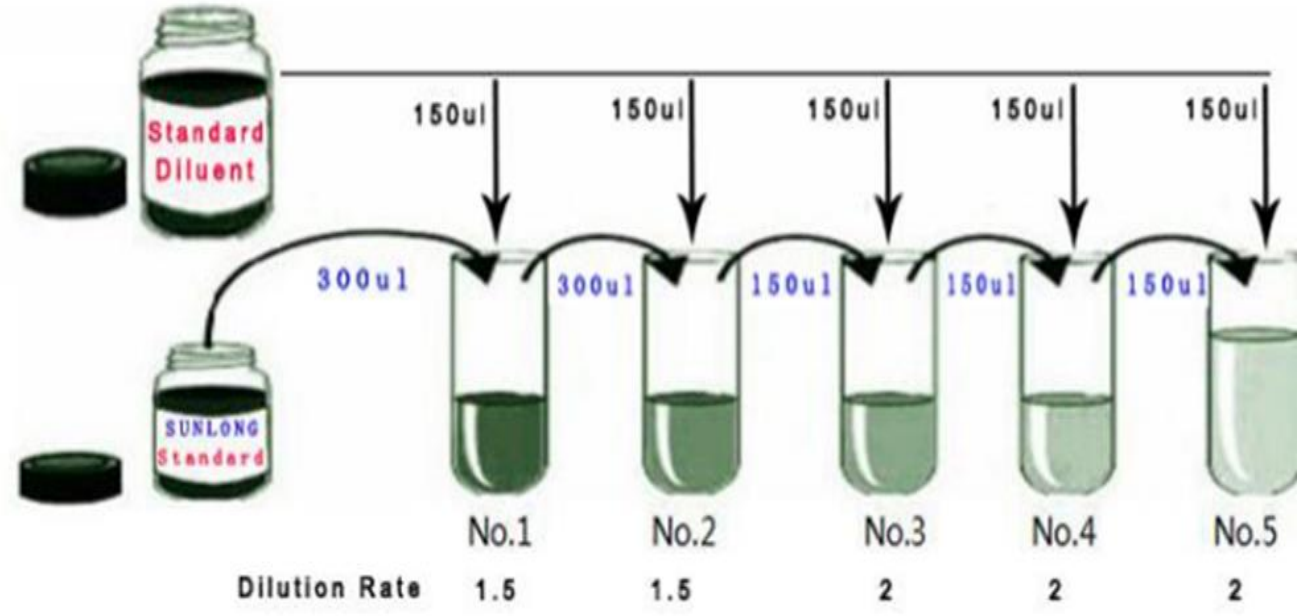
3.2.2.3.1 The principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate providedin this kit has been pre-coated with an antibody specific to Ang I Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Ang I is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain Ang I and HRP conjugated Ang I antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of Ang I,You can calculate the concentration of Ang I in the samples by comparing the OD of the samples to the standard curve.

3.2.2.3.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2**.** The control well should be left empty on the micro Elisa strip plate, the bottom should be loaded with samples of the sample wells without touching the wall of the well, then gently shaken to properly blend with the 40 µl of dilution buffer and 10 µl of sample (the factor of dilution is 5) that are added.

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. Incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, The OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.2.4 Determination of Angiotensin II:

Angiotensin II was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human angiotensin II kit (Chappell *et al.*, 2021).

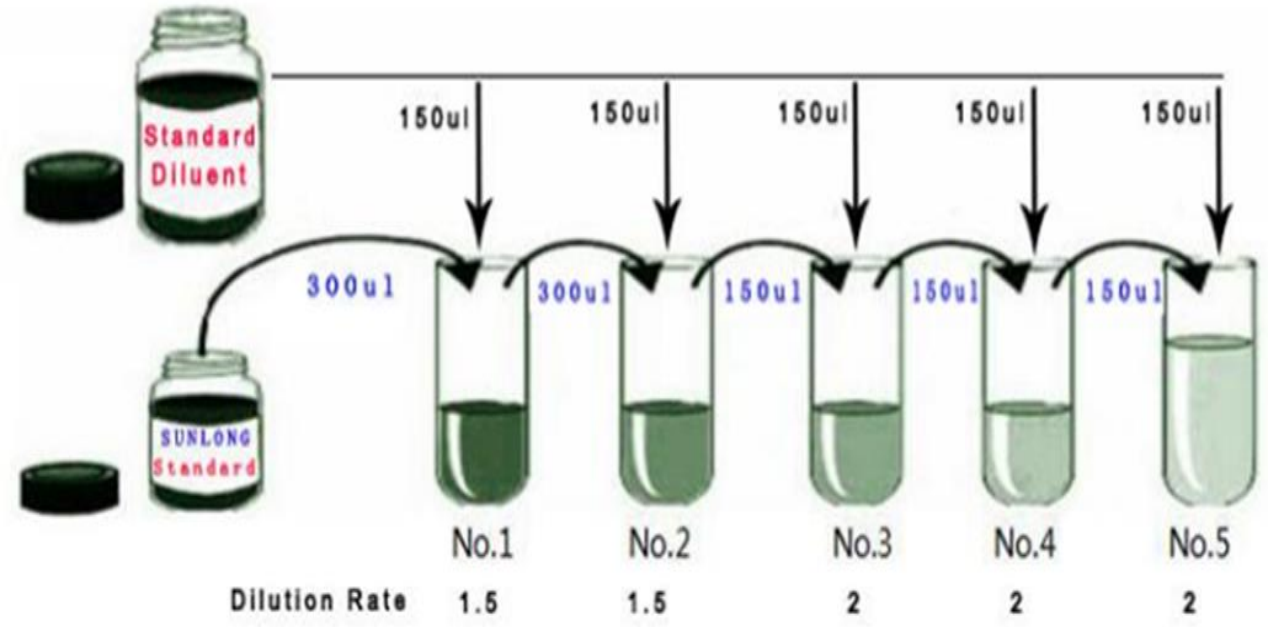
3.2.2.4.1 The principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate providedin this kit has been pre-coated with an antibody specific to Ang II Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Ang II is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain Ang II and HRP conjugated Ang II antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of Ang II, you can calculate the concentration of Ang II in the samples by comparing the OD of the samples to the standard curve.

3.2.2.4.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. The control well should be left empty on the micro Elisa strip plate, the bottom should be loaded with samples of the sample wells without touching the wall of the well, then gently shaken to properly blend with the 40 µl of dilution buffer and 10 µl of sample (the factor of dilution is 5) that are added.

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. Incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, the OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.2.5 Determination of Aldosterone (ALD):

ALD was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human ALD kit (Lun *et al.,* 1983; Stabler *et al.*, 1991).

3.2.2.5.1 The principle:

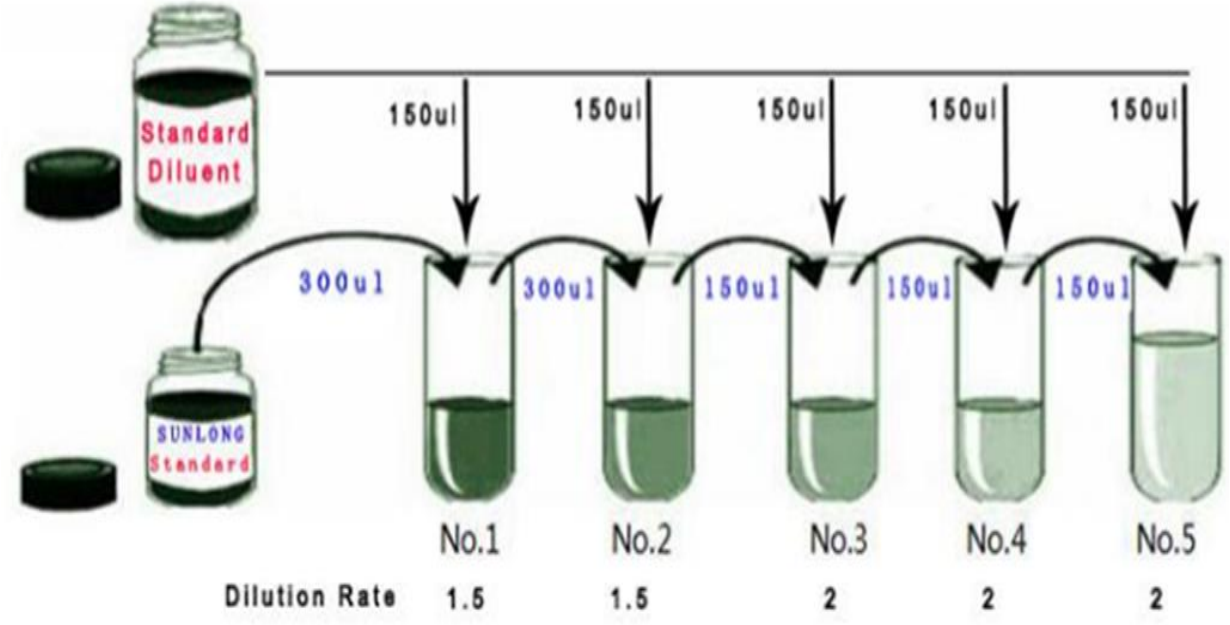
This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to ALD Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for ALD is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is

added to each well. Only those wells that contain ALD and HRP conjugated ALD antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of ALD, you can calculate the concentration of ALD in the samples by comparing the OD of the samples to the standard curve.

3.2.2.5.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. The control well should be left empty on the micro Elisa strip plate, the bottom should be loaded with samples of the sample wells without touching the wall of the well, then gently shaken to properly blend with the 40 µl of dilution buffer and 10 µl of sample (the factor of dilution is 5) that are added.

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. Incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, The OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.3 Determination of Biochemical and Hormonal parameters:

3.2.3.1 Determination Vasopressin (AVP):

Vasopressin was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human AVP kit (Mohamed *et al.*, 2015; Jeong *et al.*, 2020).

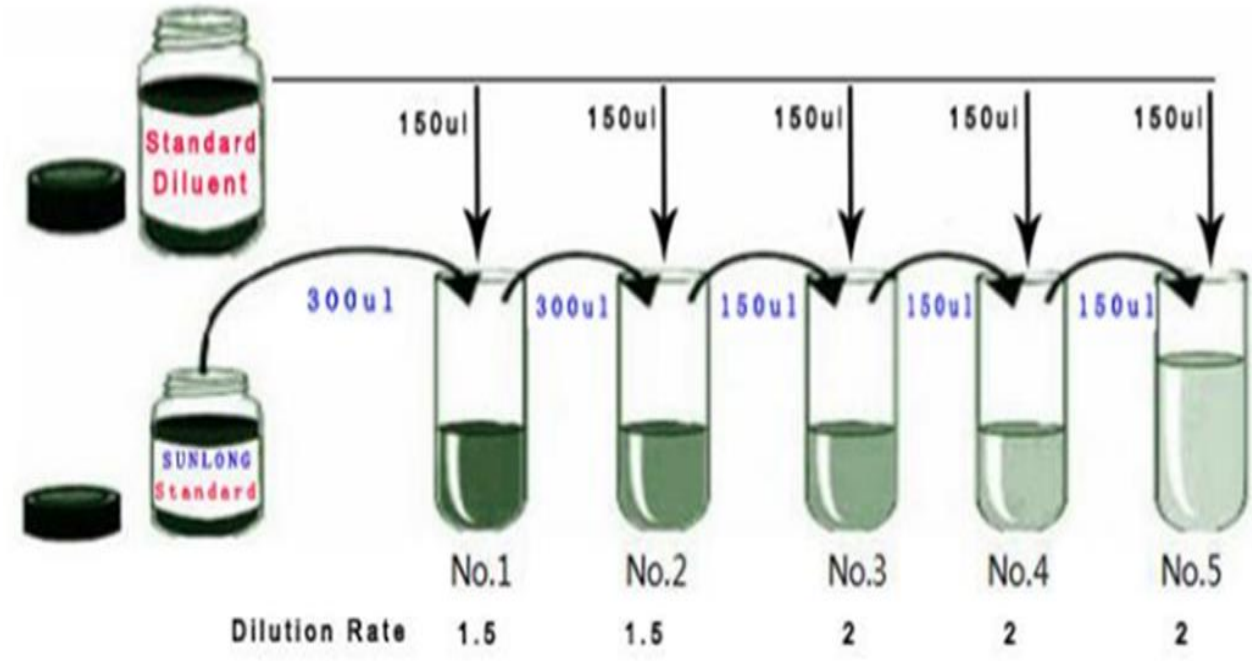
3.2.3.1.1 The principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to AVP Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for AVP is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain AVP and HRP conjugated AVP antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of AVP, you can calculate the concentration of AVP in the samples by comparing the OD of the samples to the standard curve.

3.2.3.1.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. In the microelisa stripplate, leave a well empty as blank control. In sample wells, 40 µl sample dilution buffer and 10 µl of sample are added (dilution factor 5). Sample should be loaded onto the bottom without touching the wall of the well, Mix well with gentle shaking .

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. Incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, The OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.3.2 Determination of ET-1:

ET-1 was evaluated by using enzyme-linked-immunosorbent-assay (ELISA) system, with human ET-1 kit (Bakrania *et al.*, 2018; Yoshida *et al.*, 2020).

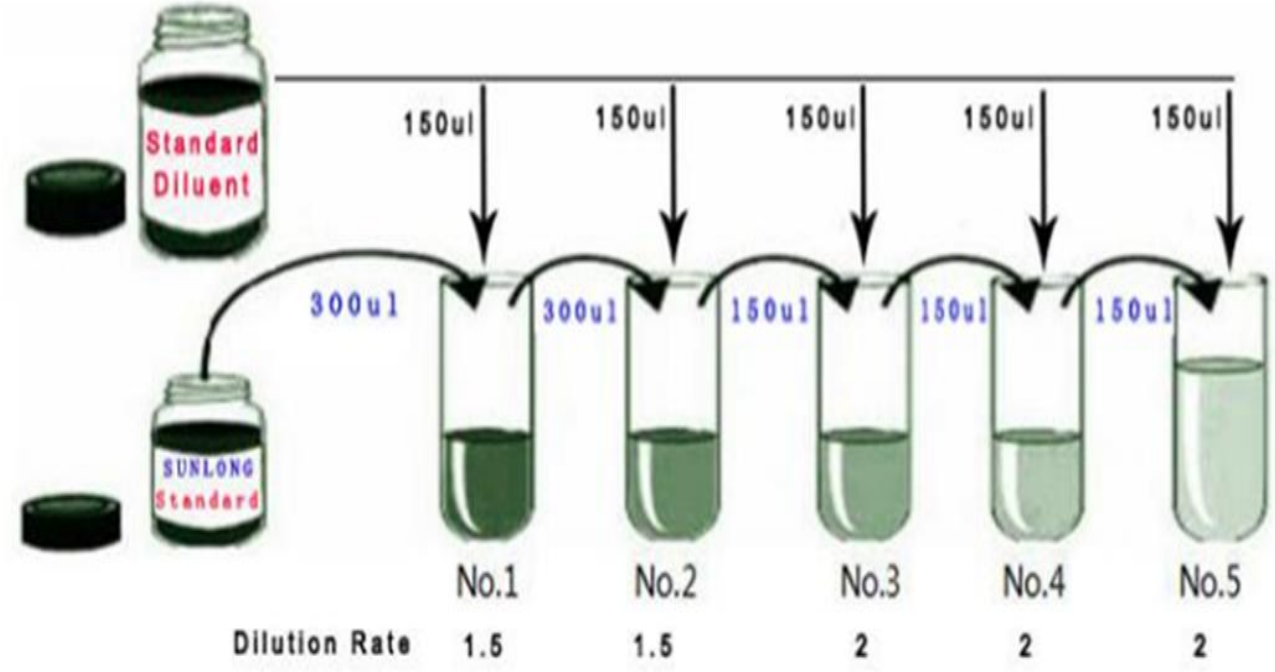
3.2.3.2.1 The principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to ET-1 Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for ET-1 is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain ET-1 and HRP conjugated ET-1 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of ET-1 You can calculate the concentration of ET-1 in the samples by comparing the OD of the samples to the standard curve.

3.2.3.2.2 The procedure:

1. The standards dilution: Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

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



2. The control well should be left empty on the micro Elisa strip plate, the bottom should be loaded with samples of the sample wells without touching the wall of the well, then gently shaken to properly blend with the 40 µl of dilution buffer and 10 µl of sample (the factor of dilution is 5) that are added.

3. Thirty minutes of incubation at 37 °C once, the membrane on the closure plate has been shut up.

4. Diluting: To diluted the concentrated washing buffer, use distilled water (20 times for 48T and 30 times for 96T).

5. Cleaning: From the closure plate, take off the membrane aspirate gently and refill with this solution, discard the wash solution after it has settled for 30 seconds during the washing procedure, five times.

6. Fifty µl of HRP-Conjugate reagent should be added to every well, excluding the blank control one.

7. Incubated, as stated in step three and cleaned, as stated in step five.

8. For coloring, add 50 µl of chromogen solution A and 50 µl of chromogen solution B to each well, gently stir, and incubate for 15 minutes at 37 °C and turn off the light during coloring.

9. To halt the process, add 50 µl of stop solution to every well, the color of the well must change from blue to yellow.

10. In order to read the absorbance O.D, at 450 nm, use a microtiter plate reader, The OD value for the empty control well is zero, the assay must be finished right away once the stop solution has been added.

3.2.4 Other related enzyme:

3.2.4.1 Determination of total protein:

Total protein was evaluated by using spectrophotometer BioSystem automated, with human total protein kit (Gornall *et al.,* 1949).

3.2.4.1.1 The principle:

In an alkaline solution, protein within the sample combines with the ion copper II to produce a colorful complex that may be detected by spectrophotometry.

3.2.4.1.2 Preparing the reagent:

Standard and reagent are offered prepared for usage.

3.2.4.1.3 The procedure:

1. Pipette into labelled test tubes:

	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. 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.

3.2.4.1.4 Calculations:

The following formula is used to calculate the sample's protein concentration**:**

A sample / A standard x C standard = C sample

3.2.4.2 Determination of Serum Albumin:

Albumin was evaluated by using spectrophotometer BioSystem automated, with human albumin kit (Doumas *et al.,* 1971).

3.2.4.2.1 The principle:

Albumin in the sample reacts with bromocresol green in acid medium forming a colored complex that can be measured by spectrophotometry.

3.2.4.2.2 Preparing the Reagent:

Standard and reagent are offered ready for use.

3.2.4.2.3 The procedure:

1. Pipette into labelled test tubes.

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

2. Mix thoroughly and let stand the tubes for 1 minute at room temperature.

3. Read the absorbance (A) of the Standard and the Sample at 630 nm against the blank, the color is stable for 30 minutes.

3.2.4.2.4 Calculations:

The amount of albumin in the sample is calculated using the general formula below:

C sample / A standard = A sample x C standard

3.2.4.3 Determination of LDH Enzyme:

LDH was evaluated by using spectrophotometer BioSystem automated, with human LDH kit (Maftouh *et al.*, 2014; Ariceta *et al.*, 2021).

$$\text{Lactate} + NAD^{+} \xrightarrow{LDH} \text{Pyruvate} + NADH$$

3.2.4.3.1 The principle:

LDH enables NAD+ to oxidize lactate, producing pyruvate and NADH. The rate at which NADH appears is measured at 340 nm, and this value is used to calculate the catalytic concentration.

3.2.4.3.2 Preparing the reagent:

Active reagent: Empty the reagent B with the contents of reagent B a container. Shake gently: If you want to prepare other volumes, mix 4 mL of reagent A plus 1 mL of reagent B, in that order, three days of stability at 2 to 80ºC.

3.2.4.3.3 The producer:

1. Prewarm working reagent and instrument to reaction temperature.

2. Pipette into a cuvette**.**

Working Reagent Sample	**1.0Ml** **25 Ml**

3. Mix and insert the cuvette into the photometer. Start the stopwatch.

4. After 30 seconds, record the initial absorbance and take new readings every minute for 3 minutes.

5. Calculate the average absorbance increase per minute (ΔA/min).

3.2.4.3.4 Calculations:

The following general formula is used to determine the amount of LDH present in the sample:

$$\Delta A / min \times vt \times 10^6 / \varepsilon x1 \times Vs = U / L$$

The sample volume (Vs) is equal to 0.025 mL, the total reaction volume (Vt) is equal to 1.025 mL, the molar absorption coefficient (ε) of NADH at 340 nm is 6300, and the light path (l) is 1 cm. The subsequent elements are deducted to calculate the catalyst concentration:

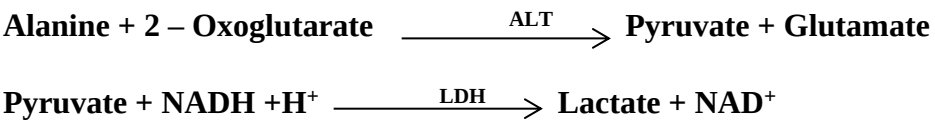
ΔA/min	**x 6508 = U /L** **x 108 = µkat /L**

3.2.4.4 Determination of ALT enzyme:

ALT was evaluated by using spectrophotometer BioSystem automated, with human ALT kit (Gella, 1985; Clínica *et al.*, 1987).

3.2.4.4.1 The principle:

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction.



3.2.4.4.2 Preparing the reagent:

- Working reagent: Fill the reagent A bottle with the reagent B's contents. Mix slowly. The following ratio can be used to prepare other volumes: four mL reagent (A + 1) mL reagent B. stable around 2 to 8 ºC for two months.

- Pyridoxal phosphate working reagent (Note 1): combine as follows: working reagent (10 ML) with reagent C (0.1 mL) (cod 11666). 6 days of stability at 2 to 8 ^{o}C**.**

3.2.4.4.3 The technique:

1- Warm up the instrument and the working reagent to reactional temperatures.

2- into a cuvette using a pipette**.**

Reaction temperature	**37 ºC**	**30 ºC**
Active Reagent	**1.0 Ml**	**1.0 Ml**
Sample	**50 µL**	**100 µL**

3. Place the cuvette inside the photometer after mixing, set the stopwatch to start.

4. Start the record the first absorbance after 1 minute (Note 1) and then every 1 minute after that for 3 minutes.

5. Calculate the average absorbance difference per minute (A/min) and the difference between two successive absorbances.

3.2.4.4.4 Calculations:

The ALT/GPT concentration in the sample is calculated using the following general formula:

$$\Delta A / min \times vt \times 106 / \varepsilon x1 \times Vs = U/L$$

The sample volume (Vs) is 0.05 at 37°C and 0.1 at 30°C, and 1 U/L are 0.0166 kat/L. The whole reaction volume (Vt) is 1.05 at 37°C and 1.1 at 30°C, and the molar absorbance of NADH at 340 nm is 6300. The following formulas are derived in order to determine the catalytic concentration:

	37 ℃	30 ℃
Δ A/min	x 3333 = U/L x 55.55 = µ kat/L	x 1746 = U/L x 29.1 = µ kat/L

3.2.4.5 Determination of AST enzyme

AST was evaluated by using spectrophotometer BioSystem automated, with human AST kit

The Principle (Gella *et al.*, 1985; Clínica *et al.*, 1987).

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2- oxoglutarate, forming oxaloacetate and glutamate, the catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

Aspartate + 2 – Oxoglutarate $\xrightarrow{\text{AST}}$ **Oxaloacetate + Glutamate**

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ **Malate + NAD^+**

3.2.4.5.1 Preparing the reagent:

Reagents are offered in a functional, ready state.

- Fill the reagent A bottle with the reagent B contents, mix slowly, you can prepare additional volumes in the following ratio: reagent A: 4 mL; Reagent B: 1 mL. 1 month of stability at 2 to 8 °C.

- Working with pyridoxal phosphate in reagent (Note 1): Combine as follows: working reagent 10 mL + reagent C 0.1 mL (cod 11666). enduring for six days at 2-8ºC (Clínica *et al.*,1987).

3.2.4.5.2 The technique

1- Raise the temperature of the working reagent and the instrument to that of the reaction.

2- Within a cuvette, pipette.

Reaction temperature	**37 °C**	**30 °C**
Active Reagent	**1.0 mL**	**1.0 Ml**
Sample	**50 µL**	**100 µL**

3- After mixing, in the photometer place the cuvette. Put the stopwatch on.

4- Start recording the first absorbance, after 1 minute (Note 1) and then every 1 minute after that for the next 3 minutes.

5- Determine the average absorbance difference per minute (A/min) and the difference between two successive absorbances.

3.2.4.5.3 Calculations:

The following general formula is used to determine the amount of AST present in the sample:

$$\Delta A / min \times vt \times 106 / \varepsilon x1 \times Vs = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37ºC and 1.1 at 30ºC, the sample volume (Vs) is 0.05 at 37ºC and 0.1 at 30ºC, and 1 U/L are 0.0166 µ kat /L. The following formulas are deduced for the calculation of the catalytic concentration:

	37 ℃	30 ℃
ΔA/min	x 3333 = U/L x 55.55 =μ kat/L	x 1746 = U/L x 29.1 = μ kat/L

3.2.4.6 Determination of ALP enzyme:

ALP was evaluated by using spectrophotometer BioSystem automated, with human ALP kit

(Rosalki *et al.,* 1993).

3.2.4.6.1 The principle:

ALP catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol, the catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm (Rosalki, 1993).

4 Nitrophenylphosphate + AMP $\xrightarrow{\text{ALP}}$ **AMP – phosphate + 4 Nitrophenol**

3.2.4.6.2 Preparing the Reagent:

Active reagent:

- Cod: 11593 and 11592; Carefully combine the contents of a bottle of reagent A with one vial of reagent B. and prepare more quantities according to the following ratio: reagent A: 4 mL, reagent B: 1 mL stable around 2 to 8 ºC for two months.
- Cod. 11598: pour 25mL from one vial of reagent B into a bottle of reagent A, then mix gently and prepare more quantities according to the following ratio: reagent A: 4 mL; reagent B: 1 mL. stable around 2 to 8 ºC for two months.

3.2.4.6.3 The procedure:

1- Obtain reaction temperature for the tool and the working reagent and pipette within a cuvette.

The Sample of working Reagent	**1.0 mL** **20 µL**

3. Mix and insert the cuvette into the photometer.

4. Record initial absorbance and at 1-minute intervals thereafter for 3 minutes.

5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

3.2.4.6.4 Calculations:

The ALP catalytic: the following formula is used to determine the concentration in the sample:

ΔA / min x vt x 106 / εx1 xVs = U / L

The following formulas are derived for the determination of the catalytic concentration from the following data: the absorbance of molar (ε) of 4-nitrophenol at 405 nm is 18450, the light path (l) is 1 cm, the total reaction volume (Vt) is 1.02, the volume of the sample (Vs) is 0.02, and 1 U/L are 0.0166 µ kat/L.

ΔA / min	**x 2764 = U / L** **x 46.08 = µ kat / L**

3.3 Statistical Analysis:

The results are expressed as mean ± standard deviation (SD). Statistical analysis was performed by IBM SPSS statistics, version 26 was used to conduct this analysis (IBM Co., Armonk, NY, USA) and by one - way analysis of variance (ANOVA) and by used Duncan's test at (p-value ≤ 0.01) significant (Steel *et al*., 1997).

Chapter Four

Results

4.1 Angiotensin converting enzyme2:

Results revealed: ACE2 decreased significantly ($P \leq 0.01$) in second (7.200 ±0.695 p g/ml), third (6.050 ±0.944 p g/ml) and fourth groups (5.950 ±0.887 p g/ml) in comparison with the first group (12.450 ±0.887 p g/ml). ACE2 decreased significantly in both third and fourth groups when compared to a second group and declined not significantly in the fourth group compared to the third group. (Table (4.1), figure (4.1)).

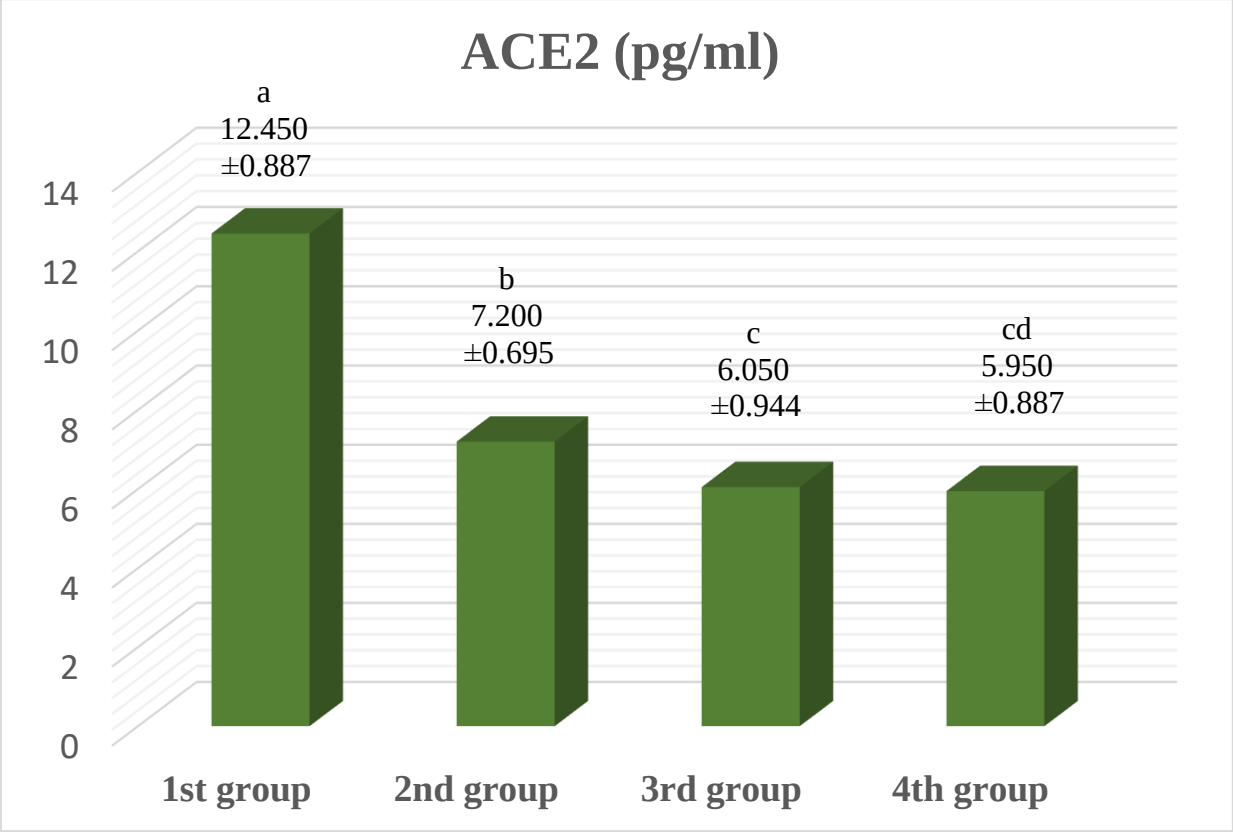


Figure (4.1) The levels of ACE2 enzyme (p g/ml) in hypertensive men among groups.

- 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.

4.2 RAAS components

4.2.1 Renin

The results revealed: Renin decreased significantly ($P \leq 0.01$) in third (13.000 ±0.858 p g/ml) and fourth groups (11.800 ±0.951 p g/ml) and not significantly in second group (16.850 ±0.933 p g/ml) in comparison with the first group (17.100 ± 0.911 p g/ml) and decreased significantly in both third and fourth groups in comparison with the second group in addition, when compared to the third group, fourth group decreased significantly. (Table (4.1), figure (4.2)).

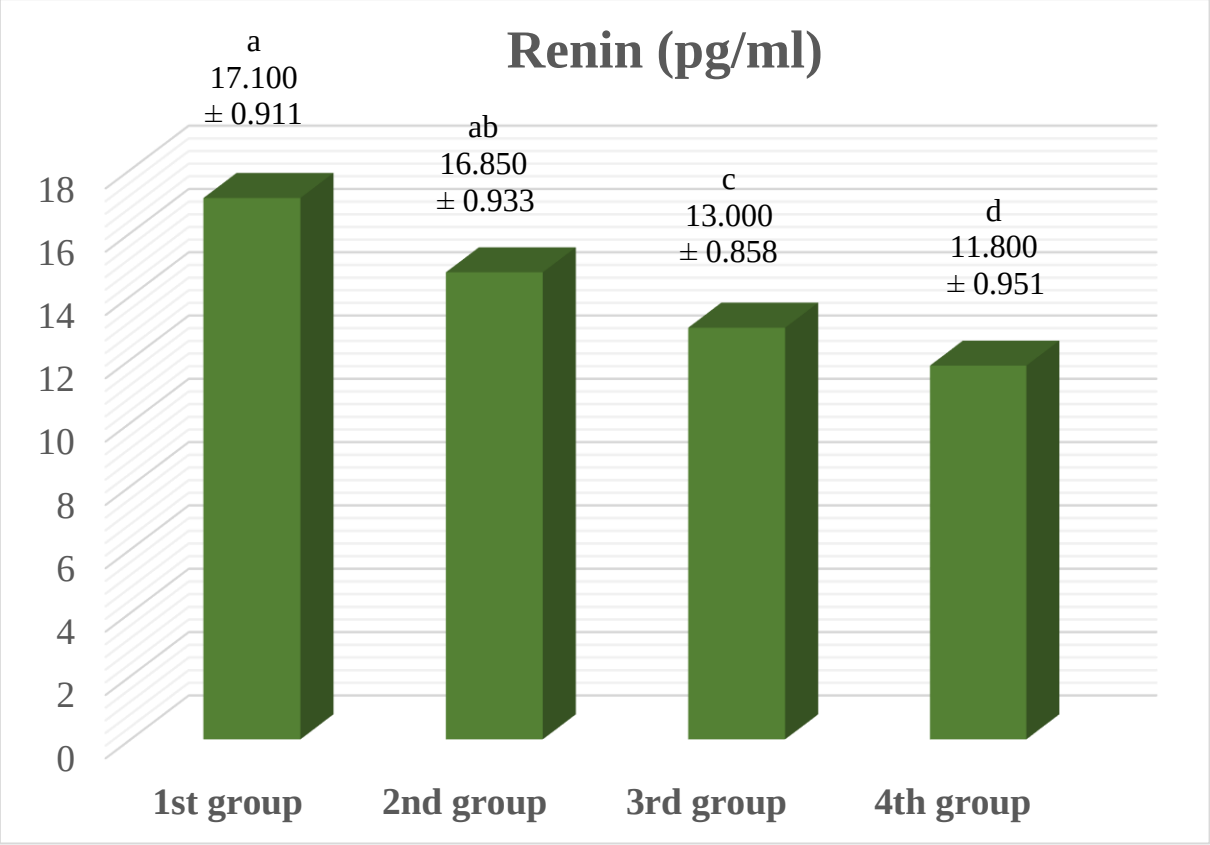


Figure (4.2) The levels of renin enzyme (p g/ml) in hypertensive men among groups.

- 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.

4.2.2 Angiotensin I:

The results revealed: Angiotensin I decreased significantly ($p \leq 0.01$) in second (23.800 ±0.833 p g/ml), third (19.950 ±0.887 p g/ml) and fourth groups (18.800 ±0.894 p g/ml) in comparison with the first group (24.850 ±0.812 p g/ml) and decreased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group decreased significantly. (Table (4.1), figure (4. 3)).

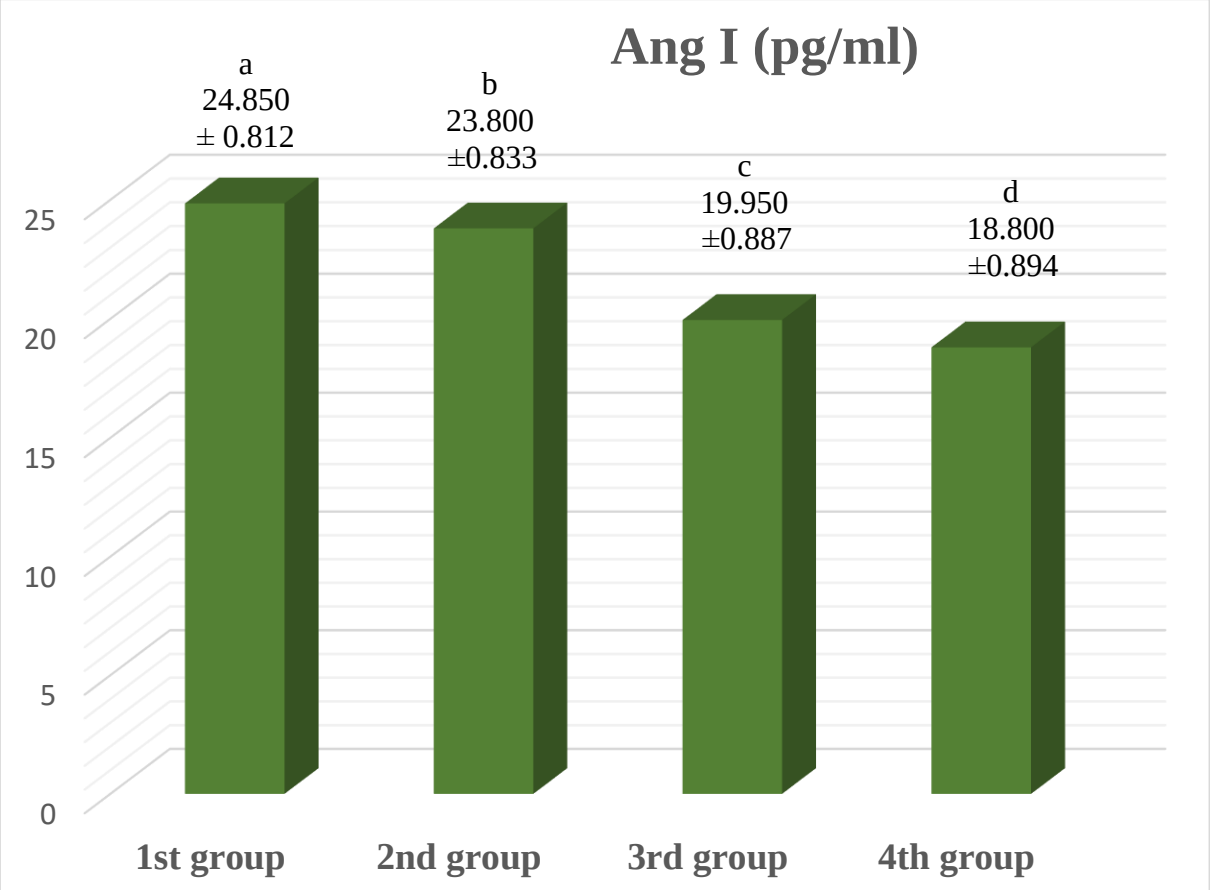


Figure (4.3) The levels of angiotensin I (p g/ml) in hypertensive men among groups.

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

4.2.3 Angiotensin II:

The results revealed: angiotensin II increased significantly ($P \leq 0.01$) in second (21.300 ±0.923 p g/ml), third (27.850 ±0.933 p g/ml) and fourth groups (31.500 ±0.945 p g/ml) in comparison with the first group (17.800 ±0.894 p g/ml) and increased significantly in both third and fourth groups in comparison with the second group in addition, when compared to the third group, fourth group increased significantly. (Table (4.1), figure (4. 4)).

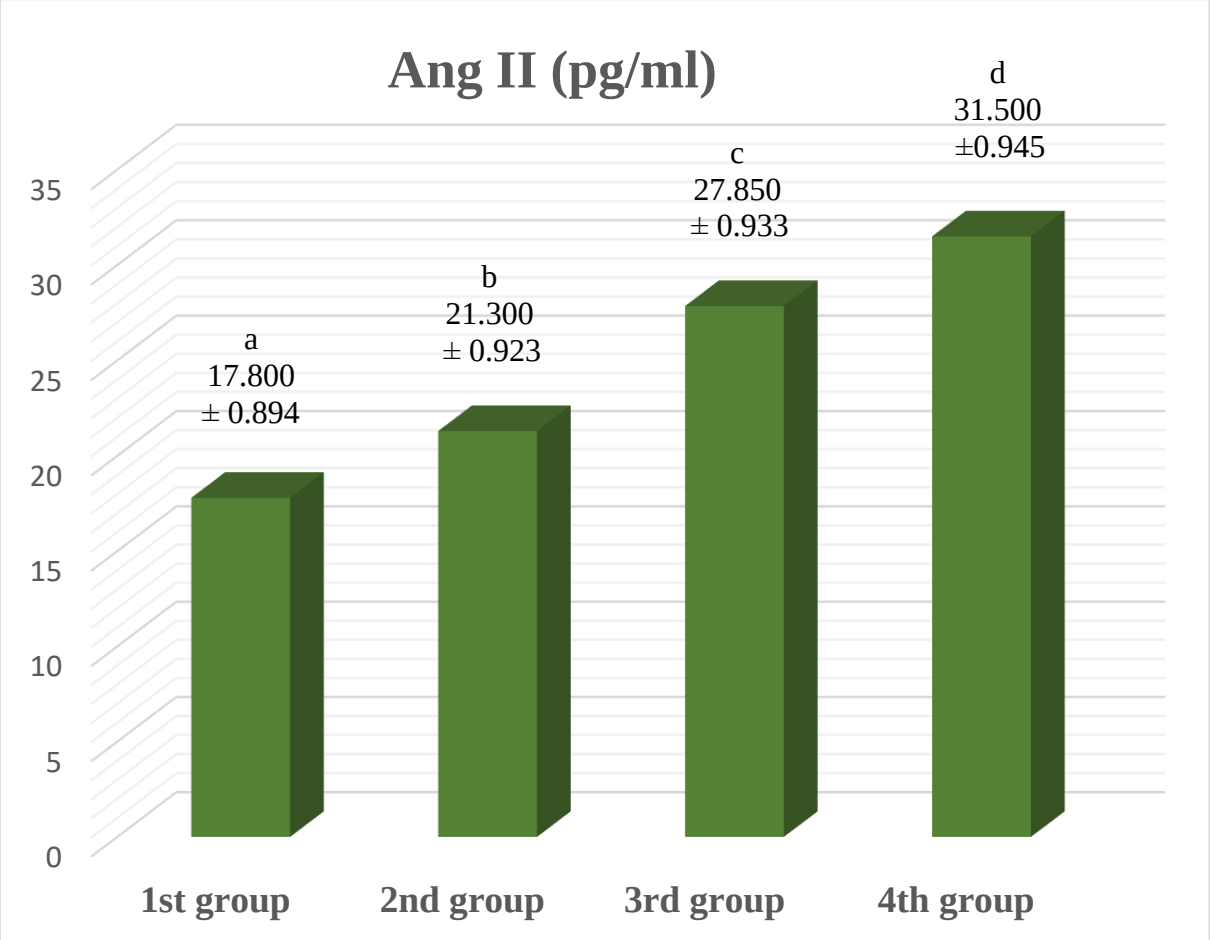


Figure (4.4) The levels of angiotensin II (p g/ml) in hypertensive men among groups.

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

4.2.4 Aldosterone:

The results revealed: aldosterone increased significantly ($p \leq 0.01$) in second (18.250 ± 0.910 p g/ml), third (37.700 ± 0.864 p g/ml) and fourth groups (37.900 ±0.911 p g/ml) in comparison with the first group (16.200 ± 0.894 p g/ml) and increased significantly in both third and fourth groups in comparison with the second group in addition, when compared to the third group, fourth group increased no significantly. (Table (4.2), figure (4.5)).

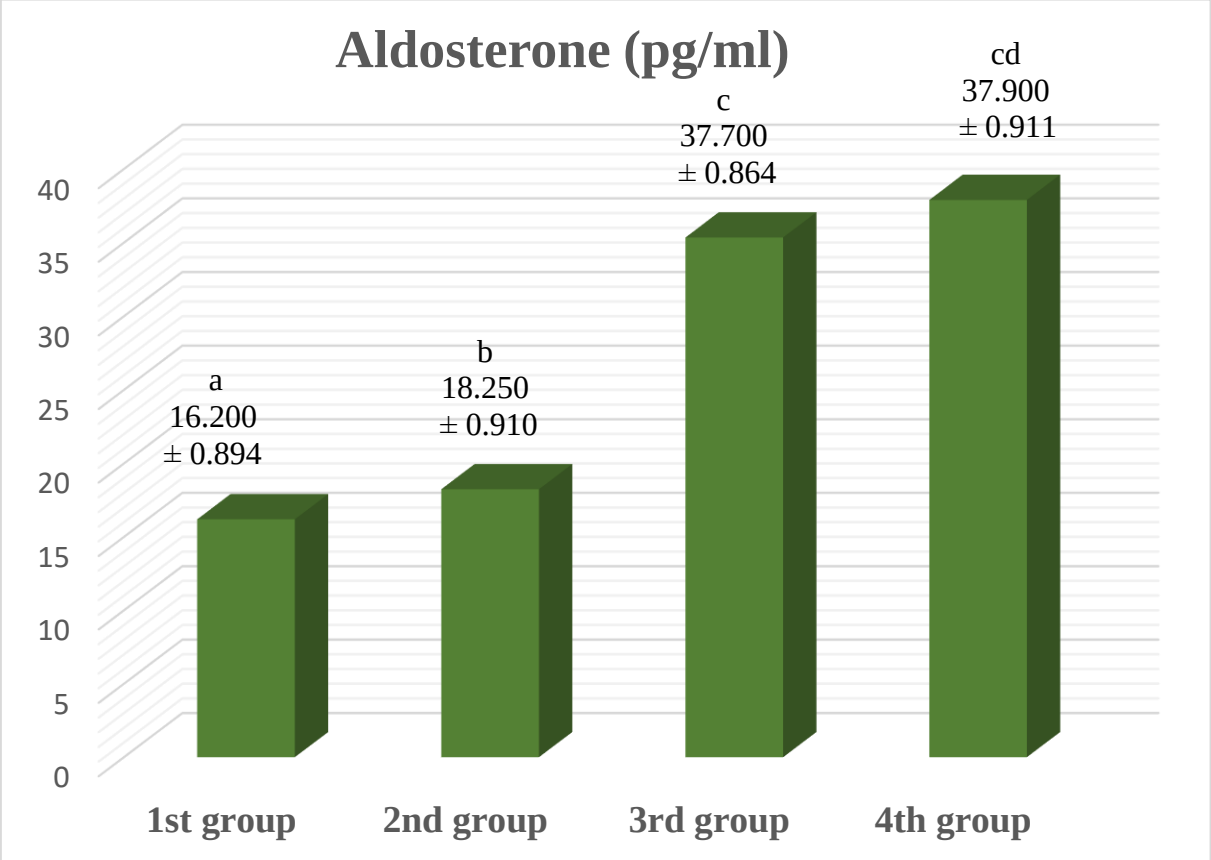


Figure (4.5) The levels of aldosterone hormone (p g/ml) in hypertensive men among groups.

- 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.

Table (4.1) The Levels of ACE2 and RAAS Components in Hypertensive Men during Different Groups.

Groups	Parameters				
	ACE2 p g/ml	Renin p g/l	Angiotensin I p g/ml	Angiotensin II p g/ml	Aldosterone p g/ml
First group 30-39 y	a 12.450 ±0.887	a 17.100 ± 0.911	a 24.850 ±0.812	a 17.800 ±0.894	a 16.200 ± 0.894
Second group 40-49 y	b 7.200 ±0.695	ab 16.850 ±0.933	b 23.800 ±0.833	b 21.300 ±0.923	b 18.250 ± 0.910
Third group 50-59 y	c 6.050 ±0.944	c 13.000 ±0.858	c 19.950 ±0.887	c 27.850 ±0.933	c 37.700 ± 0.864
Fourth group 60-69 y	cd 5.950 ±0.887	d 11.800 ±0.951	d 18.800 ±0.894	d 31.500 ±0.945	cd 37.900 ± 0.911

- 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.

4.3 Hormonal and Biochemical Parameters

4.3.1 Vasopressin (AVP):

The results revealed: AVP increased significantly ($P \leq 0.01$) in second (4.050 ±0.998 pg/ml), third (5.800 ±0.894 pg/ml) and fourth groups (6.000 ±0.917 pg/ml) in comparison with the first group (2.950 ±0.759 pg/ml) AVP increased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group increased not significantly. (Table (4.2), figure (4.6)).

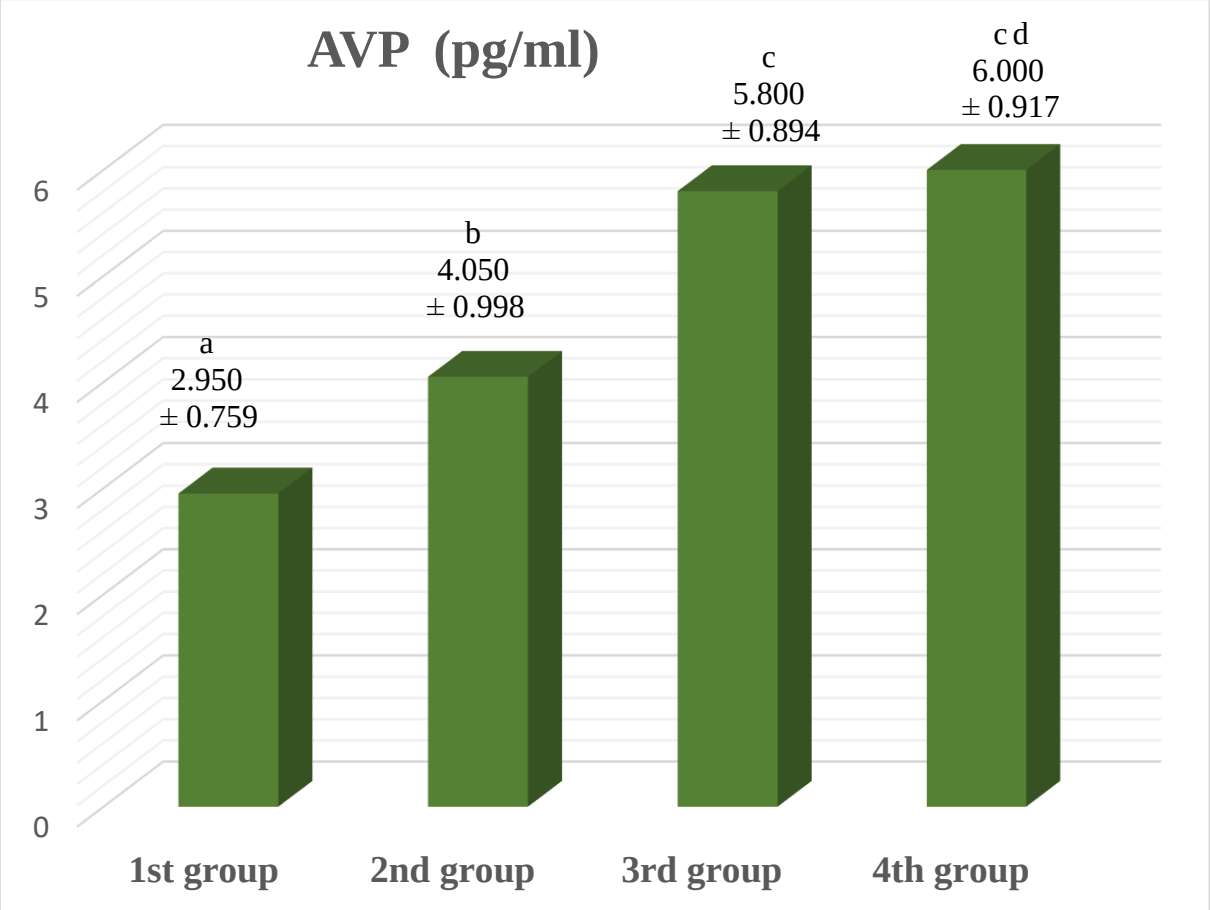


Figure (4.6) The levels of AVP hormone (p g/ml) in hypertensive men among groups.

- 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.

4.3.2 Endothelin -1 (ET-1):

The results revealed: ET-1 increased significantly ($P \leq 0.01$) in second (26.250 ±0.966 p g/ml), third (26.800 ±0.951 p g/ml) and fourth groups (29.800 ±0.833 p g/ml) in comparison with the first group (22.000 ±0.858 p g/ml) and increased not significantly in third groups in comparison with the second group, in addition, when compared to the both second and third groups the fourth group increased significantly. (Table (4.2), figure (4. 7)).

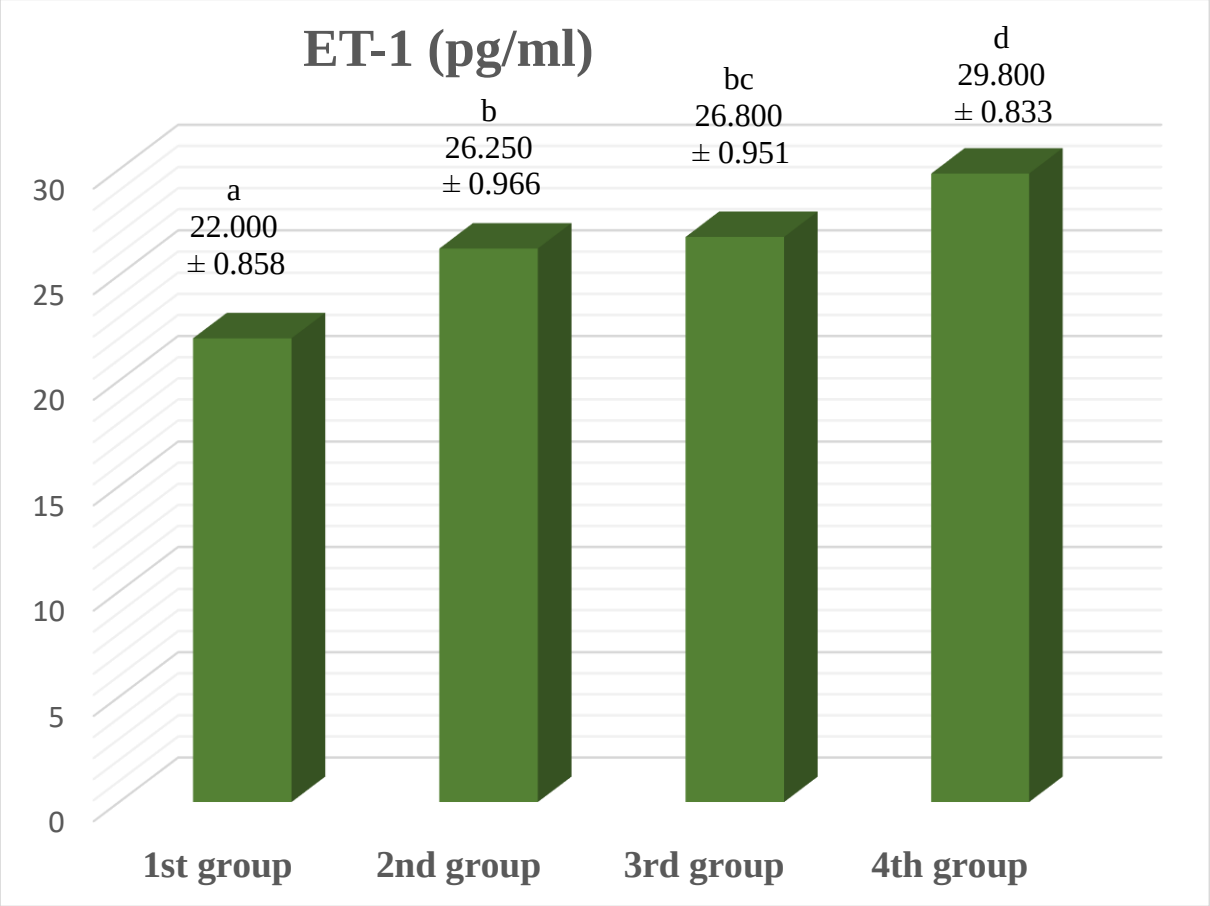


Figure (4.7) The levels of ET-1 (p g/ml) in hypertensive men among groups.

- 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.

4.3.3 Total protein (TP):

The results revealed: Total protein decreased significantly ($P \leq 0.01$) in 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, when compared to the third group the fourth group decreased significantly. (Table (4.2), figure (4.8)).

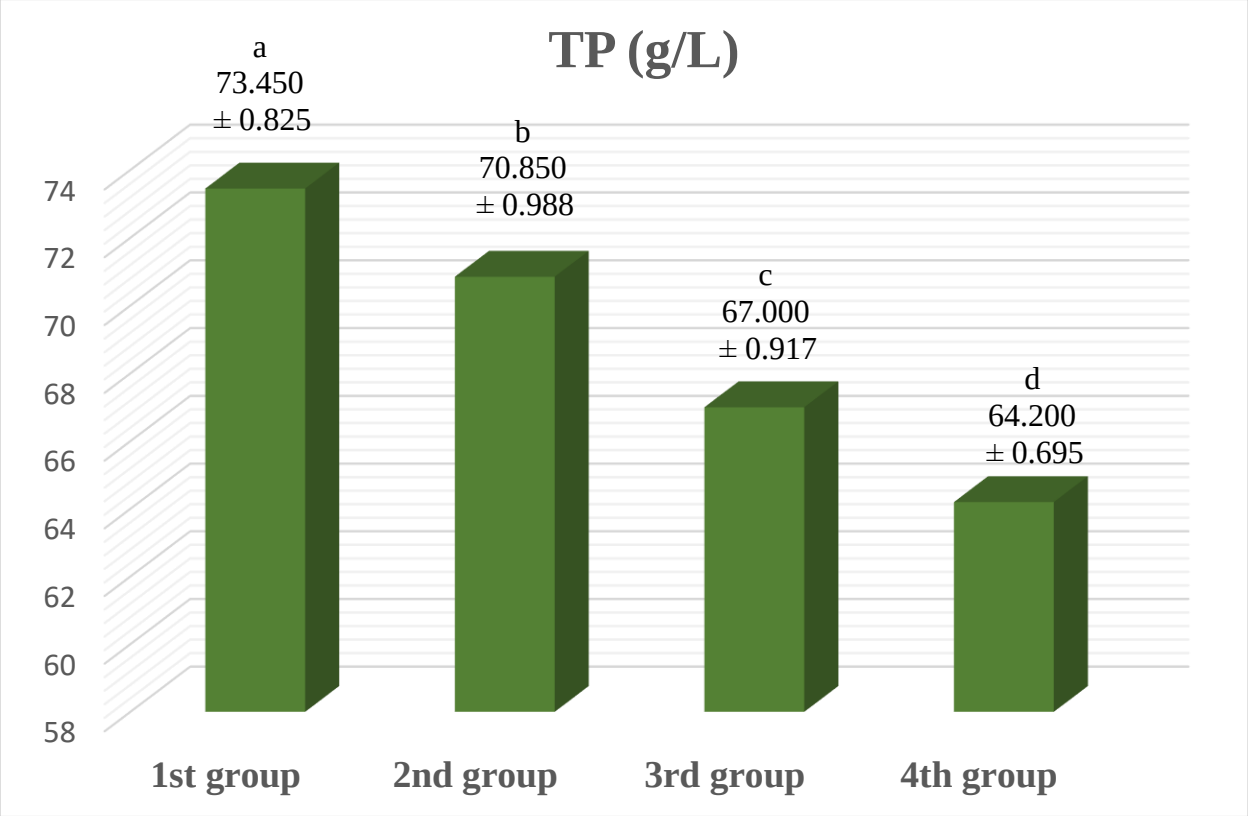


Figure (4.8) The levels of total protein (g/L) in hypertensive men among groups.

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

4.3.4 Serum Albumin:

The results revealed: serum albumin decreased significantly ($P \leq 0.01$) in 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, when compared to the both second and third groups, fourth group decreased significantly. (Table (4.2), figure (4.9)).

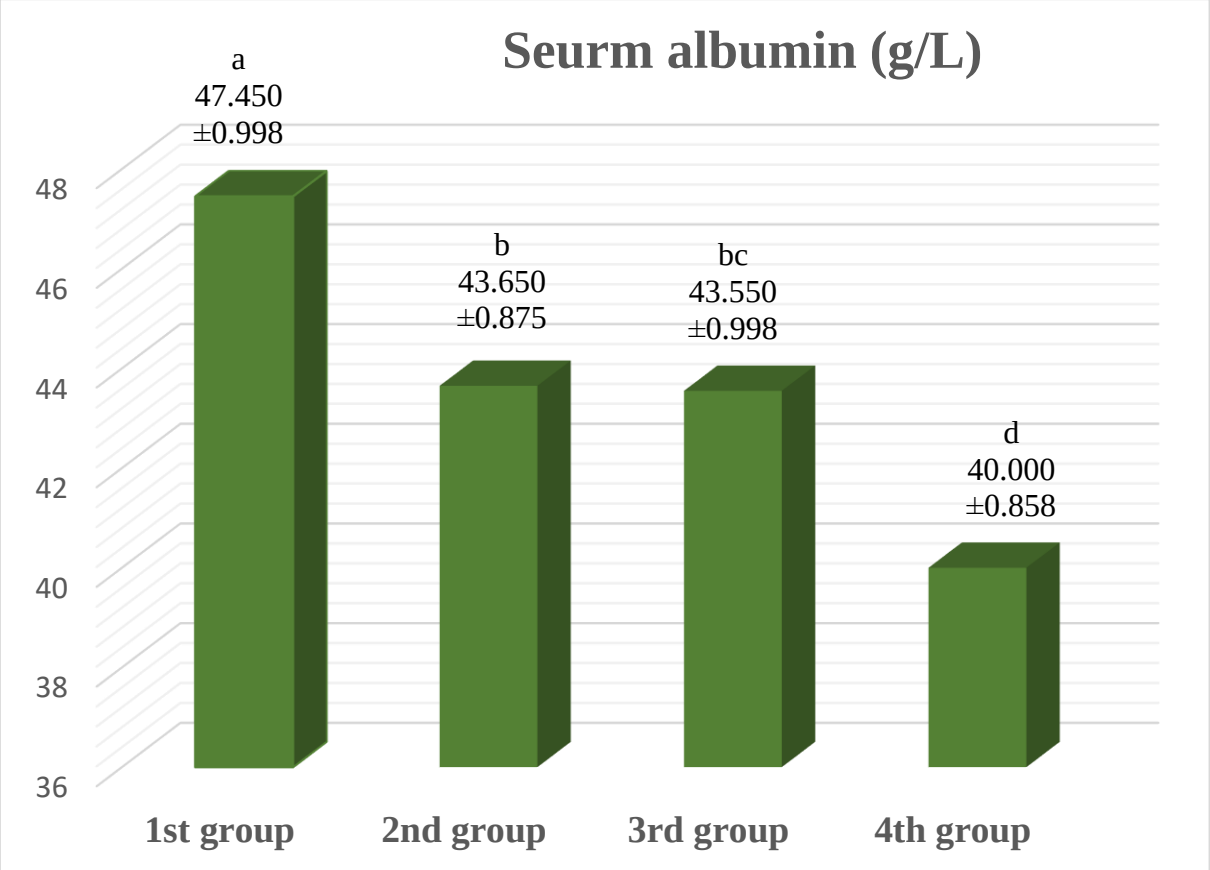


Figure (4.9) The levels of serum albumin (g/L) in hypertensive men among groups.

- 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.

Table (4.2) The Levels of AVP, ET-1, Total Protein and Serum Albumin in Hypertensive Men during Different Groups.

Groups	Parameters			
	AVP p g/ml	ET-1 p g/ml	T. P g/L	Serum albumin g/L
First group 30-39 y	a 2.950 ±0.759	a 22.000 ±0.858	a 73.450 ± 0.825	a 47.450 ± 0.998
Second group 40-49 y	b 4.050 ±0.998	b 26.250 ±0.966	b 70.850 ±0.988	b 43.650 ± 0.875
Third group 50-59 y	c 5.800 ±0.894	bc 26.800 ±0.951	c 67.000 ±0.917	bc 43.550 ± 0.998
Fourth group 60-69 y	cd 6.000 ±0.917	d 29.800 ±0.833	d 64.200 ±0.695	d 40.000 ± 0.858

- 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.

4.4 LDH and Liver Enzymes:

4.4.1 Lactate Dehydrogenase (LDH):

The results revealed: LDH increased significantly ($P \leq 0.01$ U/L) in second group (375.200 ± 2.440 U/L), third (415.700 ± 2.451 U/L) and fourth groups (494.200 ± 2.930 U/L) in comparison with the first group (302.600 ± 2.927 U/L) and increased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group increased significantly. (Table (4.3), figure (4.10)).

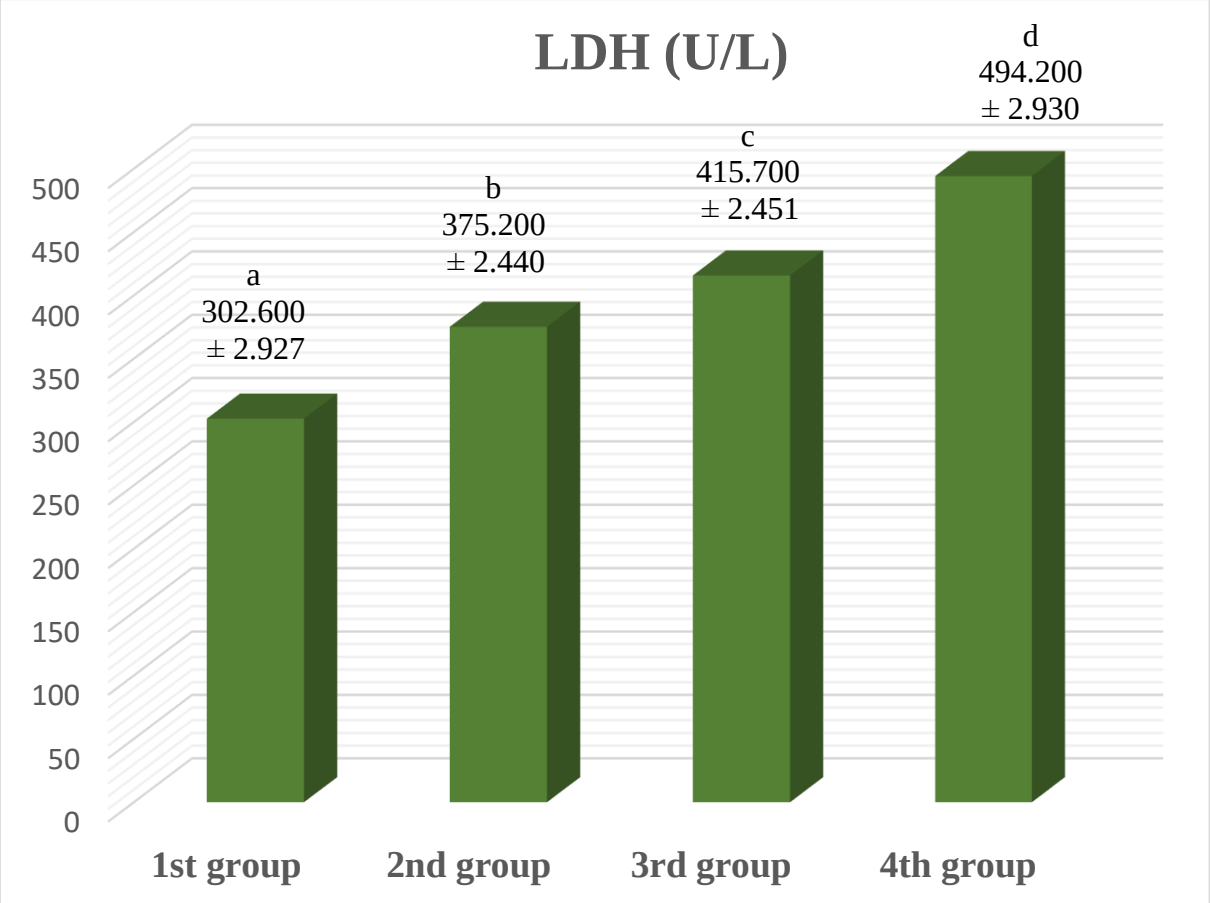


Figure (4.10) The levels of LDH enzyme (U/L) in hypertensive men among groups.

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

4.4.2 Alanine Transaminase (ALT):

The results revealed: ALT increased significantly ($P \leq 0.01$) in third (32.000 ± 0.858 U/L) and fourth groups (34.400 ± 0.994 U/L) and not significantly in second group (27.800 ± 0.894 U/L) in comparison with the first group (27.300 ± 0.923 U/L) and increased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group increased significantly. (Table (4.3), Figure (4.11)).

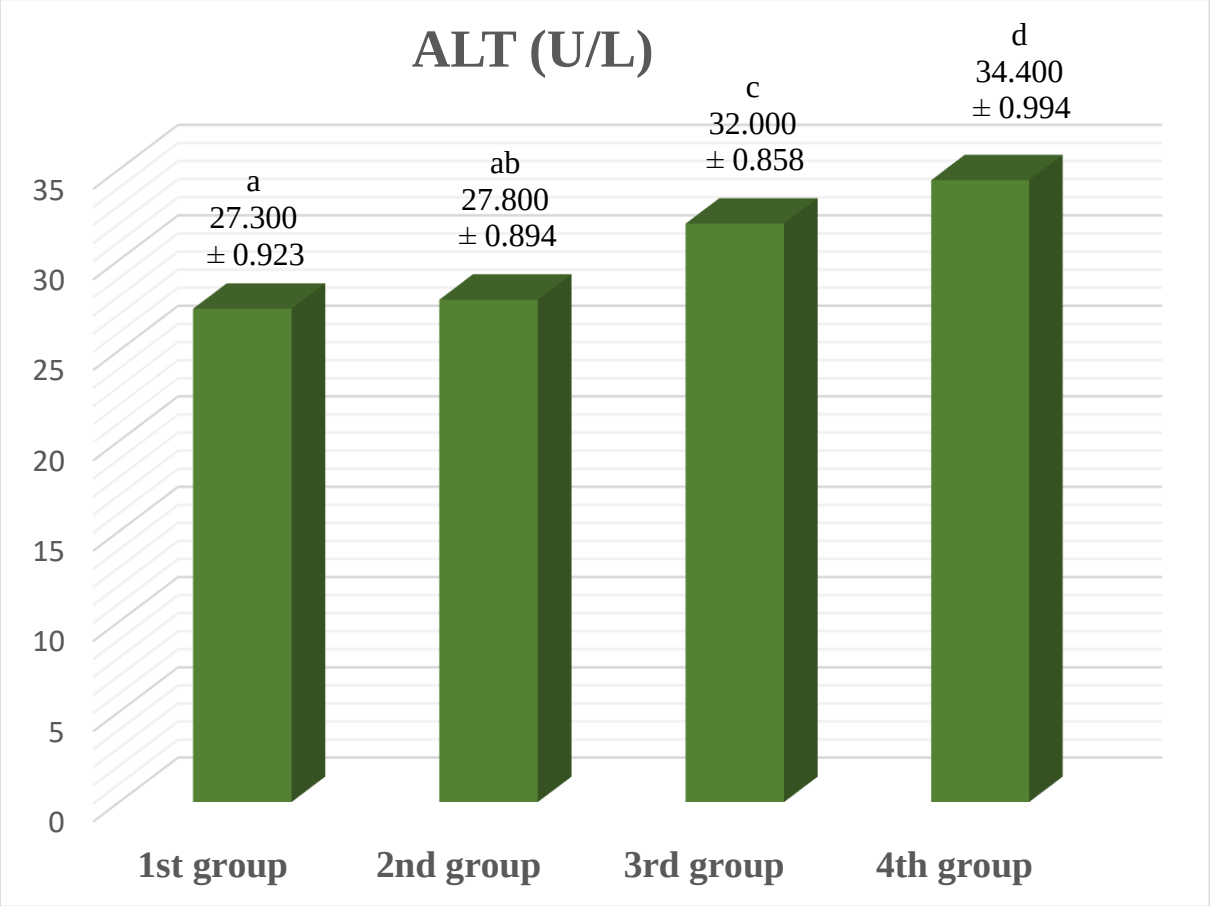


Figure (4.11) The levels of ALT enzyme (U/L) in hypertensive men among groups.

- 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.

4.4.3 Aspartate Transaminase (AST):

The results revealed: AST increased significantly ($P \leq 0.01$) in third (33.650 ± 0.875 U/L) and fourth groups (35.500 ± 0.945 U/L) and not significantly in second group (29.800 ± 0.951 U/L) in comparison with the first group (29.400 ± 0.940 U/L) and increased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group increased significantly. (Table (4.3), figure (4.12)).

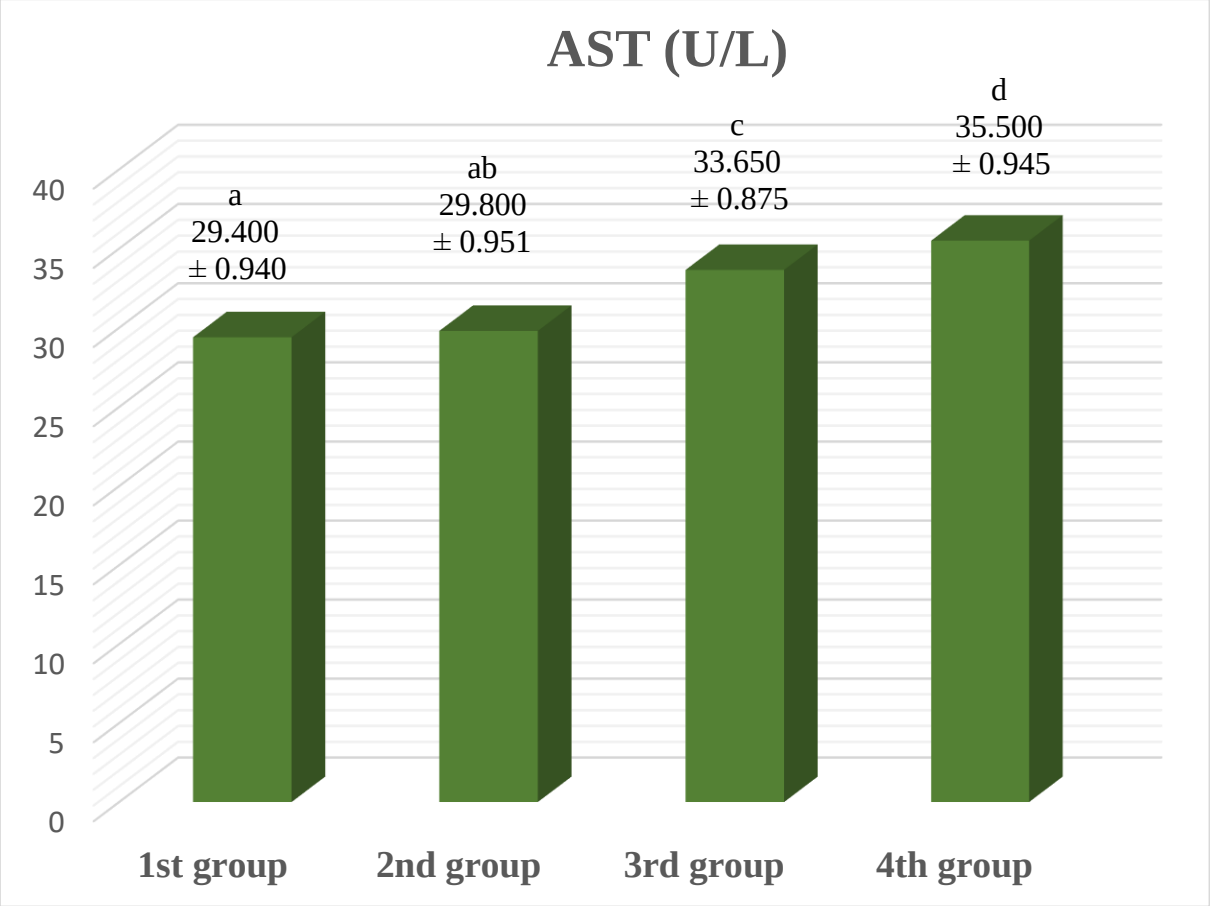


Figure (4.12)The levels of AST enzyme (U/L) in hypertensive men among groups.

- 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.

4.4.4 Alkaline Phosphatase (ALP) :

The results revealed: ALP increased significantly ($P \leq 0.01$) in second (120.500 ± 1.318 U/L), third (123.550 ± 1.190 U/L) and fourth groups (123.900 ± 1.651 U/L) in comparison with the first group (117.950 ± 0.759 U/L) and increased significantly in both third and fourth groups in comparison with the second group, in addition, when compared to the third group, fourth group increased no significantly. (Table (4.3), figure (4.10)).

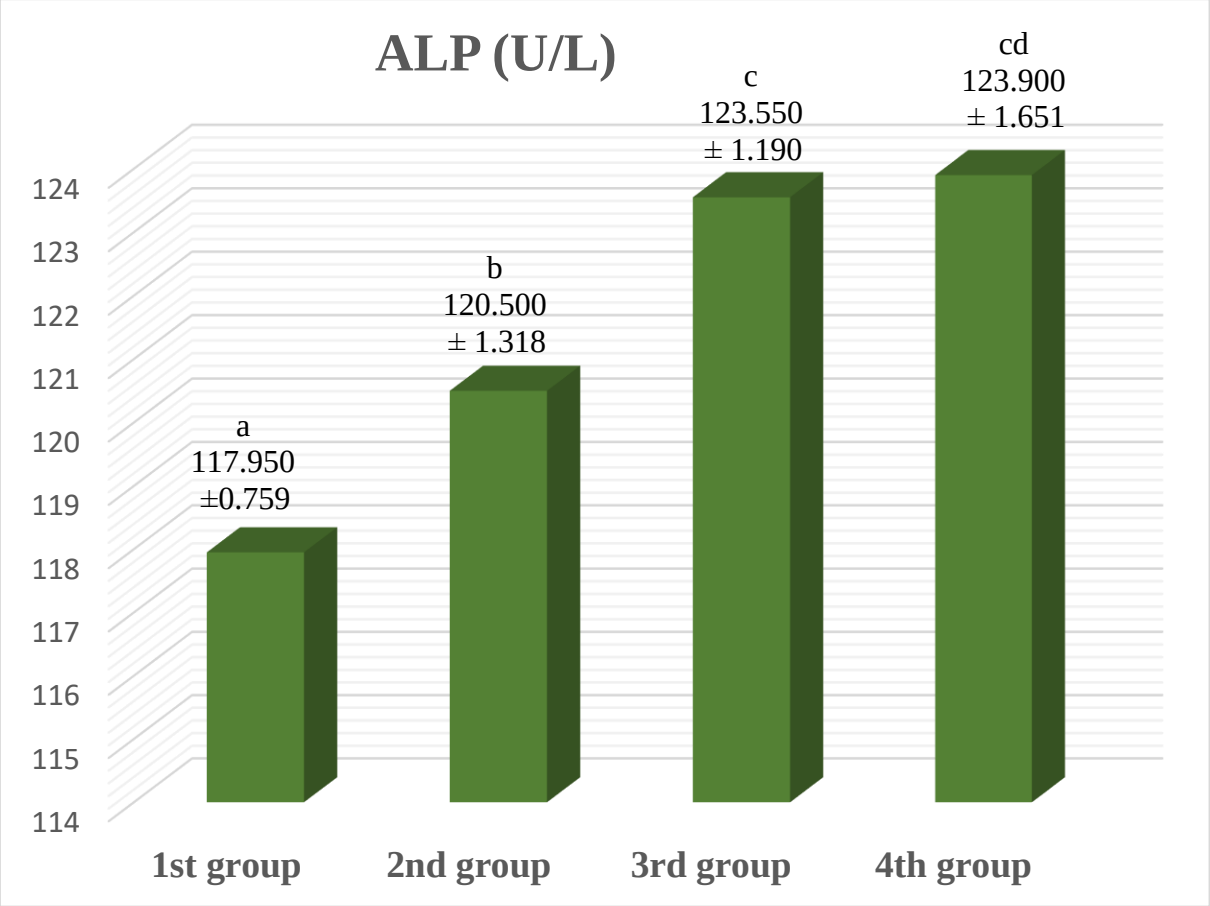


Figure (4.13) The levels of ALP enzyme (U/L) in hypertensive men among groups.

- 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.

Table (4.3) The levels of LDH and Liver (ALT, AST and ALP) Enzymes in Hypertensive Men during Different Groups.

Groups	Parameters			
	LDH U/L	ALT U/L	AST U/L	ALP U/L
First group 30-39 y	a 302.600 ± 2.927	a 27.300 ± 0.923	a 29.400 ± 0.940	a 117.950 ± 0.759
Second group 40-49 y	b 375.200 ± 2.440	ab 27.800 ± 0.894	ab 29.800 ± 0.951	b 120.500 ± 1.318
Third group 50-59 y	c 415.700 ± 2.451	c 32.000 ± 0.858	c 33.650 ± 0.875	c 123.550 ± 1.190
Fourth group 60-69 y	d 494.200 ± 2.930	d 34.400 ± 0.994	d 35.500 ± 0.945	cd 123.900 ± 1.651

- 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.

Chapter Five

Discussion

5.1 ACE2

The present results revealed that the ACE2 levels decreased significantly ($P \leq 0.01$) (except fourth group against third group) in different groups. Table (4.1), figure (4.1).

The deficiency of the current enzyme may be explained the impact of both oxidative stress and hypertension that appear or worsen as a result of progressive age and aging, oxidative stress and the endothelial dysfunction enhanced by the present high levels of Ang II during progressive age. (Table 4.1, figure 4.4) and by the ACE2 deficiency, whereas, that ACE2 enzyme plays as a protector agent against the effects of Ang II and its detrimental changes by converting Ang II to Ang (1–7). In addition, the current deficiency in ACE2 during progressive age may be caused by the Ang II action via it's triggered the cleavage of ACE2. Also, the high levels of the current ET-1, (table 4.2, figure 4.7) may be targeted the downregulation of ACE2 levels via its dysregulation effect on the endothelial cells (the site of ACE2 synthesis). On the other hand, the present low levels of albumin (table 4.2, figure 4.9) might be enhanced the upregulation of ACE and downregulation of ACE2.

The present findings and ideas are in agreements with the following studies. Oxidative stress has been postulated to acts a crucial role during progressive age and aging and ranges from physiological to toxic levels of molecular or organelle damage (Chandrasekaran *et al*., 2017; Sies *et al.,* 2017; Liguori *et al.,* 2018; Luo *et al.,* 2020).

Oxidative stress considered as an important factor in the endothelial damage, vascular dysfunction, cardiovascular remodeling and pathophysiology of hypertension thereby, oxidative stress leading to impaired hypertension coordinated regulatory systems (Hurr and Young, 2016; Tain and Hsu, 2022).

Ding and his team (2020) find that increased Ang II is one of the most common oxidative stress-induced factors which causing excessive ROS, resulting in endothelial dysfunction.

Lee and his colleagues (2019) find that Ang II induced oxidative stress by activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and enhances ROS production by binding to AT1R, which mediates the major biological effects of Ang II.

Liguori and his team 2018 noted that progressive age and aging were accompanied with productions of oxidative stress due to the imbalance between reactive oxygen and nitrogen species (RONS).

ACE2 converted Ang I to Ang (1–9) which subsequently converted to Ang (1–7) by a non-ACE2 mediated process also converted Ang II directly to Ang (1–7) which binds to Mas R and then promoting vasodilatory, anti-inflammatory, and anti-fibrotic effects, so ACE2 protects against the inflammatory effect of Ang II. (Karnik *et al*., 2015; Te Riet *et al.*, 2015; Gheblawi *et al*., 2020).

Moreover, ACE2 levels were elevated with hypertensive patients whom taking ACE i and ARB drugs (Reynolds *et al.*, 2020).

Furthermore, many studies indicated that ACE2 levels reduced significantly during progressive ages and aging by creating a proinflammatory changes in renin angiotensin signaling caused by the high levels of Ang II that be detrimental vascular and endothelial factor with advancing age signaling (Verdecchia *et al.*, 2020; Wang *et al.*, 2020; Gu *et al.*, 2021), similarly ACE2 deficiency resulted in patients with hypertension were accompanied by upregulation of Ang II levels and downregulation of Ang1-7 (Basu *et al.*, 2017; Bártová *et al.*, 2020).

On the other hand, Gheblawi and his team (2020) find that a soluble version of ACE2 was released from the membrane by TNF-converting enzyme (metalloproteinase) in response to high levels of Ang II.

Moreover, Zhang and his colleagues (2013) whom showed that ET-1 decreased the ACE2 level significantly, due to the endothelial dysfunction action of ET-1.

Liu and his colleagues (2009) mentioned that the low levels of the albumin, contribute to the provoked amplification of intrarenal RAAS by increasing ACE levels and decreasing ACE2 levels.

Nevertheless, Fang and his co-worker (2020) mention that cellular expression of ACE2 was increased in hypertensive patients whom taken antihypertensive drugs such as ACE inhibitors and Ang II type I receptor blockers.

5.2 Renin

The present results revealed that the renin levels decreased significantly ($P \leq 0.01$) (except the second group against first group) in different groups. Table (4.1), figure (4.2).

This present reduction may be explained via the excessive of oxidative stress caused by both hypertension and progressive age also, enhanced by the current high levels of Ang II, (table 4.1, figure 4.4), these serial reactions promote proinflammatory markers such as the present high levels of ET-1, (table 4.2, figure 4.7), that caused an inhibition and stimulation action on renin and aldosterone release, respectively, thereby oxidative stress may be a main contributor in RAAS functions, specifically during the renin levels decline , in addition to the effects of, the present high levels of the Ang II (table 4.1, figure 4.4) and AVP (table 4.2, figure 4.6), which decreased the activity of renin by the negative feedback mechanism.

These present findings and thoughts are in consents with many studies concerning the role of the activity, such as, the study of Jang and his colleagues (2018) they find that renin levels decreased in elderly hypertensive individuals due to impaired tissue function and increasing Ang II which plays an paramount role in kidney's aging process, due to its ROS and oxidative stress production.

Moreover, Ang II suppresses renin synthesis and release in JG cells by negative feedback mechanism (Peti-Peterdi and Harris, 2010; Quadri *et al.*, 2016).

On the other hand, hypertension and progressive age are companied with increasing oxidative stress and endothelial dysfunction which leads to increasing cytokines such as ET-1 (Guzik and Touyz, 2017).

Rossi and his colleagues (1997) note that ET-1 had an inhibition action on renin synthesis and stimulation action on aldosterone secretion via its effect on adrenal cortex in both animals and humans.

Moreover, hypertensive patients have an inversely correlation between the high levels of ET-1 with low of renin activity, where there was a significant negative correlation between endothelin and plasma renin activity in their patients and this could be explained by endothelin-induced inhibition of renin release (Elijovich *et al.*, 2001).

Also, Komers and Plotkin (2016) note that ET-1 have an inhibition action on the release of renin enzyme from isolated juxtaglomerular apparatus in hypertensive individuals. On the other hand, using of ET-1 receptors antagonist may be increase the levels of renin and Ang II in hypertensive rats (Caires *et al.*, 2019).

Furthermore, the declines in the renin levels and the increased in the aldosterone secretion a companied strongly with the progressive age and aging in hypertensive men (Inoue *et al.*, 2020; Joseph *et al.*, 2021).

Aging and hypertension considered as main factors that influenced on the renin angiotensin aldosterone system and by their impacts, they reduced the activity of the renin enzyme both in synthesis and release by the kidney's juxtaglomerular cells due to kidney renin mRNA is down-regulated, and this reduction in tissue renin mRNA precedes the later fall in renin concentration (Baudrand and Vaidya, 2018; Joseph *et al*., 2021).

Moreover, renin levels and the plasma renin activity were declined with age in hypertensive patients (O'Sullivan *et al.*, 2017).

Moreover, AVP suppresses renin synthesis and release in JG cells by negative feedback mechanism when it acts on the V1a receptors (V1aR) in the macula densa cells in hypertensive individuals (Aoyagi *et al.*, 2009).

Reid and his team (1983) find that high levels of AVP inhibited the renin secretion by direct action on the juxtaglomerular apparatus.

5.3 Angiotensin I

The present results revealed that the Ang I decrease significantly ($P \leq 0.01$) in different groups. Table (4.1), figure (4.3).

This deficiency may be explained by many trends, firstly, the present significant decline of renin levels during progressive age (table 4.1, figure 4.2), secondly, the possible influence of progressive age, hypertension, oxidative stress, endothelial dysfunction and inflammation on the increment of ACE activity, thirdly, the negative feedback mechanism by the present parameters … Ang II, aldosterone and AVP (tables 4.1, 4.2, figures 4.4, 4.2, 4.6) on renin secretion.

These results are in agreement with many studies concerning the role of these parameters. The renin catalyzes the generation of Ang I by acting on the substrate

angiotensinogen (Bellomo *et al*., 2020; Liu *et al.*, 2020; Kuriakose *et al.*, 2021; Laghlam *et al.*, 2021).

Amraei and Rahimi (2020) mention that renin converts angiotensinogen to Ang I, therefore, the deficiency in renin levels may be also leads to Ang I reduction.

Aging, hypertension, oxidative stress, endothelial dysfunction and inflammation indirectly influence positively ACE activity, therefore, Ang I converted to Ang II by ACE (Zhu *et al.*, 2020).

Moreover, hypertension has been well-known to elevated inflammation cytokines (Chen *et al.*, 2020), this elevation of cytokines (such as ET-1) a associated with progressive age (Yanes and Reckelhoff, 2011) and a vasoconstriction action that originated to the pathogenesis of hypertension (Stauffer *et al.*, 2008).

In addition, both of aging and inflammation caused the endothelial dysfunction and associated with high levels of ET-1 that influenced on ACE levels found mostly in endothelial surface of some organs (lung, kidney) (Guignabert *et al.*, 2018).

Furthermore, the high levels of Ang II inhibit Ang I, whereas, Ang II acts as antagonist for both Ang I and it is receptor (Ong *et al.*, 2010; Bellomo *et al.*, 2020).

Moreover, the high levels of Ang II inhibit renin activity via a bio feedback loop leading to Ang I decrease (Bellomo *et al.*, 2020; Liu *et al.*, 2020).

Moreover, renin was reduced by some other substances such as aldosterone and AVP hormones through their negative feedback mechanism and this reduction may be the cause for Ang I deficiency (Arnold *et al.*, 2013; Rehan *et al.*, 2015; Gonzalez *et al.*, 2016; Leung *et al.*, 2017).

5.4 Angiotensin II

The present results revealed that the Ang II increased significantly ($P \leq 0.01$) in different groups. Table (4.1), figure (4.4).

It's worth mentioning that the current high levels of Ang II is a sine qua none to explain and discuss the findings of this study, particularly, Ang II is a result of aging, hypertension, oxidative stress, endothelial dysfunction and inflammation, it has a detrimental impacts on some current findings such as ACE2, renin and Ang I (table 4.1, figures 4.1, 4.2, 4.3), ET-1 (table 4.2, figure 4.7), some liver enzymes (ALT, AST and ALP) (table 4.3, figures 4.11, 4.12, 4.13) and it's a companied with aldosterone and AVP (tables 4.1, 4.2 , figures 4.5, 4.6) as stimulator agent for their releasing.

These results and findings are in agreement with the observations of many studies. Ang II levels increased in aging and hypertensive mice associated with oxidative stress, endothelial dysfunction and inflammation (Incalza *et al.,* 2018; Jia *et al.,* 2019; Birk *et al.,* 2021).

Moreover, high levels of Ang II activity in hypertensive rats is a sequence results to the oxidative stress and endothelial dysfunction by its role in upregulation of Ang II receptor (AT1R) (Bhatt *et al.,* 2014).

Furthermore, Ang II levels increased with progressive age and hypertension, due to the ACE2 deficiency whereas, this enzyme was responsible of the transformation of Ang II to Ang 1-7, and that resulting in decreased in Ang 1-7, also, the Ang 1-7 age-related deficiency had been shown to be associated with a concomitant enhanced Ang II production (Chen *et al*., 2020; Cook and Ausiello, 2021; Lissoni *et al*., 2021).

ACE2 deficiency result in patients with hypertension accompanied by upregulation of Ang II levels and downregulation of Ang 1-7 (Basu *et al.,* 2017; Bártová *et al.,* 2020).

Furthermore, the high levels of Ang II triggered the cleavage and shedding of a soluble form of ACE2 from the membrane by enhancing the TNFα-converting enzyme (metalloproteinase) (Patel *et al.,* 2014).

Jang and his colleagues 2018 noted that renin levels decreased in elderly hypertensive patients due to the high levels of Ang II which plays an essential role in kidney's aging process, due to its ROS and oxidative stress production.

On the other hand, progressive age and hypertension considered as a low-grade inflammation that associated with releasing of cytokines such as ET-1 (Underwoods and Adler, 2013; Kostov, 2021).

Moreover, Ang II elevated in hypertensive rodents associated with endothelial dysfunction and inflammation, leading to triggered ET-1 release, in turn, increase the Ang II activity (Ortiz *et al.,* 2001; Jerkic and Letarte, 2015).

Furthermore, progressive age, hypertension and oxidative stress associated with the elevation of ET-1 and disruption of ACE2 activity (De Silva *et al*., 2020; Calzerra *et al.,* 2018), therefore, the increment of ET-1 increased the production of Ang II via boosting ACE that converts Ang I to Ang II (kurbel, 2021; Bukowska *et al*., 2022).

Domenig and his team 2016 noted that Ang II increased at the expense of Ang I decrease, whereas ACE converts Ang I to Ang II in experimental hypertensive rats.

Moreover, the vasopressinergic action of Ang II a combined with hypertension stimulus to AVP secretion (Szczepanska-Sadowska *et al.,* 2018; Proczka *et al.,* 2021).

Furthermore, Rieder and his team 2021 mentioned that Ang II acted as a stimulator for aldosterone secretion and they noted a positive correlation between Ang II elevation and the aldosterone secretion in hypertensive patients.

On the other hand, the increment of Ang II that associated with liver injury and fibrosis caused an elevation in the liver enzymes (ALT, AST and ALP) (Colmenero *et al.,* 2009; Grace *et al.,* 2012).

5.5 Aldosterone

The present results revealed that the aldosterone increased significantly ($P \leq 0.01$) (except fourth group against third group) in different groups. Table (4.1), figure (4.5).

This elevation may be attributed to the current increases in the Ang II, AVP and ET-1 (tables 4.1, 4.2, figures 4.4, 4.6, 4.7) and decreases in renin and albumin (tables 4.1, 4.2, figures 4.2, 4.9) during the progressive age and hypertension.

The present findings and ideas are in agreements with following studies. The elevation of aldosterone levels may be caused due to the high levels of ang II that positively correlated with the synthesis and secretion of aldosterone in hypertensive patients (Nanba *et al.,* 2018; Rieder *et al.,* 2021).

Rajamohan and his team (2012) found that the high levels of aldosterone associated with oxidative stress caused by the increment of Ang II which can either facilitate ROS production or attenuate ROS degradation contributing to elevated cellular ROS levels in human and rat.

Moreover, the bad effect of Ang II and their consequences of oxidative stress and inflammation production caused by the reduction of nitric oxide bioavailability (Birk *et al.,* 2021).

On the other hand, the high levels of AVP in hypertensive elderly people have an effect on synthesis and secretion of aldosterone, whereas, AVP reduced sodium excretion and improved water intake by increasing the water permeability of the renal collecting duct (Sparks *et al.,* 2014), moreover, atrial natriuretic peptide, dopamine, somatostatin and NO that decline during hypertension and progressive age, considered as an inhibitor agent for aldosterone synthesis and secretion (Aoyagi *et al.,* 2009; Bollag, 2011; Choi *et al.,* 2021).

Furthermore, ET-1 is an independent stimulator for aldosterone secretion in hypertensive patients whereas, this stimulation might be directly or indirectly through ET-1 or renin actions respectively (Azamian *et al.,* 2012). In addition, ET-1 increased with hypertension and progressive age (Barton, 2014).

ET-1 involved in the aldosterone secretions via its receptors (endothelin A and endothelin B), in turn, aldosterone controlled the kidneys' tubular reabsorption of both electrolytes and water (Andreis *et al.,* 2002).

Moreover, aldosterone elevated in contrast with low levels of renin, this elevation suppressed the levels of renin via a negative feedback mechanism (Barrett *et al.,* 2019), during progressive age and hypertension (Ma *et al.,* 2018; Alnazer *et al.,* 2021).

Moreover, Spence (2018) note that aldosterone elevated in elderly hypertensive patients, associated with salt and water retention and suppressed plasma renin activity, so, plasma aldosterone was high and renin was low.

On the other hand, albumin deficiency played a pathological role in kidneys performance and changed negatively GFR, thereby aldosterone is in a high amount and being hypertension (Catena *et al.,* 2017; Mirfakhraee *et al.,* 2021).

5.6 Vasopressin (AVP)

The present results revealed that the AVP levels increased significantly ($P \leq 0.01$) (except fourth group against third group) in different groups. Table (4.2), figure (4.6).

The current study appeared that Ang II, aldosterone and ET-1 increased (tables 4.1, 4.2, figures 4.3, 4.5, 4.7) and renin levels decreased (table 4.1, figure 4.2) in hypertensive men with progressive age, respectively. These changes are probably beyond the high levels of AVP secretion in hypertensive men with progressive age.

These results are in agreement with many studies. Ang II increased the AVP secretion by reinforces the response to hypovolemia and hypotension by acting on the circumventricular organs to increase AVP levels in hypertensive patients (Gonzalez *et al.,* 2020; Proczka *et al.,* 2021).

Moreover, the high levels of aldosterone associated with the increment of AVP activity, whereas aldosterone has a complex interaction with AVP (Szczepanska-Sadowska *et al.,* 2018).

AVP levels increased significantly in hypertensive rats due to the high levels of ET-1 which increased the activity of hypothalamus to AVP synthesis (Rossi, 2004).

On the other hand, renin deficiency that associated with hypertension and progressive age considered as a main cause for high levels of AVP production and this deficiency in renin release caused by a high levels of ET-1 which enhanced also the release of aldosterone in hypertensive and elderly people (Birder *et al.,* 2019; Yilmaz *et al.,* 2019)

AVP concentrations be high in aged rats (Sauvant *et al.*, 2014) and increased significantly in elderly hypertensive patients due to low levels of sodium (Tamma *et al.*, 2015; Aleksandrowicz *et al.*, 2020).

5.7 Endothelin -1 (ET-1)

The present results revealed that the Endothelin -1 levels increased significantly ($P \leq 0.01$) (except third group against second group) in different groups. Table (4.2), figure (4.7).

Aging and hypertension represent a low-grade inflammation, accompanied with oxidative stress and endothelial dysfunction and characterized with an elevation of blood inflammatory markers and cytokine release, that explained a high level of ET-1 in the different groups of the current study. Also, the increasing in ET-1 levels may be due to the present high levels of Ang II, aldosterone, AVP (tables 4.1, 4.2, figures 4.4, 4.5, 4.6) and low levels of ACE2 (table 4.1, figure 4.1).

These results are in agreement with many studies concerning the role of ET-1 and its relation with other parameters.

Aging and hypertension represent as a low-grade inflammation, associated with oxidative stress and endothelial dysfunction and resulted in inflammatory markers and cytokines release , such as ET-1 that increased significantly in elderly and hypertensive patients (Speed *et al.*, 2015; Puzianowska-Kuźnicka *et al.*, 2016; Subhi *et al.*, 2019; Jankowich and Choudhary, 2020; Mesquita *et al.*, 2021).

Moreover, both of aging and inflammation caused the endothelial dysfunction and oxidative stress, so, they contribute to arterial inflammation and age-related arterial remodeling (Avolio *et al.*, 2011).

Faramarzi and his coworkers 2012 mentioned that ET-1 increased significantly in elderly hypertensive persons due to lack of proper functioning of

endothelial cells which led to endothelial dysfunction and alterations in ET-1 signaling pathways, so released more of ET-1.

Spontaneously hypertensive rats have been shown a significant increase in ET-1 associated with endothelial dysfunction, inflammation and oxidative stress (Bukowska *et al.,* 2022).

On the other hand, Ang II enhanced the production of ET-1 by boosting the endothelin converting enzyme activity in hypertensive elderly people (Komers and Plotkin, 2016).

ET-1 secretion was elevated as a response to high levels of Ang II, which stimulates ET-1 release and expression in several kinds of cells, such as renal cells in hypertensive patients (Albertoni *et al.,* 2010; kurbel, 2021).

Furthermore, endothelial damage that induced by both aldosterone and AVP caused ET-1 secretion in hypertensive rodents (Wong *et al.,* 2007).

ET-1 levels increased significantly in elderly hypertensive patients due to the ACE2 deficiency, whereas the high concentrations of ACE2 acted as an anti-inflammatory activity by its an inhibitor action on several of the inflammatory cytokines secretion, therefore, both of angiotensin imbalance and endothelial dysfunction jumped to the front after this deficiency and leading to high levels of ET-1 secretion during aging and hypertension (Lakatta, 2018; Lissoni, 2021; Cook and Ausiello, 2022).

5.8 Total Proteins and Serum Albumin

The present results revealed that the total proteins and serum albumin decreased significantly ($P \leq 0.01$) (except third group against second group for total proteins) in different groups. Table (4.2), figures (4.8), (4.9).

Progressive age and hypertension related with oxidative stress, endothelial dysfunctions and inflammation have been the major cause for these current changes, beside that the high levels of current findings including Ang II, aldosterone, ET-1, LDH, liver enzymes and low levels of renin (tables 4.1, 4.2, 4.3, figures 4.4 , 4.6 , 4.7, 4.10 , 4.11, 4.12, 4.13) might be caused a decline in kidneys and liver functions thereby a reduction in serum albumin and total proteins.

These results and ideas are agreement with many studies. Total proteins and serum albumin decreased significantly in elderly hypertensive individuals due to high levels of Ang II which promoted ROS production and inflammation, in addition increased glomerular pressure caused a hypertrophy and renal fibrosis, resulting in a vascular permeability increase , thus, an increase in urinary albumin excretion (Han *et al.*, (2020, a); Alnajdi, 2021; Choi *et al.*, 2021; Myette *et al.*, 2021; Li *et al.*, 2022).

Moreover, the high levels of aldosterone and low levels of renin in elderly hypertensive patients caused a high kidney's damage and impairment of reabsorption in proximal tubular that leading to urinary albumin excretion thereby, albumin reduction (Tian *et al.*, 2014; Kalaitzidis *et al.*, 2015; Fernández-Argüeso *et al.*, 2021).

Furthermore, high levels of ET-1 enhanced glomerular albumin permeability, proteinuria and albumin reduction in both vitro and vivo during progressive age in hypertensive patients and mice (Komers and Plotkin, 2016; Zou *et al.*, 2020).

Moreover, the high concentration of LDH related with abnormal in GFR and cell membrane permeability caused kidney and glomerular endothelial dysfunction that associated with albumin reduction in elderly hypertensive patients (Cai *et al.*, 2021).

On the other hand, hypoalbuminemia caused by impermeant in synthesis, high catabolism, vascular permeability and renal and enteral loss in plasma albumin concentrations which a combined with the high levels of liver enzymes (ALT, AST and ALP) during hypertension and progressive age (Arques, 2018; Cai *et al.*, 2021).

5. 9 Lactate dehydrogenase (LDH)

The present results revealed that the LDH levels increased significantly ($P \leq 0.01$) in different groups. Table (4.3), figure (4.10).

The detrimental effects of progressive age and hypertension … i.e.. excessive of oxidative stress, endothelial dysfunction, inflammation and organs damage besides the current high levels of Ang II, ET-1 (tables 4.1, 4.2, figures 4.4, 4.7) and low levels of ACE2 (table 4.1, figure 4.1), are beyond the LDH elevation in different groups.

These results are in agreement with many studies concerning the role of LDH and its relationship with other parameters.

Many researchers pointed an elevation for this enzyme in hypertensive rats after the oxidative stress, renal and cardiac spoilage and dysfunction in vascular endothelial layer (Jena *et al.*, 2015; Alghamdi *et al.*, 2016; Oyagbemi *et al.*, 2017).

The high significant levels of LDH represented a pro-inflammatory state due to endothelial dysfunction which related to the pathophysiology of hypertension in elderly people (Martínez-Urbistondo *et al.*, 2022; Okay *et al.*, 2020; Ribeiro and Uehara, 2022).

Moreover, the increase of LDH levels in elderly and hypertensive men considered as a sensitive indicator of cell injury, thus, endothelial cells impairment (dysfunction) and LDH release (Cai *et al.*, 2021; Gu *et al.*, 2021).

Many authors indicated that LDH represented as systemic inflammatory indicator because of its association with liver's cirrhosis and inflammatory diseases during progressive age in hypertensive men (Chen and Wu, 2021).

Hu and his colleagues 2015 assessed the possible potential role of LDH as a biomarker and mediator in pathogenesis of idiopathic hypertension and its activity proportioned with the clinical severity of the disease, moreover, LDH released in response to endothelial cells dysfunctions.

Moreover, high levels of Ang II enhanced more oxidative stress and endothelial dysfunction thereby, LDH releasing considered as a clear sign of kidneys and blood vessels damages in elderly hypertensive patients (Kij *et al.*, 2021; Wang *et al.*, 2021).

Furthermore, inflammation related with endothelial cells dysfunctions promote the high levels of ET-1 that be associated with hypertension complications and leading to high significant of LDH in elderly and hypertensive men (Gupta *et al.*, 2018; Zeng *et al.*, 2020; Bukowska *et al.*, 2022).

Furthermore, ACE2 deficiency was beyond the high levels of LDH in elderly hypertensive patients via the ACE2 action to facilitated the degradation of Ang II to Ang 1-7 generation (Underwood and Adler, 2013; Verma *et al.*, 2019; Verdecchia *et al.*, 2020).

5.10 Liver enzymes (ALT, AST and ALP)

The present results revealed that the liver enzymes increased significantly ($P \leq 0.01$) (except second group against first group for both of ALT and AST) in the different groups. Table (4.3), figures (4.11), (4.12) and (4.13).

The present elevation might be attributed to some physiological dysfunctions in liver's enzymes release caused by hypertension, inflammation and their complications, particularly, these enzymes associated with the bad effects

of progressive age and hypertension such as the present low levels of ACE2, high levels of Ang II, ET-1 and LDH (tables 4.1 , 4.2 , 4.3, figures 4.1, 4.4 , 4.7, 4.10).

The present findings and thoughts are in consist with many studies, ALT and ALP increased significantly in elderly hypertensive individuals and indicated as an important marker for liver dysfunction (Liu *et al.,* 2018; Wong *et al.,* 2018; Gu *et al.,* 2021; Wang *et al.,* 2021).

Moreover, AST and ALT concentrations increased significantly with inflammation resulted by progressive age and hypertension in male and female patients (Sookoian and Pirola, 2015; Mahady *et al.,* 2017; Rahman *et al.,* 2020; Gu *et al.,* 2021; Petroff *et al.,* 2021).

Furthermore, ALP was associated significantly with hypertension whereas its activity increased in atherosclerosis and peripheral vascular disease, so, it was an inflammatory marker (Shimizu *et al.,* 2013) in addition, it's an early indicator of hypertension due to its high levels during hypertension (Khalili *et al.,* 2022), however ALT listed as a highest activity of liver's enzymes due to its high duration of half-life or a higher specificity of ALT to liver diseases (Chen *et al.,* 2016; Gaeini *et al.,* 2020).

Many researchers showed that the elevation of ALP enzyme caused by GFR reduction as a result of kidney damage during progressive age and hypertension. (Zhang *et al.,* 2021; Khalili *et al.,* 2022).

Furthermore, ACE2 played a crucial role in protection against organ damage, including renal and liver organs, therefore, the ACE2 deficiency that associated with hypertension, aging and inflammation caused a liver dysfunction and increased in AST and ALT release (Furuhashi *et al.,* 2021).

On the other hand, Kawagishi and his colleagues 2021 noted that high levels of AST and ALT A with high levels of Ang II that during with hypertension, progressive age, vascular leakage and inflammation, resulting in the liver's deterioration and abnormal enzymes release.

On the other hand, the high levels of AST and ALT linked with high levels of ET-1 caused by liver's dysfunction in elderly hypertensive patients (Zhang *et al.,* 2019; Nickel *et al.,* 2021).

Moreover, Cai and his team (2021) find that liver enzymes (ALT, AST, and ALP) correlated positively with high levels of LDH, were increased significantly during progressive age in hypertensive patients.

However, Petroff and his colleagues (2021) mention that correlation between AST levels and the age have not strongly appeared.

Chapter Six
Conclusions and Recommendations

6.1 Conclusions

The results of the present study included the following conclusions:

1. Hypertension, and progressive age related with oxidative stress considered as a state of inflammation that had been detrimental impacts on all the body's systems and mechanisms particularly the studied parameters … i..e.. RAAS deterioration and its components.

2. ACE2 deficiency may be mediate a serial of dysfunctions caused the present changes in RAAS and other parameters in response to hypertension and progressive ages.

3. Renin 's low levels and its consequences might be the cause for an imbalance between angiotensin I / angiotensin II ratio.

4. High levels of angiotensin II might be reflected the disruption of many organs and systems

5. High levels of aldosterone and AVP hormones and their abilities in reabsorption of Na, water and blood vessels constrictions might be indicated a maintain and continues hypertension in those people whom had a hypertension with progressive age.

6. ET-1 might be represented a bio marker for a low-grade inflammation (hypertension and progressive age).

7. Renal and liver dysfunctions, impairment of reabsorption in proximal tubular and enhanced glomerular permeability to albumin might be reflected the lowest levels of serum albumin and total protein.

8. More LDH activity might be pointed more need for oxygen nutrition in damage endothelial cells.

9. High levels of liver enzymes and low albumin concentrations might be indicated that the liver was to be a supporting player in regulating and adjustment the blood pressure.

6.2 Recommendations

The present study recommended the following:

1. Further studies with more samples and other vital parameters related with RAAS and its components are required.

2. Studying the interaction between RAAS system with some other physiological aspects such as fertility, reproduction and the immunological system in both male and female.

3. Studying the RAAS dysfunctions in people attacked with some metabolic syndromes, i.e. insulin′s resistance, diabetes and obesity.

4. Studying the interaction between RAAS and ACE2 abnormalities and their detrimental influences on some other organ's performances.

5. Studying RAS activity in hypertensive men with progressive age in covied-19 patients.

6. Studying RAS activity in hypertensive women with progressive age.

7. Study the molecular levels of the RAS effects in hypertensive men with progressive age.

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Appendices

Questionnaire

Case No.	
Name	
Age	
Social status	
Duration of hypertension	
Are you suffer from diabetes, thyroid disease, heart and kidney failure and pituitary tumors?	
Are you taken hormonal or antihypertensive drugs?	
Are you smoker?	
Length	
Weight	
BMI	
Serum examination	
ACE2	
Renin	
Angiotensin I	
Angiotensin II	
Aldosterone	
Vasopressin	
Endothelin -1	
Total protein	
Serum albumin	
Lactate dehydrogenase	
Alanine transaminase	
Aspartate transaminase	
Alkaline phosphatase	

الخلاصة:

هدفت الدراسة الحالية إلى معرفة دور كل من الانزيم المحول للانجيوتنسين (٢) ونظام الرينين انجيوتنسين، إضافة الى بعض المعايير البيوكيميائية والهرمونية في الرجال المصابين بارتفاع ضغط الدم مع تقدم العمر في محافظة ميسان. شملت الدراسة على ٨٠ عينة لرجال مصابين بارتفاع ضغط الدم من المراجعين لمستشفى الصدر التعليمي وبعض المراكز الصحية (أعمارهم ٣٠-٦٩ سنة) ومن الذين يراجعون مستشفى الصدر التعليمي وبعض المراكز الصحية، وقسمت العينة إلى أربع مجاميع

(٢٠ رجلاً / مجموعة) حسب أعمارهم وعلى النحو الاتي:

- المجموعة الأولى: ٣٠ - ٣٩ سنة.
- المجموعة الثانية: ٤٠ - ٤٩ سنة .
- المجموعة الثالثة: ٥٠-٥٩ سنة.
- المجموعة الرابعة: ٦٠ - ٦٩ سنة.

أظهرت النتائج ما ياتي: -

١- انخفاض مستوى الانزيم المحول للانجيوتنسين (٢) معنوياً (أ ≤ ٠,٠١) (عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة) في المجاميع المختلفة مع تقدم العمر.

٢- انخفاض مستوى الرينين معنويا (أ≤ ٠,٠١) عدا المجموعة الثانية مقابل المجموعة الأولى) في المجاميع المختلفة مع تقدم العمر .

٣- انخفاض مستوى الانجيوتنسين (١) معنويا (أ≤ ٠,٠١) في المجاميع المختلفة مع تقدم العمر.

٤- ارتفاع مستوى الانجيوتنسين (٢) معنويا (أ≤ ٠,٠١) في المجاميع المختلفة مع تقدم العمر.

٥- ارتفاع مستوى هورمون الألدوستيرون معنوياً (أ≤ ٠,٠١) (عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة) في المجاميع المختلفة مع تقدم العمر .

٦- ارتفاع مستوى هورمون الفازوبريسين معنويا (أ≤ ٠,٠١) (عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة) في المجاميع المختلفة مع تقدم العمر .

٧- ارتفاع مستوى الاندوثيلين -١ معنوياً (أ≤ ٠,٠١) (عدا المجموعة الثالثة مقابل المجموعة الثانية) في المجاميع المختلفة مع تقدم العمر .

٨- انخفاض مستوى البروتين الكلي معنويا (أ≤٠,٠١) في المجاميع المختلفة مع تقدم العمر.

٩- انخفاض مستوى الالبومين معنويا (أ≤٠,٠١) (عدا المجموعة الثالثة مقابل المجموعة الثانية) في المجاميع المختلفة مع تقدم العمر .

١٠-ارتفاع مستوى انزيم اللاكتيت ديهايدروجينيز معنويا (أ≤٠,٠١) في المجاميع المختلفة مع تقدم العمر .

١١-ارتفاع مستوى انزيم الامينو ترانسفيريز معنويا (أ≤٠,٠١) (عدا المجموعة الثانية مقارنة بالمجموعة الأولى) في المجاميع المختلفة مع تقدم العمر .

١٢-ارتفاع مستوى انزيم اسبارتيت ترانسمينيز معنويا (أ≤٠,٠١) (عدا المجموعة الثانية مقارنة بالمجموعة الأولى) في المجاميع المختلفة مع تقدم العمر .

١٣-ارتفاع مستوى إنزيم الكلاين فوسفاتيز معنويا (أ≤٠,٠١) (عدا المجموعة الرابعة مقارنة بالمجموعة الثالثة) في المجاميع المختلفة مع تقدم العمر .

تمت مناقشة التأثير الفسيولوجي لهذه النتائج وفقًا لتأثيرات ارتفاع ضغط الدم وتقدم العمر على المعايير المدروسة كافة ، لا سيما وان نقص مستوى الانزيم المحول للانجيوتنسين (٢) وارتفاع مستويات الانجيوتنسين (٢) قد اشر إلى وجود خلل واضح في تنظيم ضغط الدم مع تقدم العمر.

وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم علوم الحياة

نشاط RAS وبعض المستويات البيوكيميائية والهرمونية لدى الرجال المصابين بارتفاع ضغط الدم في مختلف الفئات العمرية

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

ميثاق محمد حسن

بكالوريوس تربية / علوم حياة (٢٠٠٤)

بإشراف

أ. د. أحمد عبود خليفة

كانون الثاني ٢٠٢٣ م

جمادي الاخر ١٤٤٤ هـ