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Determination of angiotensin-converting enzyme concentration in cardiovascular patients

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***Abstract*---** cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year, More than four out of five CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age. The circulating Renin Angiotensin Aldosterone system (RAAS) comprises liver - secreted angiotensinogen (AGT) that is enzymatically converted into angiotensin I (Ang I). ACE, as a key component in RAS, converts angiotensin I to angiotensin II, Angiotensin II increase the production of adhesion molecules and chemokines stimulates LDL oxidation and foam cell formation in macrophages, Increased level of the ACE and subsequent ACE activity by raising the production of angiotensin II can lead to atherosclerosis. A number of 100 subjects were involved in this investigation, 100 patients of CVDs in AL-Saddir Hospital and AL Hkem Hospital, in AL-Najaf province, 70 of them with CVDs and the other 30 healthy individuals, were used as control.The study was conducted to find out the importance of Determination of angiotensin-converting enzyme concentration in cardiovascular patients and some clinical and biochemical variables . We record significant differences between CVDs patient and control in level of ACE enzyme was($p< 0.0001$) . We also referred to the relationship of the lipid profile and smoking in patients with an increase in the enzyme converting angiotensinogen.

***Keywords*---** angiotensin, converting, enzyme concentration, cardiovascular, patients.

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year, More than four out of five CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age (WHO,2020). The circulating Renin Angiotensin Aldosterone system (RAAS) comprises liver - secreted angiotensinogen (AGT) that is enzymatically converted into angiotensin I (Ang I) in the blood stream by kidney-derived renin, In the next step, (Ang I) is being converted by angiotensin-converting enzyme (ACE) to form Ang II, Ang II is the main effector in this system that acts either as a systemic molecule or as a locally produced factor (Nehme *et al.,* 2019).

Angiotensin - Converting Enzyme (ACE) is one of the components of the RAS and zinc - dependent metalloproteinase found widely in endothelial and epithelial cells, Moreover, the enzyme has been isolated from several sources including serum, lungs, seminal fluid, and plasma(Bernstein *et al.,*2013). ACE, as a key component in RAS, converts angiotensin I to angiotensin II, Angiotensin II increase the production of adhesion molecules and chemokine stimulates LDL oxidation and foam cell formation in macrophages, Increased level of the ACE and subsequent ACE activity by raising the production of angiotensin II can lead to atherosclerosis (Sztechman *et al.,* 2018). It is undoubtful that RAAS plays an important role in the regulation of thermogenesis. This understanding gave rise to a variety of investigations highlighting the not only beneficial impact of ARBs and ACE inhibitors on blood pressure, but also their effects on the cardiovascular system due to their anti-inflammatory activities(Nathaniel,2020) . The mechanism by which another RAAS element, aldosterone, acts during the athero-genesis, is not clear, However, the importance of aldosterone is doubtful.

Material and Methods

Study population from AL-Najaf province in Iraq, we taken 70 patients with CVDs and 30 healthy persons as control, they were >50 years of age, were referred to Al-Sadder Hospital (located in kufa, Iraq) for heart center and Al Hakim Hospital in AL-Najaf province from November 2021 to February 2022., patients and control groups were approved for sampling and then informed of the results The experimental design. Evaluation criteria for patients history are considered conventional risk factors for CVDs based on high blood pressure (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg) , lipid profile (Cholesterol > 225 mg /dl , Triglyceride > 200 mg /dl , LDL >129 mg /dl , HDL <60 mg /dl) , White blood cell (WBC) 4000-11000 (cell × / µL)

Detection of Angiotensin I-converting enzyme

ACE concentrations in serum detected by ELISA kit from Elabscience, cat.no.E-EL-H0002, which applies to the in vitro quantitative determination of human ACE concentrations in serum, plasma and other biological fluids. according to the manufacturer's instructions. Samples and standards were transferred to 96-well microplates pre-coated with specific antibodies and incubated for 1 hour. After

the plates were washed and decanted, biotinylated detection antibodies were added to each well and incubated for 60 minutes. Then, an avidin-horseradish peroxidase (HRP) conjugate was added to each well, and the plates were thereafter incubated for 30 min. A substrate reagent was added, and the plates were incubated for 15 minutes. The stop solution was then added to each well, and the absorbance was read at 450 nm using the ELISA Human-reader.

Statistical Analysis

Mega stat software used to statistically analyzed to obtained mean ± standard deviation (SD),the odds ratio (OR) and $P < 0.0001$ were considered significant.

Result and Discussion

A number of clinical and biochemical parameters are affected in CVD patients compared with healthy individuals in Table 1.

Table 1
Clinical and biochemical parameters analysis between control and CVDs groups

Clinical & Biochemical Parameters	Control (n=30)	CVDs Patients (n=70)	P value
Age (yrs)	50.50 ± 0.8660	62.31±1.211	< 0.0001
Systolic BP	111.7 ± 2.809	127.9 ± 2.244	0.0008
Diastolic BP	70.17 ± 2.557	80.00 ± 1.467	0.0026
TG(mg/dl)	106.7 ± 2.757	183.4 ± 7.512	<0.0001
Cholesterol (mg/dl)	152.2 ± 5.201	207.3 ± 6.590	0.0001
HDL (mg/dl)	70.31 ± 1.594	40.16 ± 2.221	<0.0001
LDL (mg/dl)	143.6 ± 1.941	126.6 ± 5.200	0.1037 ns
WBC(cell × / µL)	8.939 ± 0.4546	11.88 ± 0.4078	0.0007
ACE	41.41 ± 2.462	59.74 ± 1.123	< 0.0001

Ages, on the average, showed a significant increase in the prevalence of CVDs comparing with younger. This result agreed with Rodgers *et al.,* (2019). Probable explanation is the progressive in Age is an independent risk factor for cardiovascular disease (CVD) in adults, but these risks are compounded by additional factors, including obesity, and diabetes. the risks associatedwith CVD increase with age As mentioned Fleg *et al.,* (2013). Systolic blood pressure (SBP) is an important clinical reading, it is a common syndrome in many diseases and affected by many known and unknown causes. Present results of SBP reading were significantly higher in CVDs patients compared to control ($p = 0.0008$).

A high blood pressure increases the potential for atherosclerosis, and it can destabilize vascular lesions, resulting in acute coronary events, Hence, it is crucial to achieve and maintain a healthy blood pressure. Management of hypertension among patients with coronary heart disease" concluded that a combination of an ACE inhibitor or b-blocker and possibly a thiazide diuretic is needed to achieve a target blood pressure of 130/80 in such patients, A treatment goal is to reduce the morbidity and mortality associated with hypertension and heart disease (Malik *et al.,* 2021) . Son *et al.,*(2018) Tan et al*.,* (2018) declared

same decision. Diastolic blood pressure is another clinical reading usually associated with SBP to obtain more accurate diagnostic decision, it is also influenced by variable factors. Our data, on the average, showed that patients have a significant rise in DBP compared with control (p = 0.0026). This is supported by Zhou *et al.,* (2018), who published the significant rise in CVDs patients, whereas Liu *et al.,* (2015) reported no significant difference between both groups.

The results of the present study indicated that high level of triglyceride was found in the serum of patients and significantly more than in control < (p = 0.0001) , Results by Talayero & Sacks,(2011) and Aberra et al., (2020) reached to the same conclusion, but Sun,(2021) found no significant difference in the level of TG between CVDs patients and healthy individuals. Although cholesterol plays a fundamental role in a plethora of intracellular mechanisms, it is known that individuals with highplasma cholesterol concentration are at increased risk of atherosclerotic heart disease(Zhang *et al.,*2021) . We found significant increase in blood cholesterol of CVDs patients compared to its level in control (p = 0.0001). This finding was supported by Avci *et al.,* (2018), but not by Berger *et al.,* (2015). body but Our results not showed a significant different in LDL among patients compared with healthy individuals (p = 0.0749). This result is in agreement with the reports published by Ravnskov et al., (2016) but in disagreement with Boren *et al.,* (2020) and Ference *et al.,* (2017). While The present results showed a significant decrease in HDL levels in patients compared to control (p > 0.0001) which is similar to the finding of Ben-Aicha et al., (2020) and Nicholls & Nelson (2019) whereas Rosenson *et al.,* (2018) and Casula *et al.,* (2021) have claimed no significant difference between patients and control. These variations may due to sort of mediations and kind of food habits. WBC reading were significantly higher in CVDs patients compared to control (p <0.0001). Kim et al., (2017) and Haybar et al., (2019) declared same decision of significance, whereas Lassale et al., (2018) ended up with no difference in WBC between both groups.

Angiotensin-converting enzyme (ACE) compared between CVDs patients and control

Indicted ACE has an important impact on cardiovascular structure and function (Wilson et al., 2016). Plasma ACE levels are less than 40 nmol/mL/min . ACE in our investigation was significantly higher than in blood of healthy individuals (p <0.0001). This is a similar result reported by Zhou et al., (2020) and Nouryazdan *et al.,*(2018).

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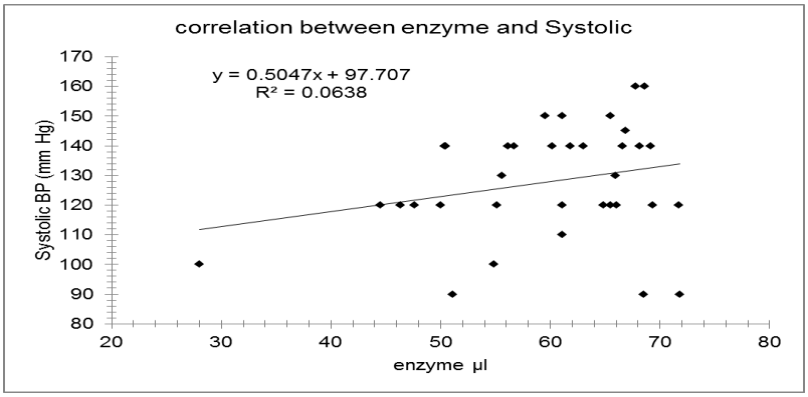

Figure 1. Correlation between Angiotensin-converting enzyme and Systolic BP

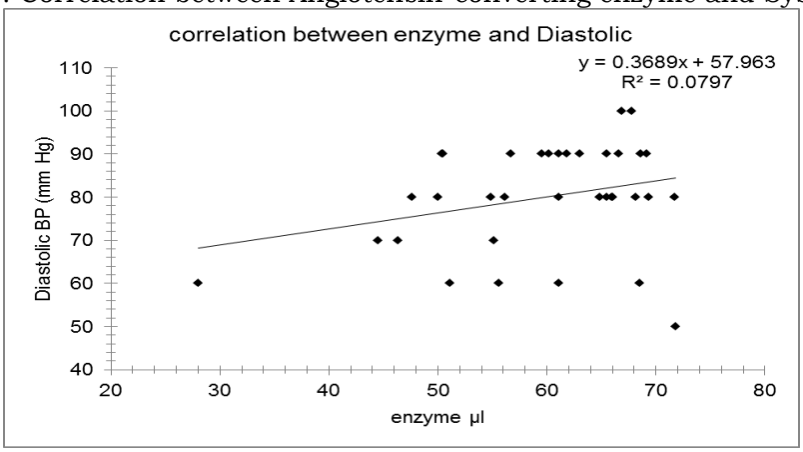

Figure 2. Correlation between Angiotensin-converting enzyme and Diastolic BP

The results indicate that there is a direct relationship between Hypertension and Angiotensin-converting enzyme , In other words, the higher the angiotensin-converting enzyme, the higher the systolic pressure . And the reason for that is Angiotensin-converting enzyme (ACE) converts AngI to angiotensin II (AngII), which is a strong vasoconstrictor. Increased production of AngII results in constriction of arterial blood vessels and induces the release of aldosterone from adrenaline and affect the activity of several key sodium transporters and the induction of sodium and water retention resulting in the elevation of BP . Gared (2010) also reported that some studies of the association of hypertension with the ACE positively associated.

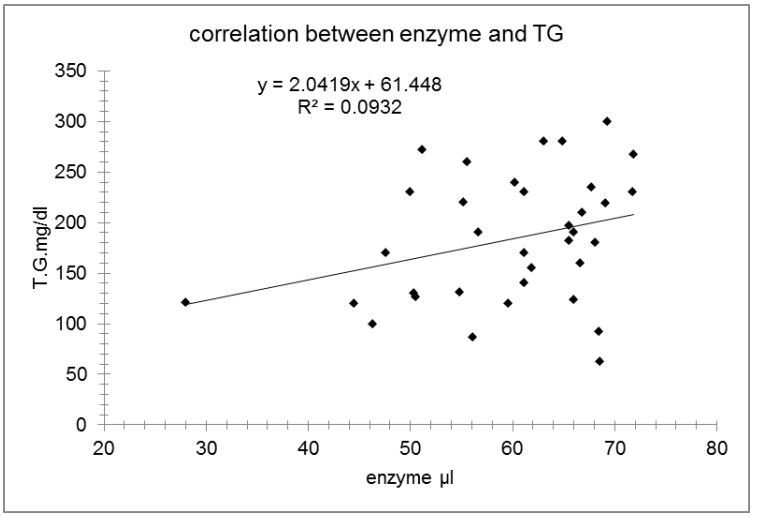

Figure 3. Correlation between Angiotensin-converting enzyme and triglyceride (TG)

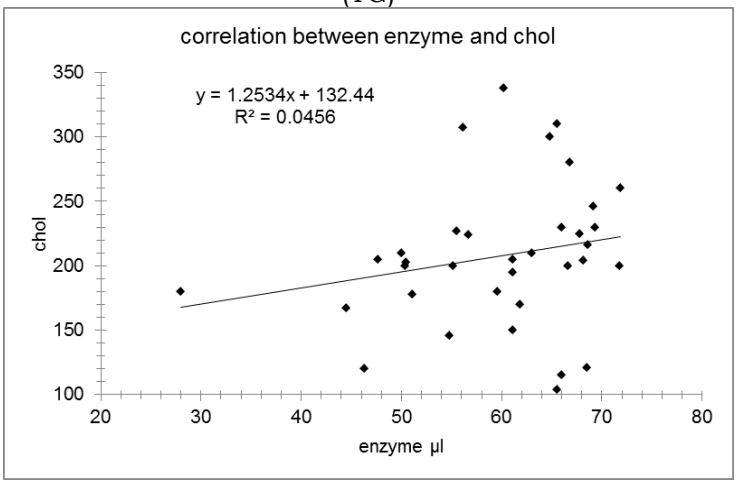

Figure 4. correlation between Angiotensin-converting enzyme and cholestrol(chol)

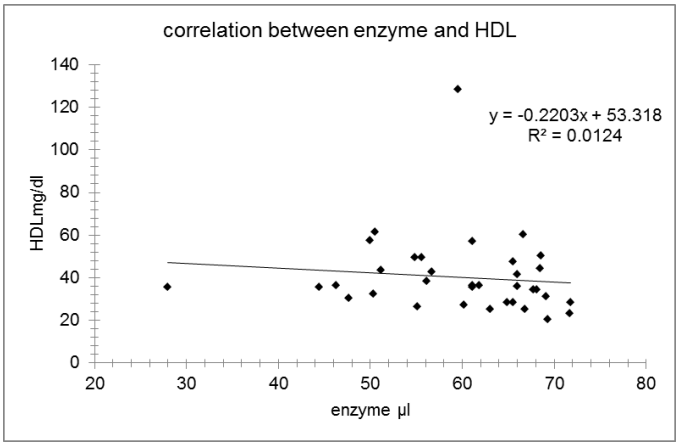

Figure 5. correlation between Angiotensin-converting enzyme and High-density-lipoprotein (HDL)

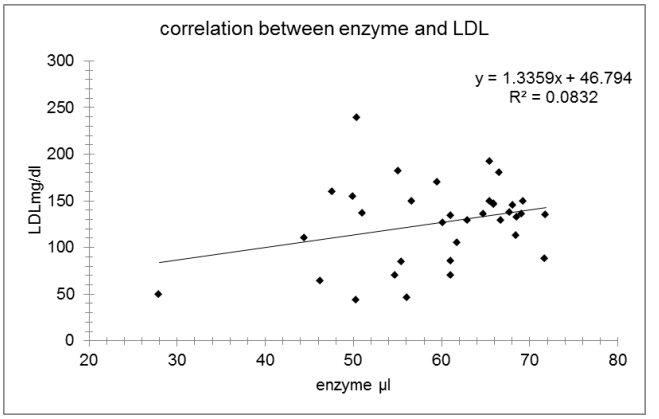

Figure 6. correlation between Angiotensin-converting enzyme and low -density-lipoprotein (LDL)

Correlation between angiotensin-converting-enzyme show a Positive relationship with triglyceride, cholesterol and Low-density-lipoprotein (LDL) in figure 3,4,6 That is, the higher the ACE, the higher the cholesterol , triglyceride and LDL levels**.** Increased activity of the ACE enzyme can contribute to an increased risk of disease CVDs by raising angiotensin II production , Ang II stimulates cholesterol synthesis and decreases high-density lipoprotein (HDL) cholesterol-Induced cholesterol efflux, The last effect is probably common for cholesterol membrane transport in other cells, Moreover, Ang II has a stimulatory effect on LDL-C oxidation and LDL-C degradation by macrophages, which is more pronounced in patients with arterial hypertension, our results corroborate with that of Pizoń et al., (2018) and Susilo et al., (2022).

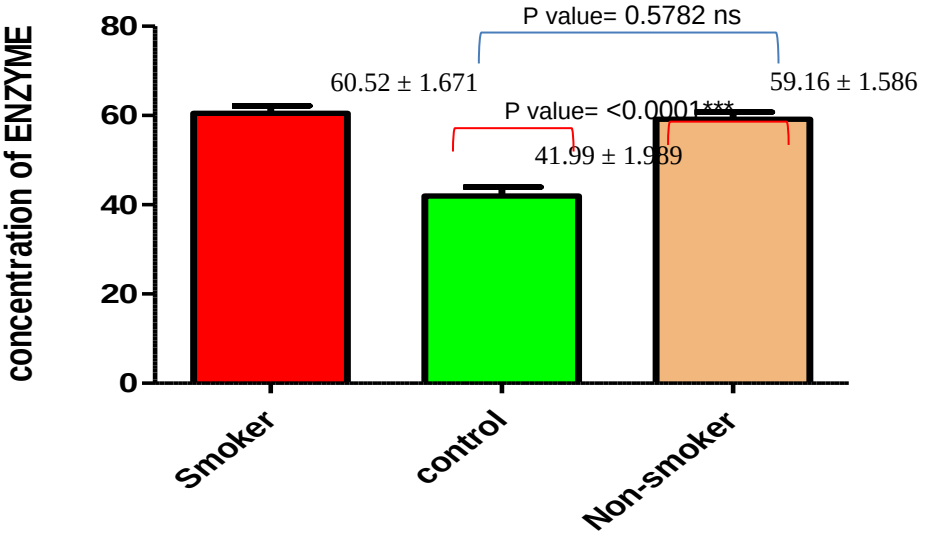

Figure 7. comparing between concentration of Angiotensin-converting enzyme with smoking in patients and control

Smoking was one of the risk factors for CVDs examined in relation to plasma ACE, Subjects were classified as smokers or nonsmokers based on their own statements , forty individuals stated that they were smokers (57%) and fifteen individuals stated that they were non smokers (21%). This study did not show any significant differences between smoking and non-smoking patients (p 0.5782) , but we did find significant differences($p<0.0001$) between non-smoker controls and smokers or non-smokers from patients in the angiotensin-converting enzyme level. Previous studies have shown that smoking increases serum ACE activity by Ljungberg (2009) and Ljungberg & Persson (2008).

Conclusion

There was relationship of the lipid profile and smoking in patients with an increase in the enzyme converting angiotensinogen.

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