JANUARY 2019 M.Sc. in Biochemistry Science and Technology HATAW ADIL MOHAMMED

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PEROXYNITRITE AND TRACE ELEMENTS IN PATIENTS WITH AORTIC VALVE SCLEROSIS

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HATAW ADIL MOHAMMED

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Peroxynitrite and Trace Elements in Patients with Aortic Valve Sclerosis

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Hataw Adil MOHAMMED

ABSTACT

PEROXYNITRITE AND TRACE ELEMENTS IN PATIENTS WITH AORTIC VALVE SCLEROSIS

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Aortic valve sclerosis (AVSc), is thickening and calcification of the aortic valve leaflets. The objective of this study was to investigate the relationship between AVSc and serum of peroxinitrite, zinc, selenium, copper, and iron. The study included 40 AVSc patients (75% females and 25% males) and were compared to same gender ratio of healthy control groups. The diagnosis of AVSc underwent based on comprehensive echocardiographic. Blood samples were taken for the determination peroxynitrite level by spectrophotometric method. Zinc and copper concentrations were determined by use of an atomic absorption spectroscopy. Selenium by utilize of inductively coupled plasma mass spectrometry device and iron by use of Beckman Coulter instrument were measured. There were significant differences in body mass index, blood pressure, and diabetes between the patient and the healthy subjects (p<0.05). The mean serum of peroxinitrite concentrations were significantly higher in AVSc subjects (6.05±1.83 µmol/L) than that of the control levels (2.61±0.97 µmol/L) (p=0.000). Differences in serum Fe, Se, and Cu levels were found between the patients and the healthy donors (p>0.05). A significant decrease was found in the serum zinc of patients group when the compared with the control group (p<0.01). Serum peroxinitrite concentrations could be markedly in patient with AVSc. Likewise, they had imbalance in some of the trace elements in their blood. An elevation in serum copper levels and a decreases of serum Se, Zn, and Fe concentrations in the valves subjectss as compared with the normal valves. The AVSc patients had higher prevalence of diabetes, hypertension, and obesity.

Keywords: Aortic valve sclerosis, Peroxynitrite, Selenium, Zinc, Copper, Iron

ÖZET

AORTIK VALF SKLEROZU OLAN HASTALARDA PEROKSINITRIT VE İZ ELEMENTLERİ

MOHAMMED, Hataw Adil
Yüksek Lisans Tezi, Biyokimya Bilimi ve Teknolojisi
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Aort kapak sklerozu (AKS), aort kapak yapraklarının kalınlaşması ve kalsifikasyonudur. Bu çalışmanın amacı AKS ile peroksinitrit, çinko, selenyum, bakır ve demir serumu arasındaki ilişkinin araştırılmasıdır. Çalışmaya 40 AKS hastası (% 75 kadın ve % 25 erkek) dahil edilmiş ve sağlıklı kontrol gruplarının aynı cinsiyet oranı ile karşılaştırılmıştır. AKS tanısı, kapsamlı ekokardiyografiye dayanarak yapılmıştır. Spektrofotometrik yöntemle peroksinitrit düzeyini belirlemek için kan örnekleri alınmıştır. Çinko ve bakır konsantrasyonları atomik absorpsiyon spektroskopisi, selenyum ile indüktif eşleşmiş plazma kütle spektrometresi cihazı ve demir düzeyi Beckman Coulter cihazı kullanılarak belirlenmiştir. Vücut kitle indeksinde, kan basıncında ve diyabette hastalar ile sağlıklı kişiler arasında anlamlı fark olmuştur (p<0,05). AKS deneklerinde (6,05±1,83 μmol/L) ortalama serum peroksinitrit konsantrasyonları kontrol düzeylerinden (2,61±0,97 μmol/L) anlamlı derecede yüksek olmuştur (p=0,000). Hasta ve sağlıklı donörler arasında serum Fe, Se ve Cu düzeylerindeki farklılıklar (p>0,05) bulunmuştur. Hasta grubunun serum çinko grubunda kontrol grubu ile karşılaştırıldığında anlamlı bir azalma saptanmıştır (p<0,01). AKS'li hastalarda serum peroksinitrit konsantrasyonları belirgin bir şekilde işaretlenmiştir. Aynı şekilde, kanlarındaki bazı eser elementlerde dengesizlikler vardı. Serum bakır seviyelerindeki bir yükselme ve normal valflere kıyasla valf hastalarında serum Se, Zn ve Fe konsantrasyonları azalmıştır. AKS hastaları daha yüksek diyabet, hipertansiyon ve obezite prevalansına sahip olmuştur.

Anahtarkelimeler: Aort kapak sklerozu, Peroksinitrit, Selenyum, Çinko, Bakır, demir

To my grandmother,

I would like to dedicate this Master dissertation to my grandmother. She has been the source of my strength throughout all my study stages. So there is no doubt in my mind that without her continued support and counsel I could not have completed my studies and could not get to this point.

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LIST OF SYMBOLS/ABBREVIATIONS

µg/dL	Microgram per Deciliter
µg/L	Microgram Per Liter
µmol/L	Micromole Per Liter
8-NG	8-nitroguanine
ACE	Angiotensin Converting Enzyme
ADP-ribose	Adenosine Diphosphate-Ribose
AIF	Apoptosis-Inducing Factor
Ao	Arterial Blood
Apo	Apolipoprotein
AS	Aortic Stenosis
Asc	Aortic Sclerosis
AV	Aortic Valve
AVC	Aortic Valve Calcification
AVC	Aortic Valve Calcium
AVS	Aortic Valve Stenosis
AVSc	Aortic Valve Sclerosis
BAV	Bicuspid Aortic Valves
BMI	Body Mass Index
CAT	Catalase
CAVD	Calcified Aortic Valve Disease
cGMP	Cyclic Guanosine Monophosphate
coenzyme Q-10	Ubiquinol
CS	Sinus Effluent
EBCT	Electron Beam Computed Tomography
EC	Endothelial Cell
FDA	Food and Drug Administration
GIT	Gastrointestinal Tract
GPx	Glutathione Peroxidase
GRx	Glutathione Reductase
GSH	Glutathione
GSH-Px	Glutathione Peroxidase
H^+	Hydron
HLA –DR	Human Leukocyte Antigens
HOCl	Hypochlorous Acid
M	Molar
M/cm	Mole Per Centimeter
$M^{-1}S^{-1}$	Second Order Reactions
mA/h	MilliAmpere Per Hour
M-CSF	Macrophage Colony Stimulating Factor

mg/L	Milligram Per Liter
mmHg	Millimeter of Mercury
mmol/L	Millimoles Per Liter
MMP	Matrix Metalloproteinase
Msrs	Methionine Sulfoxide Reductases
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
ng/ml	Nanogram Per Milliliter
Nm	Nanometer
NOS	Nitric Oxide Synthases
ONOOH	Peroxynitrous Acid
PARG	Poly (Adenosine Diphosphate Ribose) Glycohydrolase
PARP-1	Poly (Adenosine Diphosphate Ribose) Polymerase-1
PDF	Platelet-Derived Factor
PDGF	Platelet-Derived Growth Factor
PN	Peroxynitrite
RANK	Receptor Activator of Nuclear Factor Kappa B
RONS	The Reactive Oxygen Nitrogen Species
ROO•	Peroxyl Radical
RUNX2	Runt-Related Transcription Factor 2
S2	The Second Heart Sound
Se/day	Selenium Per Day
Sec	Selenocysteine
sGC	Soluble Guanylate Cyclase
TGF-b	Transforming Growth Factor b
TLR	Toll Like Receptor
TNF-a	Tumor Necrosis Factor a
TPN	Total Parenteral Nutrition
TPTZ	2,4,6-Tri-(2-pyridyl)-5-triazine
TrxR	Thioredoxin Reductase
Tsat	Transferrin Saturation
TTE	Transesophageal Echocardiography
Ul	Microliter
v/v %	[(Volume of Solute)/(Volume of Solution)] x 100%
Vitamin P	Flavonoids
E	Extinction Coefficient
µg/day	Microgram Per Day
µg/L	Microgram Per Liter
µL	Microliter

CHAPTER 1

INTRODUCTION

1.1 The Heart

The heart is a biological pump consisting of atrial and ventricular chambers and four heart valves whose synchronized movements cause blood to flow from the heart (Ilyas and Shah, 2017). The heart consists of four heart chambers, as shown in Figure 1.1. It contains two chambers that collect the incoming blood, left atrium, and right atrium.

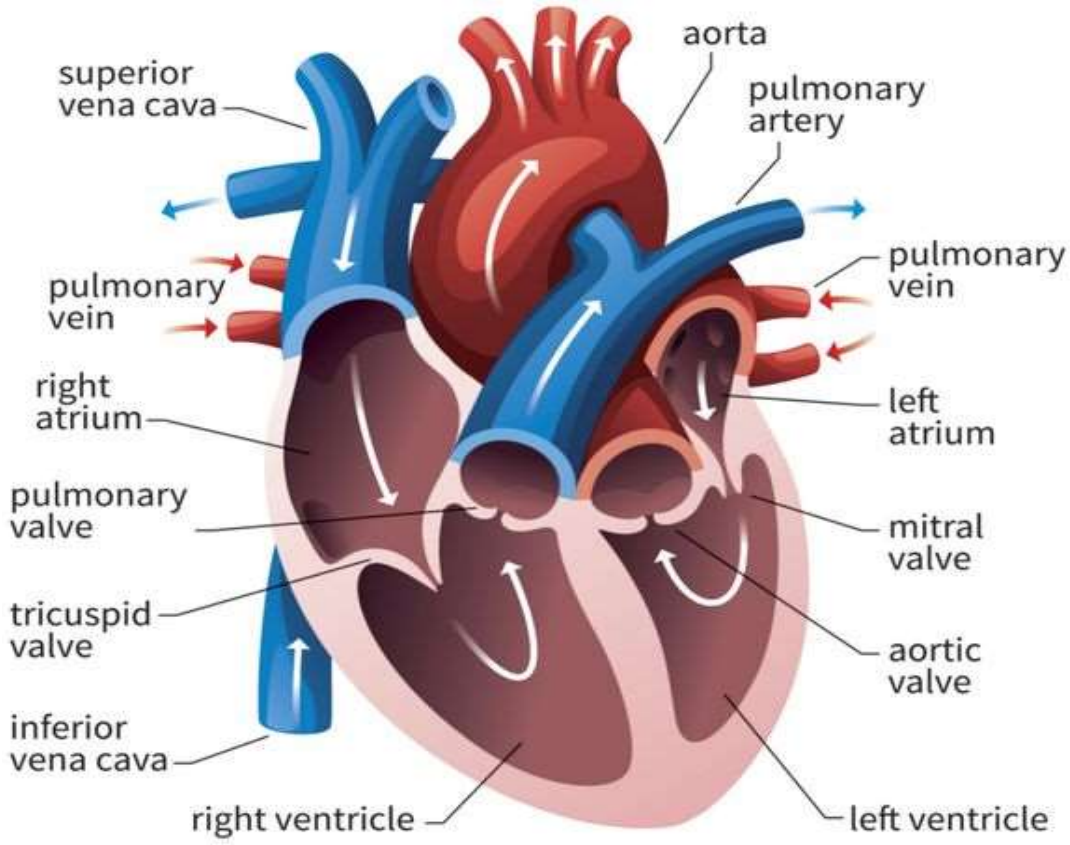

Figure 1.1 Drawing of the heart showing its chambers and valves. The arrows indicate the direction of blood flow (https://www.thoughtco.com/evolution-of-the-human-heart-1224781)

The blood is transferred from the atria to the corresponding ventricles. After the electrical stimulation, the right ventricle pumps blood into the lungs to deliver oxygen. The oxygen-rich blood is then collected in the left atrium. It leaves the heart through the left ventricle, but this time the blood flows into all organs to provide them with nutrients and oxygen and carry away their waste products. Four valves are present in the heart to maintain unidirectional blood flow (Guyton and Hall, 1996). Cardiovascular diseases are a heterogeneous group of disorders affecting the blood vessels and the heart (Mimić-Oka et al., 1999). In the advancing regions about 50% of the deaths caused by cardiovascular diseases (CVD) (Townsend et al, 2016).

1.2 Heart Valves

The heart valves are complex soft tissue structures responsible for unidirectional blood flow in the heart (Cheung et al, 2015). The task of all the heart valves which they are four valves (the pulmonary and tricuspid valves in conjunction with the right atrium and ventricle, the mitral and aortic valves in conjunction with the left atrium and ventricle), (see more detail in Figure 1.1), is intended to maintain unidirectional flow through the heart. The ability of the valves to allow unrestricted forward flow depends on the flexibility and smoothness of their blades (Wallby, 2008). So, one can say that the valves of heart have two jobs: opening state, the way in which the blood flows are controlled, and close state, the pressure differences are allowed to exist in a closed system. Any Abnormality in the valve functions results in either pressure overload because of restricted opening or volume overload due to insufficient occlusion (Novaro, 2014). Valvular heart disease (VHD) is an increasing form of cardiovascular disease whose prevalence increases with age. A significant proportion of newborns (1-2% of all live births) are affected by congenital heart diseases, the most common are the valves, which are associated with 44,000 cases annually (Cheung et al., 2015). Dysfunction of the heart valve is a relatively popular health issue among the people, while a significant heart valve disease is rather unusual. The cause of various heart valve diseases depends on which valve is affected (Wallby, 2008). A defect or damage in the any of the four heart valve caused the VHD. It is a serious health trouble affecting older people especially. A congenital defects or acquired pathology caused the VHD (Zeng el al., 2016). Heart valve disease is an important clinical problem worldwide and responsible for significant

mortality and morbidity (Stam et al., 2017). Heart valve diseases account for almost 20,000 deaths in the US alone (Garg, 2006).

1.3 Aortic Valve

The trileaflet semilunar valve consisting of right, left, and noncoronary leaflets is called the aortic valve (AV) (Poppers et al., 2013) Figure 1.2. The AV consists of three equal sized leaflets or bumps that are shaped like half-moons, hence the name "semiarunate" valve (Merryman and Schoen, 2013). Each tip is several hundred microns thick and consists of three layers: fibrosa with its dense connective tissue provides strength, cancellous bone with its loose matrix of glycoproteins provides a cushion for the mechanical forces and ventricularis provides elasticity for changes in shape during opening and closing, three aortic valve layers are avascular and innervated by adrenergic and cholinergic neuronal networks (Alexopoulos et al., 2011).

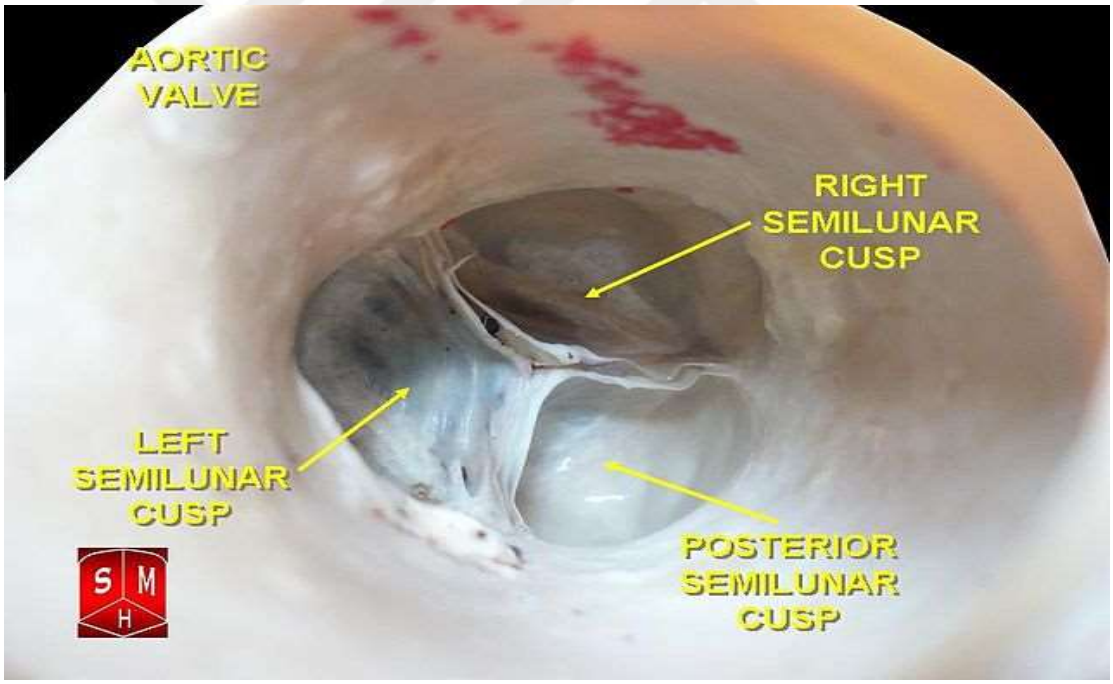

Figure 1.2 Show the aortic valve (AV) cusps (https://www.kid-facts.com/2016/01/aortic-valve.html)

An aortic valve is usually tricuspid, although it is found congenitally bicuspid in 1% of the population (Alexopoulos et al., 2011). The AV is a passive tissue whose movement is controlled by blood flow. When the heart contracts during systole, blood flows from the left ventricle through the aortic valve into the aorta. During

diastole, back pressure closes the aortic valve and prevents oxygen-rich blood from flowing back into the left ventricle (Rajamannan et al., 2011; Jaeggli, 2015).

The normal aortic valve keeps unidirectional blood flow from the left ventricle into the aorta. It is a flexible membrane which closes and opens most than 100,000 times a day with each heartbeat. The healthy aortic valve consists of three wings and is located at the junction among the aortic root and the left ventricular outflow tract (Lerman et al., 2015). The aortic valve sits within the aortic root. The aortic root, located between the interventricular septum and the anterior mitral valve leaflet, extends from the aortic valve to the sinotubular junction (Poppers et al., 2013), Figure 1.3.

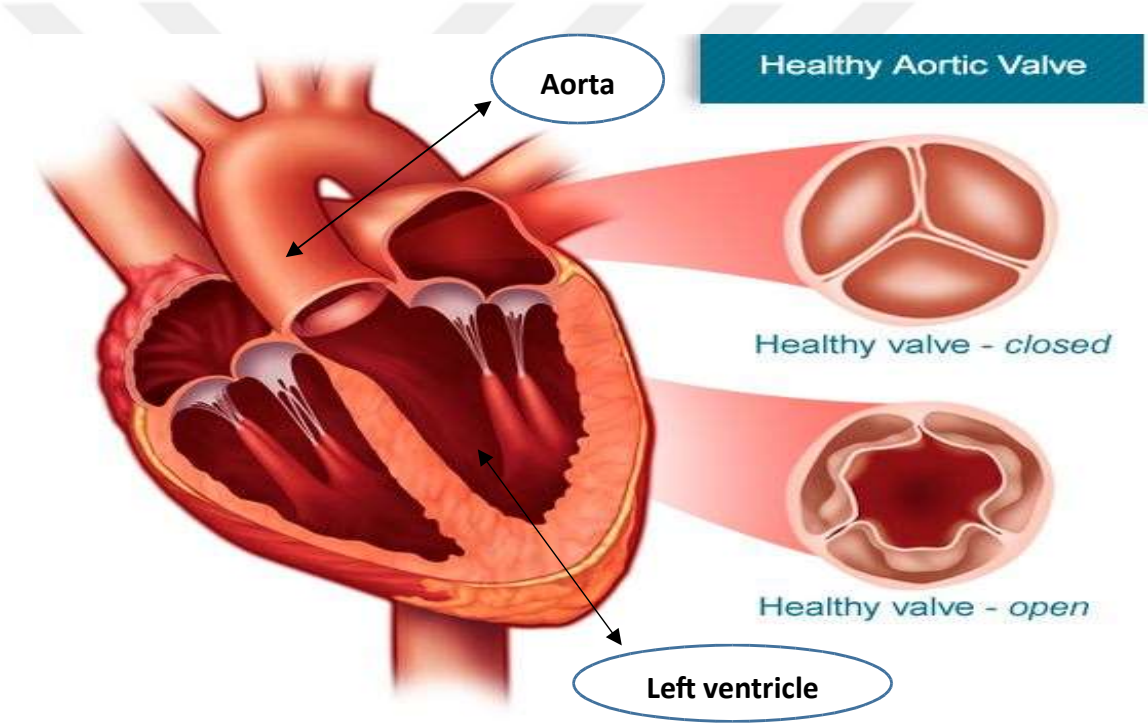

Figure 1.3 Shows the healthy aortic valve (https://www.sjchs.org/media/file/diseased-valve-large.jpg)

Typically, the leaflets are ≤1 mm thick (Chen and Simmons, 2011; Leopold, 2012). The aortic valve thickens over time, averaging 0.67 mm thick in those under 20 years and 1.42 mm in those over 60. This size is surprisingly thin given the tensions on the tissue (Jaeggli, 2015). Aortic valve calcification (AVC) (as active aortic valve) risk factors and molecular pathways leading to disease progression and eventually complications, AVC a predictive predictor of adverse clinical events in patients with

the cardiovascular disorder and generalized AVC has also been identified as a danger factor for complications following implantation of the aortic valve (Yousry et al., 2015). AVC is a common disease that ranges from 21% to 26% in adults over 65 (Yu et al., 2009). Calcifying aortic valve disorder has been classified into two functional classes: aortic valve stenosis (AVS) and aortic valve sclerosis (AVSc). 10 to 15 years have been this both types of disease are identified as an active process (O'brien, 2006). The degenerative aortic valve disorder is characterized by thickening and calcification of the cusps of the aortic valve with normal function at the beginning. It is called aortic valve sclerosis (Olszowska, 2011).

1.4 Definition of Aortic Valve Sclerosis (AVSc)

Aortic valve sclerosis (AVSc) is a kind of aortic valve disorder (Milin et al., 2014). It is defined as thickening and calcification of the aortic valve leaflets (at minimum one abnormal leaflet per valve) (Agmon et al., 2001), in the absence of obstruction of ventricular outflow (Khilla et al., 2018), with (gradient <20 to 25 mm Hg) (Turi, 2008). AVSc is characterized by increased stiffness, and calcification of the aortic leaflets without fusion of commissures (Taylor et al., 2005; Marmelo et al., 2014). In developed countries, AVSc is the most common valve disorder (Markus et al., 2015). It is present in about 25% of people aged 65 (Beckmann et al., 2010) and increases to 50% at the age of 80 (Marechaux et al., 2009; Sverdlov a et al., 2012). The normal aortic valve consists of a dense layer of aligned collagen fibers with few fibroblasts, the so-called fibrosa. On the ventricular side of the valve is a layer of elastic tissue, the ventricularis. Between fibrosis and ventricularis there is a layer of loose connective tissue, the so-called cancellous bone, predominantly in the basal third of the valve. Endothelium lines both sides of the valve leaflets. With age, the number of fat cells in the cancellous bone increased and leaf thickness increased slightly Figure 1.4. In contrast, in aortic sclerosis, focal subendothelial lesions are present on the leaflet aortic side, spreading the disease process to adjacent fibrosis (Lee et al., 2005). These early aortic valve lesions are characterized by lipid deposition (Topcu et al., 2017), macrophage and lymphocyte infiltration, basement membrane disorder, and microscopic calcification (Lee et al., 2005). AVSc is the late outcome of a long-lasting inflammatory process due to various pathophysiological mechanisms and not a result of normal ageing (Nordström et al., 2003), possibly leading to life-threatening injuries (Yang et al., 2015). Aortic sclerosis can be identified by either on

echocardiography by the qualitative thickening of the aortic valve or calcification of tissue of the aortic valve as demonstrated by computed tomography (Small et al., 2017). The conventional classification of AVSc severity depends on the evaluation of the calcification amount by the echocardiography at the aortic valve and is therefore a subjective measurement. A comparison of the early stages of AVSc can be hard as the evaluation depends on the judgment of the reading cardiologist. In addition to the severity, the location and type of calcification of the aortic valve are also significant (Milin et al., 2014).

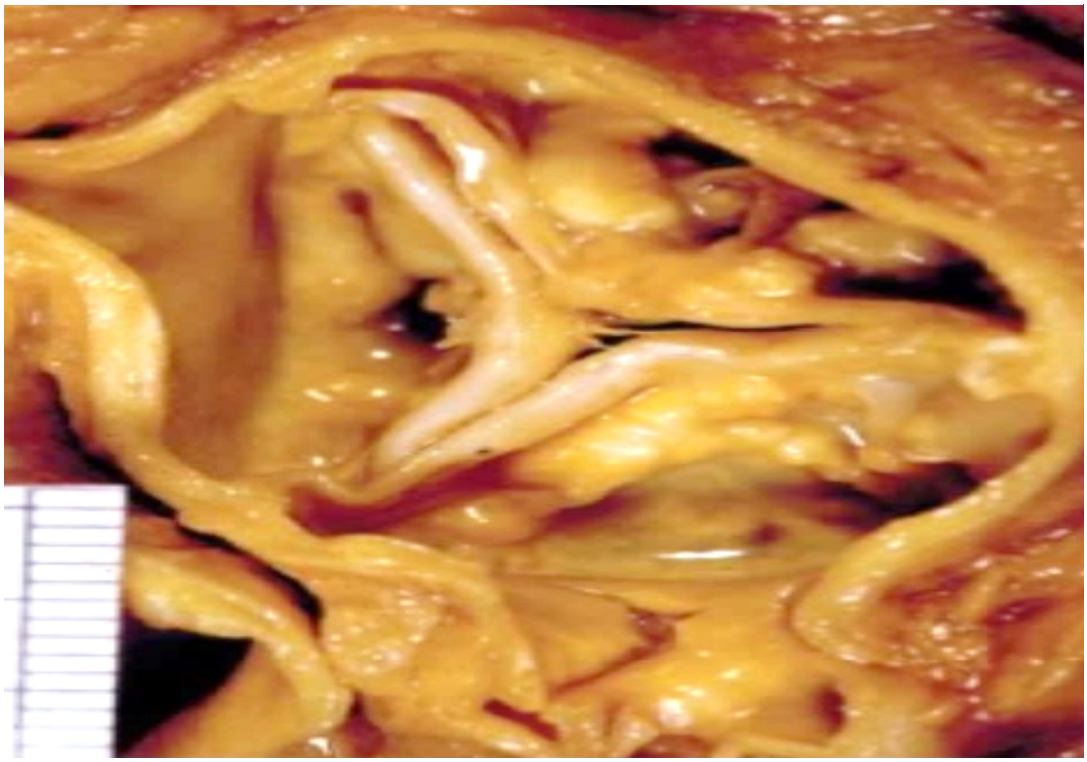

Figure 1.4 Shows the aortic valve sclerosis (https://www.google.com/search?rlz=1C1CAFB_enTR713TR713&biw=1366&bih=626&tbm=isch&sa=1&ei=yHxLXLHOHsGVsAGZ55uwDQ&q=aortic+valve+sclerosis&oq=aortic+valve+sclerosis&gs_l=img.12...0.0..1935...0.0..0.0.0.....0.....gws-wiz-img.T4ipB6kZgJk#imgrc=Oz6n2eHaijn2BM:)

Light, moderate and severe AVSc were classified as the thickness of a given hump of 2-4 mm, > 4 mm, and> 6 mm (Hsu et al., 2005), with a transaortic flow rate <2.5 m/s (Topcu et al., 2017).

Therefore, five stages were classified for AVSc (from stage 0 to stage 4) according to Tolstrup et al. (2002):

Stage 0: no thickening of the aortic valve leaflets, normal reflection;

Stage 1: slightly accentuated reflection, thickness <2 mm;

Stage 2: slightly accentuated reflection, thickness 2-4 mm;

Stage 3: generalized over-reflection, thickness> 4 mm;

Stage 4: clearly overshadowing masses, thickness> 6 mm (Khilla et al., 2018).

The essential significance of AVSc is that it provides a window on the overall presence of vascular disorders, particularly coronary heart disorder. Therefore, all patients with AVSc treated in intensive care should have a high index of suspected vascular disorder (Turi, 2008). Most studies have proved that patients with AVSc have an increased incidence of cardiovascular events (London et al., 2000; Shah et al., 2007; Ngo et al., 2009; Bonapace et al., 2014), and mortality (Taylor et al., 2005; Völzke et al., 2010; Korkmaz et al., 2013). AVSc is a hallmark of most of the heart diseases (Lucena and Santos, 2015), ranging from chronic heart failure and myocardial infarction (Picardo et al., 2016), to calcification of AVS (Poggio et al., 2013). Increase the hazard of stroke, congestive heart failure, angina pectoris, and death due to cardiovascular causes (Otto et al., 1999). For example, the cardiovascular health study showed that death rates from cardiovascular causes or deaths in patients with aortic sclerosis were twice as high as in patients with normal aortic valves (Otto et al., 1999; Prasad and Bhalodkar, 2004). It is also associated with endothelial dysfunction in humans (Sverdlov a et al., 2012). In fact, about 50% of patients with AVSc are also affected by mitral sclerosis (Palmiero et al., 2007). Therefore, AVSc is a common disease, particularly in the elderly, and can no longer be considered as an innocent murmur because by 50% it increases the hazard of myocardial infarction or cardiac death (Nightingale and Horowitz, 2005).

1.5 History of Aortic Valve Sclerosis

As early as the 17th century calcifying-AVD was recognized. At the beginning of the 20th century, it was assumed that venous calcifications happen due to degeneration or rheumatic disorders. The prevalence of rheumatoid etiology in developed countries has declined due to the decrease in rheumatic fever, while the disease of the aortic valve with degenerative etiology has increased due to longer life expectancy

(Prasad and Bhalodkar, 2004). The pathogenesis of AVC has been controversial since its original description by Monckeberg in 1904 (Sell and Scully, 1965). Although calcification was originally attributed to endocarditis, Möenckeburg wrote the first detailed description of AVC in 1904 (Akat et al., 2009; Sathyamurthy and Alex, 2015). Who reported that this pathology probably occurred as a result of calcium deposition on sclerotic valve leaflets. He described two mechanisms to explain this phenomenon: degeneration in the valvular veins near the sinus veins of valsalva, which extended to the tips of the humps, or sclerotic changes in the aorta that extended to the valve leaflets (Leopold, 2012).

In the 1970s, it was recognized by the work of Roberts and Pomerance that a third etiological group existed, namely a degenerative, wear-related form of aortic stenosis (AS), which affects preferably the older part of the population. In the early 1990s, several studies were published that showed signs of inflammation in tricuspid stenosis, which had both epidemiological and histopathological similarities to atherosclerosis (Wallby, 2008).

1.6 Epidemiology of Aortic Valve Sclerosis

The epidemiology of AVSc suggests that it occurs in about 30% of 65- to 74-year-olds (Stewart et al., 1997; Stritzke et al., 2009; Beckmann et al., 2010; Poggio et al., 2013; Sider et al., 2014), and about 50% of persons older than 84 years (Sverdlov a et al., 2012). The main popular heart valve disorder in the US (Roger et al., 2011), and Europe (Iung et al., 2003), is calcified aortic valve disease (CAVD). Recent estimates indicate that aortic valve disease is common in the United States adult population and contributes to more than 28,000 deaths and 48,000 hospitalizations annually (Leopold, 2012). CAVD (Rajamannan et al., 2011), includes both sclerosis, which is considered a common disease and is described by leaf thickening with no left ventricular outflow obstruction, and aortic valve stenosis which is available in more than 2% of the individuals which were over 65 years, and is described by solidify leaflets, blocked flow and impaired cardiac job (Lindroos et al., 1993; Stewart et al., 1997; Sider et al., 2014). The epidemiological risk factors for calcified-AVD are similar to those of atherosclerosis and involve hypertension, diabetes, age, male gender, smoking, and high serum concentrations of (LDL) low density lipoprotein (Lindroos et al., 1994; Stewart et al., 1997; O'brien, 2006;

Picardo et al., 2016). Sclerosis and aortic valve calcification is a comparatively common valve disease in the developed countries (Heistad et al., 2013; Markus et al., 2015), and is prevalent in Western countries (Sverdlov a et al., 2012), that 29% of the 5,621 persons over 65 years of aortic sclerosis (echocardiography) (Otto et al., 1999). An identical study with an elderly people (the mean of the age 82 years) found a propagation of 42% (Nightingale and Horowitz, 2005), the prevalence continues to increase with a higher cardiovascular danger population. In a study involved 425 patients with (the mean of the age 68 years) were referred to with chest ache, aortic sclerosis was found in 50% (Chandra et al., 2004). From this it's able to conclude that AVSc is popular within the elderly and in a populace with risk factors for coronary heart disorder. One of the criticisms of these considers is that they don't indicate whether they avoided bicuspid aortic valves (BAV) from the study. It is known that the BAV calcified earlier and, with a population prevalence of up to 2%, led to a slight overestimation of spread of the sclerosis aortic valve in tricuspid valves (Nightingale and Horowitz, 2005).

1.7 Clinical Diagnosis of Aortic Valve Sclerosis (AVSc)

Aortic valve thickening (sclerosis) is an asymptomatic condition commonly referred to as systolic noise during physical examination (Picardo et al., 2016), in the aortic region, normal cleavage of the second heart sound, and the normal volume of the carotid pulse (Prasad and Bhalodkar, 2004), or echocardiography for another reason (Siscovick and Otto, 2011). The presence of systolic aortic dermatitis is often the first clue that could alert the physician to the possibility of AVSc (Nightingale and Horowitz, 2005). Especially through the physical findings: aortic sclerosis, in the absence of stenosis, usually best to hear about the right second room. In general, the noise is short and not very loud. A normal carotid pulse and a normal S2 indicate the absence of aortic stenosis. Many patients with aortic sclerosis, however, have no sound during the physical examination. In such patients, the diagnosis is usually randomized to echocardiography, which is performed for other indications. The physical examination is neither sensitive nor specific for the exclusion of aortic valve obstruction. Therefore, if cardiac symptoms are present, echocardiography should be performed to determine the severity of valve disease and determine if there is a progression to aortic stenosis or other cardiac abnormalities (Siscovick and Otto, 2011). The existence of AVSc in younger patients must be of special concern

because it's more of an inflammatory process than a degenerative process. Since the AVSc patients under the normal rate ages rarely show any symptoms, therefore it's hard to examine these young patients without underlying clinical suspicion. Youthful people with a homely history of early aortic stenosis, bicuspid valves, coronary heart disease (CHD), classical risk factors like chronic inflammatory disorders and diabetes mellitus would be appropriate starting points (Milin et al., 2014). AVSc develops over for many years. Patients may be asymptomatic for a relatively long time before symptoms (for instance, stress syncope, left heart failure, and angina pectoris) progressively appear. Current surgical and diagnostic techniques and perioperative steps, involving the required intensive care, make the prognosis favorable (Nyström-Rosander et al., 2002). Since the tissues of asymptomatic aortic sclerosis patients are mostly not ready to researchers as these valves are not surgically changed till moderate to severe stenosis happen (Branchetti et al., 2013).

The systolic ejection sound (during the middle systolic phase) could be detected and serves as an important indicator in the diagnosis of aortic sclerosis. In some cases, the cardiovascular exam could not have a heart murmur; therefore, the burden of diagnosing the condition is with radiological techniques, such as echocardiography. In patients who are asymptomatic, routine electrocardiogram follows as an essential feature. Echocardiography is the gold standard diagnostic tool to identify AVSc (Palmiero et al., 2007) Figure 1.5. Echocardiography is a cost-effective, portable, radiation-free, and simple technique with a clear possibility for the detection and quantitation of vascular and valvular calcifications (Corciu et al., 2010).

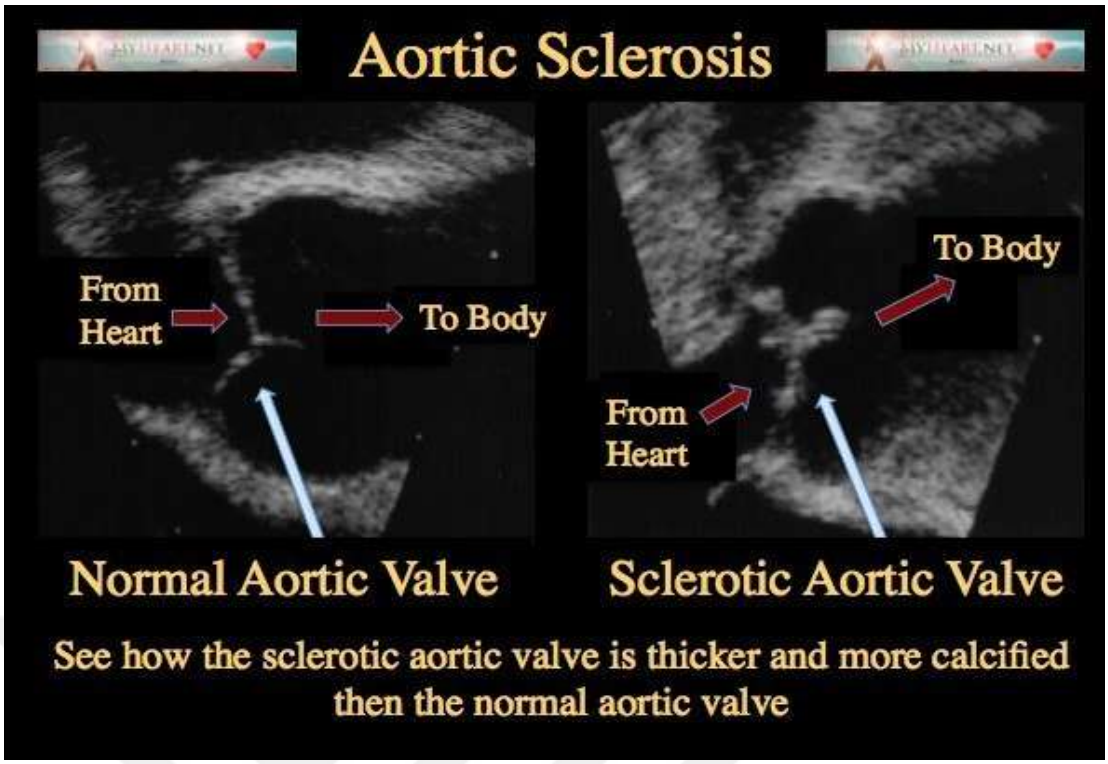

Figure 1.5 A picture illustrating a normal aortic valve and sclerotic aortic valve echo's (https://myheart.net/articles/aortic-sclerosis-is-it-dangerous/)

AVSc is defined echocardiographically by focal regions of valve thickening usually located in the center of the leaflet with commissural sparing and normal cusp motility. Diffuse leaflet thickening is not characteristic of AVSc; instead, it proposes normal aging changes, another valve pathology, or an imaging artifact. In the sclerotic valve, valve hemodynamics are within normal limits (Freeman and Otto, 2005), with an anterograde velocity of <2.5 m/s through the AV (at minimum single abnormal valve per valve) (Palmiero et al., 2007). Quantification of the severity of AVSc on a scale of 0 to 3: when 0, is normal (no participation); 1, mild (small contribution of a booklet); 2, moderate (minor participation of two cusps or dense participation of a cusp); and 3, severe (dense participation of two cusps or participation of all three cusps (Chandra et al., 2004). Others have applied a scale of 1 to 4, and basically, these ways are still subjective (Nightingale and Horowitz, 2005). Although two-dimensional echocardiography is the mainstay of the diagnosis of AVSc, particularly as it is usefulness in people-based studies, it does not quantify calcium (Prasad and Bhalodkar, 2004). Therefore, other image techniques have been suggested. For example, electron beam computed tomography (EBCT) or Ultrafast

CT, which is a relatively sensitive and accurate tool for quantification of aortic valve calcium (AVC) (Rajamannan et al., 2011), and recent multi-detector CT quantifies the grade of aortic valve calcification. These and other techniques, in addition to assessing aortic valve calcification, able to discover calcium in the coronary arteries, in the abdominal aorta, and in the mitral annulus aortic arch (Nightingale and Horowitz, 2005). Other imaging modalities such as transesophageal echocardiography (TTE), TTE could also be used to diagnose AVSc (Coffey et al., 2014). Thus, it can be concluded that, although in some cases a systolic outflow sound can be intercepted during the physical discharge, AVSc has no reliable linked clinical symptoms (Freeman and Otto, 2005).

1.8 Pathophysiological Mechanism of AVSc

Although non-rheumatic AVSc is the most popular form of valve disorder among the elderly, its pathogenesis is not fully understood (Nightingale and Horowitz, 2005). Many pathophysiological factors link to calcification of the valve and involve both environmental and genetic factors (Prasad and Bhalodkar, 2004). It can say that three key factors responsible for the development of aortic sclerosis are lipid accumulation, inflammation followed by calcification (Bhatt et al., 2015). However, these factors can be integrated into the occurrence and presence of certain risk factors. For example, abnormal lipid levels in the blood (hyperlipidemia), an increase in lipoprotein levels, male gender, smoking, hypertension, diabetes, older age group (Twardowski et al., 2015). With the increase in the age of the person, the aortic valve mobility and the syndrome of the metabolic (consisting of pentads: low-density lipoprotein, abdominal fatness, elevated triglyceride levels, elevated blood sugar levels, and elevation blood pressure are also at danger for aortic sclerosis (Siscovick and Otto, 2011). In particular, renal diseases and troubles of phosphate and calcium metabolism speed up the operation significantly (Nightingale and Horowitz, 2005). The aortic valve (AV) is equipped with the ability to dynamically move in a high pressure environment. This will produce environmental stress on the endothelium of the aortic cusp which may be considered the incentive for AVSc, a region of high disorderly flow as well as low shear stress (Goldbarg et al., 2007). The middle of the booklet has the highest mechanical load and is more repeatedly affected than the commissures. In addition, there is a preference for the participation of the non-coronal tubercle, probably due to stress over the lower shear, as the diastolic flow is

absent via this leaflet (Milin et al., 2014). The initial and early lesions in AVSc include lipid deposition (Nightingale and Horowitz, 2005), and focal sclerosis (Hansson, 2005), Figure 1.6.

AV leaflets consist of three layers which are: the ventricular (on the ventricular side of the cusp with elastin), cancellous bone a loose connective tissue in the basal third of the valve, and fibrosis (from the collagen core) (Milin et al., 2014). The deposition of lipids [apolipoprotein (apo) E, apo (a), apo B] (Prasad and Bhalodkar, 2004), happen on the aortic side of the cusps, given the above mechanical strengths. Similar to arteries, homeostasis over the trade of mechanical and biochemical signals. Comparing to the endothelial cell alignment in the vascular system (parallel to the flow direction), the endothelial cell alignment of the valve is perpendicular to the flow (Butcher et al., 2004). The area with the highest turbulent flow will have thickening in the cusps. In this area of high impacts, the endothelium will have an increased amount of the adhesive molecules with infiltration of the inflammatory genes and cells (occasional T cells, foamy macrophage, and non foamy cells); after that the inflammatory cells will infiltrate and lead to deposition of the lipid within the membrane (Goldbarg et al., 2007; Milin et al., 2014). Many factors contribute to the local damage of the endothelial layer that causes the AVSc, these factors may be agentic, inflamatory cell mediation and mechanical factors. Subsequent inflammation has been proposed as a hallmark of AVSc pathogenesis.

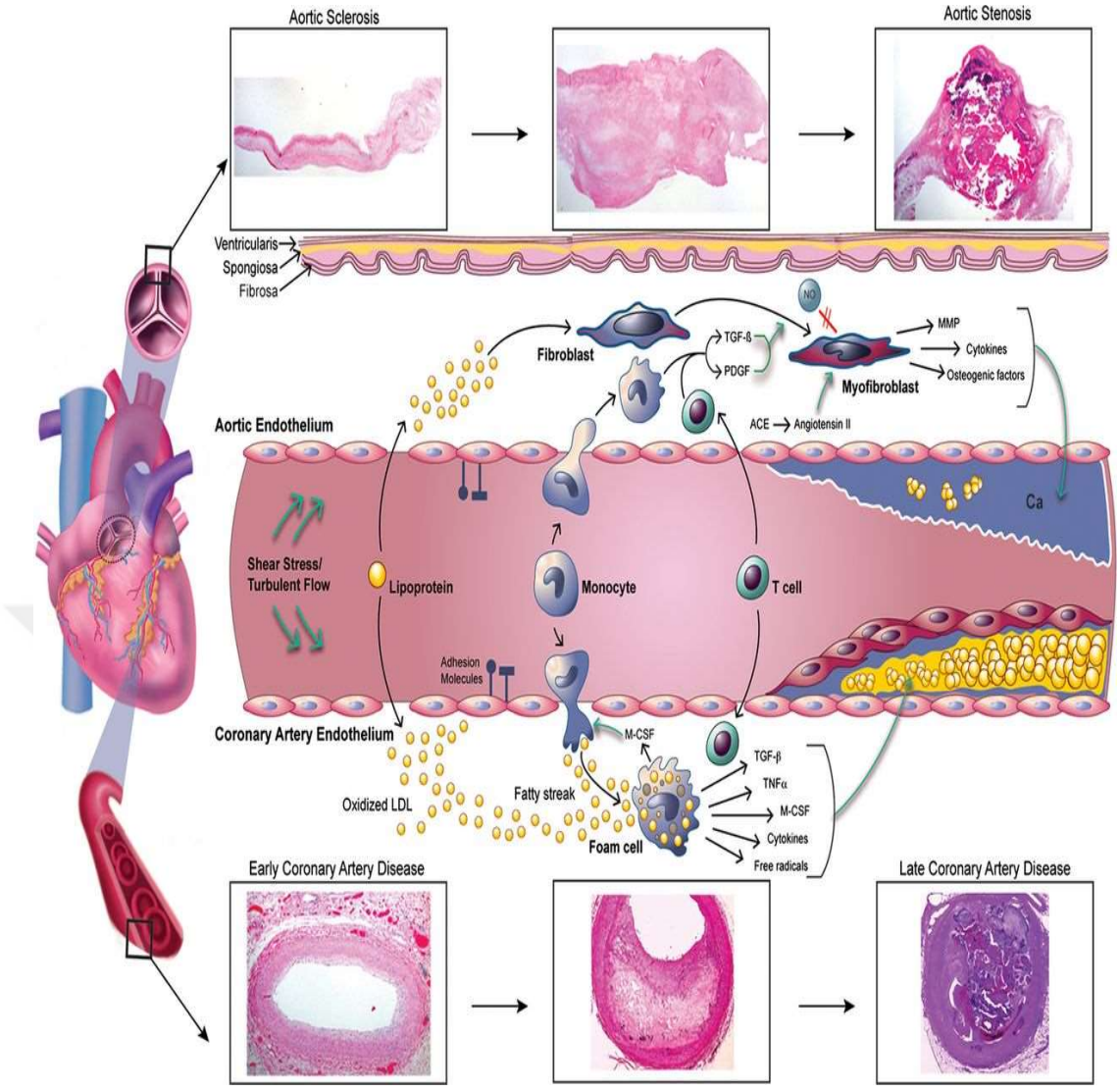

Figure 1.6 A diagram for pathophysiology in aortic sclerosis, aortic stenosis, and disease of coronary heart. Angiotensin converting enzyme is (ACE); low-density lipoprotein (LDL); nitric oxide (NO); transforming growth factor b (TGF-b); platelet-derived growth factor (PDGF); macrophage colony stimulating factor (M-CSF); calcium (Ca); tumor necrosis factor a (TNF-a); matrix metalloproteinase (MMP) (Milin et al., 2014).

The hallmark in the pathogenesis of AVSc has been proposed to result in the subsequent inflammation. There will be releasing of many mediators causing this inflammation, such as (TGF b) transforming growth factor-b, (TNF-a) tumor necrosis factor-a, and (M-CSF) macrophage colony stimulating factors (induce the maturation of monocyte in the macrophage) (Jian et al., 2003; Hansson, 2005). These monocytes will take up lipid LDL type and changed to foamy cells. This will up regulate toll like receptor causing releasing of more cytokines and free radicals that

will release more macrophage and finally more inflammation (Seneviratne et al., 2012). The major component in AVSc mesenchymal cell is the myofibroblast and release many cytokines with an increase in the expression of matrix metalloproteinases and the bone morphogenetic proteins, promoting deposition of the calcium (Kaden et al., 2003). In vitro, and by energizing of bone-specific alkaline phosphatase, matrix metalloproteinases show to work at least in portion (Clark-Greuel et al., 2007). It is believed that myofibroblasts are stimulated by the growth factors like (TGF-b), (PDF) platelet-derived factor and by low nitric oxide (Walker et al., 2004). The (ACE) angiotensin converting enzymes are present in AVSc lesions, resulting in elevation of angiotensin II which is coupled with apolipoprotein B particles, which implies an LDL in the process of inflammatory (O'brien et al., 2002). Accumulation of T cells with the expression of IL-2 receptors, fibroblast with the expression of the smooth muscle cells properties and (HLA–DR) human leukocyte antigens in the pathological area of the aortic valve (Prasad and Bhalodkar, 2004). Heterotopic calcium deposition is common in AVSc. Studies of valves with aortic stenosis showed expression of more bone-specific alkaline phosphatase, osteoclacin, osteocalcin, nuclear jB (RANK) ligands receptor activator, osteonectin and osteopontin in these calcify area of AVSc (Shetty et al., 2006). A transmembrane receptor Notch1 which regulate embryonic differentiation of osseous and calcification of AV in vitro studies and in animal models (Acharya et al., 2011). It is intracellular domain cleaves and translocates into the nucleus eventually inhibiting Runx2 when bound by the ligand (Garg et al., 2005; Hilton et al., 2008). The inhibition of those osteoblast fate transcriptional regulator caused decreasing of the calcification. So NOTCH1 gene mutation can ultimately lead to disinhibition in the calcium deposition that cause progression of AV. In addition, haploinsufficiency of NOTCH1 was well described in other families with bicuspid aortic valves (BAVs). Many growth and transcriptional factor like (TGF-b) transforming growth factor-b, ErbB, vascular endothelial growth factors, GATA and Wnt families, contribute to the BAVs pathogenisity, although their roles are less defined (Laforest and Nemer, 2012). Those informations highlights the NOTCH1 significance in the normal AV progression and their role preventing AV calcification in adult onset of the disease. Because of the durable calcium cap it consumes several years to get from AVSc to severe aortic stenosis, that can presented as syncope, angina, and heart failure. As inverse, atheroma is usually most sensitive and liable to cracking, leading to clots

and acute ischemia (Milin et al., 2014). It can be concluded that the inflammatory process of the aortic valve leads to deposition of calcium, which leads to the calcification of the aortic valve. The inflammation also leads to fibrosis of the valve. Fibrosis and calcification are responsible for the thickening of the aortic valve, which is defined as aortic sclerosis. The process, which continues unchecked and with the persistence of risk factors such as high blood pressure, leads to further progression of the disease state, ultimately leading to aortic sclerosis.

1.9 Progress of Aortic Valve Sclerosis to Aortic Valve Stenosis

Not all people with AVSc develop into aortic valve stenosis (AVS), but it was hard to prophesy which set has the highest danger of developing hemodynamically significant stenosis (Rajamannan, 2004; Novaro et al., 2007). Aortic sclerosis (ASc) represents the earliest developmental stage of aortic valve thickening observed in echocardiography (Sverdlov b et al, 2012), and could eventually lead to its most severe form of AVS (Towler, 2013). The distinction between them depend on obstruction of the left ventricular flow tract, which is missing in AVSc (morphological changes in the valve without functional oddity), and occurs in aortic valve stenosis (functional and morphological valve anomalies) (Bossé et al., 2008). Aortic sclerosis differs also of aortic stenosis due to the lack of outflow occlusion with leaflet excursion is not limited and anterograde velocity through the AV is 2.5 m/s (Naseem and Samir, 2015). Macular degeneration is sufficiently reduced to produce a measurable occlusion of flow and an important gradient from the left ventricle to the aorta (Rajamannan et al., 2011). AVSc developing approximately takes 6 decades, then it takes around one decade for a patient to progress AVS (Hurst, 2001). The initial noticeable microscopical changes in the cusps considered to be calcification or focal cusp thickening with normal valve job are called AVSc, but primary events in the process of the disease are likely to happen so earlier (Rajamannan et al., 2011). The progression of the disease is identified by an operation of thickening the wings of the valve and formation of nodules of the calcium (Shao et al., 2006). Often contain the formation of real bones and novel blood vessels that are centered near the surface of the aortic. The end-phase disorder, calcified-AS, is pathologically described by a big nodular calcification within the bumps of the aortic that stand out across the flow surfaces into the sinuses of valsalva and disrupt the leaflets opening (Rajamannan et al., 2011), Figure 1.7.

Therefore, the guidelines for grading severity of AVSc to AVS described below (Freeman and Otto, 2005).

	Antegrade jet velocity, (m/s)	The area of the aortic valve, (cm^2)
Aortic sclerosis	<2.5	Normal
Mild aortic stenosis	2.5–<3.0	>1.5
Moderate aortic stenosis	3.0–<4.0	1.0–1.5
Severe aortic stenosis	>4.0	<1.0

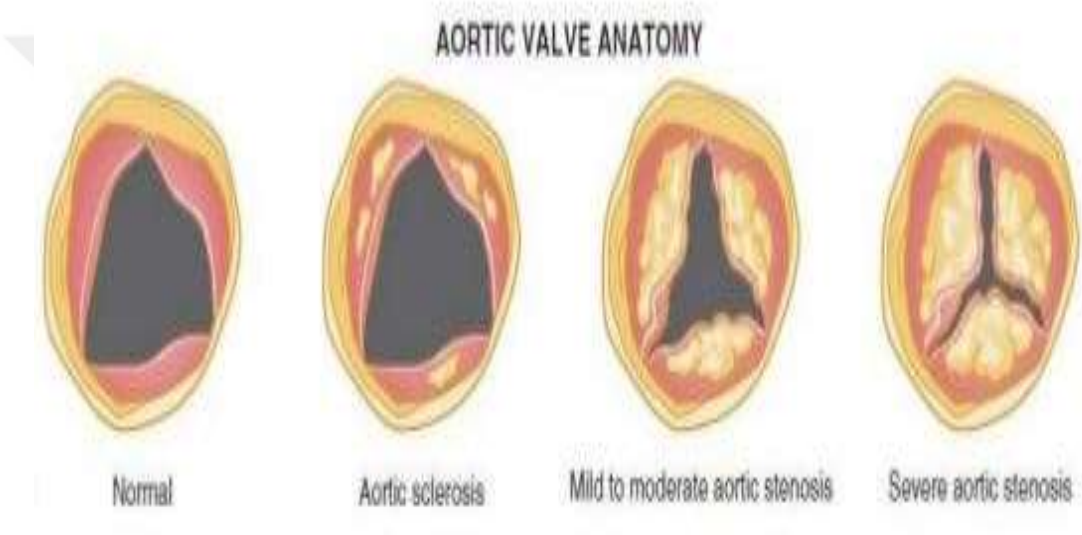

Figure 1.7 Shows the progress from aortic aalve sclerosis to aortic valve stenosis (https://www.researchgate.net/figure/Inflammatory-Process-of-Calcific-Aortic-Stenosis_fig2_288021844)

In the western universe, non-rheumatic AVSc is the major reason for AVS (Olsson et al 1994; Raslan and Mookadam, 2011). Thus, it has become the more popular signal for a referral to flap alteration surgery (Passik et al., 1987). With pronounced thickening and calcification of AVSc, the increased stiffness of the valve leaflets leads to valve narrowing, which leads to AVS in 2 to 9% of older adults (Otto et al., 1999). The prevalence of both AVSc and aortic stenosis increases with age (Turi, 2008), and is available in 48% and 4% of above 85-year-old adults, respectively (Otto et al., 1999). It is presently comprehended that stenosis of aortic valve is the terminal phase of a disorder that develops from microscopical early changes to

sclerotic of the aortic valve and afterward into a subgroup of patients to extreme biomineralization (Branchetti et al., 2013). The increased danger of cardiovascular deaths in the AVSc patients might be associated with a gradual loss of aortic mobility and evolution the stenosis of the aortic valve (Rosa et al., 2007). Therefore, one of the most important reasons for the early detection of aortic sclerosis is the risk of developing aortic stenosis and its progression by controlling cardiovascular risk factors (Palmiero et al., 2007).

1.10 Similarity of Sclerotic Aortic Valve with Atherosclerosis

Many new notings suggest a pathophysiological bind among both AVSc and atherosclerosis (Freeman and Otto, 2005; Orden et al., 2014), Figure 1.8. They also share a common etiology (Rossi et al., 2003), risk factors (Çelik et al., 2008; Topcu et al., 2017), inflammatory (Iida et al., 2008), epidemiological and histological similarities (Lindroos et al., 1994; Marechaux et al., 2009). Disease of the atherosclerosis is described by the aggregation of lipid substance in the wall of the arterial, which results from inflammatory and autoimmune mechanisms. Further than 90 percent of those adipose plaques are calcified (Libby et al., 2002; Corciu et al., 2010). The danger factors of arteriosclerosis, as well as clinical atherosclerotic cardiovascular disorder (Agmon et al., 2001), are independently linked with (AVSc), proposing that AVSc is an atherosclerosis-similar operation involving the AV (Poggianti et al., 2003; Çelik et al., 2008; Marechaux et al., 2009; Massoni et al. 2014). The mechanisms driving to the evolution of AV changes have traditionally been considered degenerative, caused by time-dependent wear and detachment of the cusps with negative aggregation of calcium in sclerosis (Lindroos et al., 1994; Marechaux et al., 2009). Additionally, histological analysis has shown atherosclerotic harms in the leaves of the aortic caused by aggregation of macrophages (Turi, 2008), T lymphocytes (Marechaux et al., 2009), extracellular lipids, and oxidized low density lipoproteins on the aortic side of the cusps (Lis et al., 2014). Also in atherosclerosis, more and more reports indicate that microorganisms, involving certain bacteria and viruses, may play a role in the initiation or maintenance of the disease process. Likewise, in the tissue of the sclerotic aortic valve, Chlamydia pneumoniae bacteria newly have been reported in about 50% of the cases. However, the pathogenic role of these microorganisms remains controversial (Nyström-Rosander et al., 2002).

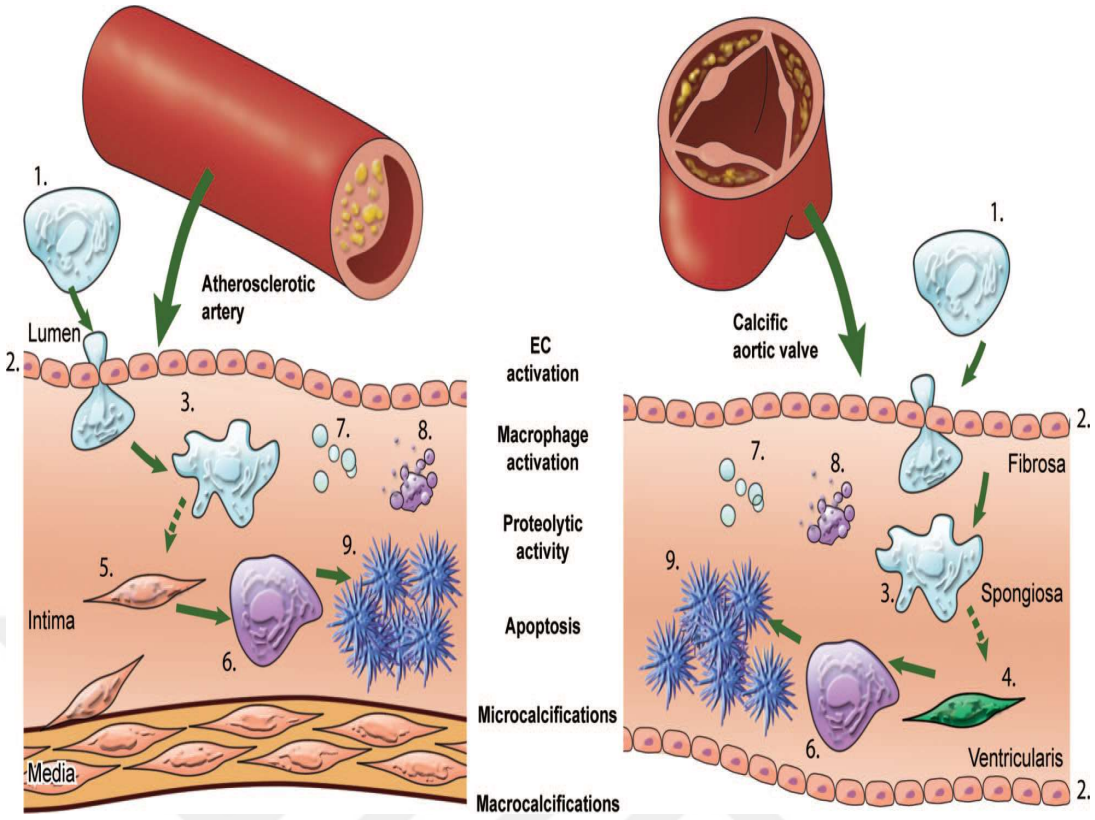

Figure 1.8 Showing of common molecular mechanisms underlying calcification of arteries and aortic valves. Also scheme shows the theory that calcification follows a similar procedure in both the aortic valve and the artery, as well as epitomizes how NIRF imaging could illustrate dynamic series of the calcification operation (New and Aikawa, 2011).

1.11 Treatment of Aortic Sclerosis

There is currently no proven effective medical treatment for the aortic sclerosis. There is also no randomized controlled trial on drug therapy in the aortic sclerosis (Lee et al., 2005). Therefore, no medical interventions are able to delay or stop the AVSc progression (Utsunomiya et al., 2014). The treatment of aortic sclerosis typically involves a surgical process to replace the aortic valve (Jaeggli, 2015). The first successful surgical replacement of diseased human heart valves was reported in 1960 (Braunwald et al., 1960; Björk, 1970; Braunwald, 2000). Heart valve replacement is open heart surgery that uses a biological or mechanical valve to replace a defective heart valve. So far, two basic types of valve replacement are most common. The biological valve replacement may be an autograft (i.e., from the patient's own body), a xenograft (i.e., from a pig or a cow), or a homograft (i.e., from a human donor). These biological valves last about 10 years. In February 2001, US

Food and Drug Administration (FDA) approved the use of a novel artificial heart valve made from a segment of a porcine aorta including its aortic valve. The second type is a mechanical valve replacement. This valve is made of synthetic plastics or metals. These valves can last over 30 years (Gawlitta, 2001). However, all of these valve substitutes, with the exception of the autograft, are considered foreign by the body, they can cause blood clots. To prevent this, patients need a regiment of anticoagulant therapy for the rest of their lives (Carpentier et al., 1969; Carpentier, 1977; Gawlitta, 2001). Except for minor stent modifications, sutures, and anti-calcification treatments currently not developed by those in the 1960s and 1970s (Zilla et al., 2008), Figure 1.9. The disease of the aortic valve is the reason for 370,000 annual valve replacement surgeries worldwide (Butany and Collins, 2005). Due to the rising and aging population, this will increase to 850,000 annual replacements by 2050 (Yacoub and Takkenberg, 2005; Jaeggli, 2015). Unfortunately, and because the current valve replacement parts have serious disadvantages that often require replacement surgery or life-long anticoagulant therapy. Therefore, we conclude that the factors predicted to be linked with the evolution of aortic valve sclerosis and its subsequent advances such as smoking, hypertension, ventricular hypertrophy, hyperlipidemia, and diabetes need to be adequately controlled and treated. Apart from risk factor modification, there is no active therapy required for aortic valve sclerosis.

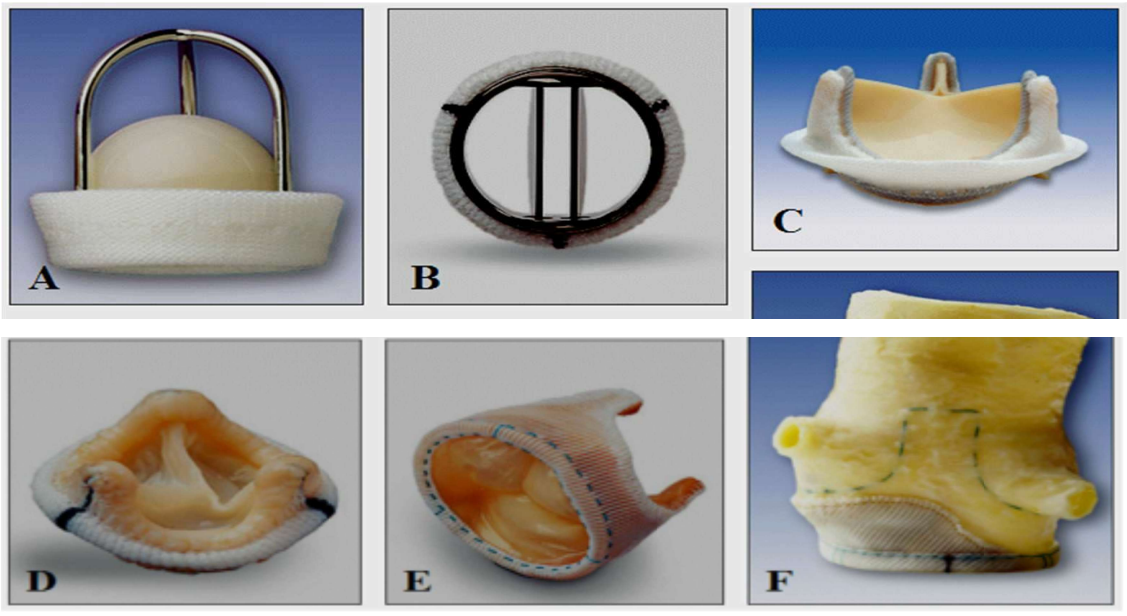

Figure 1.9 The picture illustrated artificial heart valves. Caged ball (A), hinged

leaflets (B), stented bovine pericardium (C), stented porcine valve (D), stentless porcine valve (E), and stentless porcine valve (F) (Jaeggli, 2015).

1.12 Free Radicals (FRs)

A free radical (FR) is an atom or molecule (chemical species) which contains a single or more unpaired electrons in the outer of atomic or molecular orbitals (Shah and Channon, 2004; Kalyanaraman, 2013), which is often a highly reactive (Kerksick and Zuhl, 2015), and unstable molecule (Pham-Huy et al., 2008). When an overload of (FRs) free radicals cannot progressively be broken, their aggregation in our body produce the oxidative stress; (overproduction of (ROS) reactive oxygen species (Valko et al. 2007; Pham-Huy et al., 2008; Valko et al., 2016), and nitrosative stress (Kovacic and Jacintho, 2001; Valko et al., 2001; Ridnour et al., 2005), which is overproduction of (RNS) reactive nitrogen species (Klatt and Lamas, 2000; Ridnour et al., 2004). This operation plays a significant role in the evolution of degenerative and chronic disease such as rheumatoid arthritis, cataract, and autoimmune disorders (Pham-Huy et al., 2008), diabetes (Poyton et al., 2009), cancer, physiological aging, cardiovascular and neurological diseases (Gutteridge and Halliwell, 2000; Pacher and Szabo, 2008; Bhattacharya et al., 2011), Free radicals have some various kind. Nitrogen-centered, oxygen-centered, sulphur-centered and carbon-centered radicals are various species of the FRs (Agarwal et al., 2006; Perrone et al., 2010; Ozcan and Ogun, 2015). The most two important sets of these radical molecules or reactive species are often categorized as ROS; which they are molecules containing oxygen (D'Autréaux and Toledano, 2007; Sharma et al., 2012; Kroese and Scheffer, 2014), generated as a byproduct of biochemical reactions (natural metabolism of oxygen), in cytochrome P450, peroxisomes, mitochondria, and other cellular component (Balaban et al., 2005; Gonzalez, 2005; Ozcan and Ogun, 2015), and RNS which they are a family of molecules derived from superoxide anion (O_2•-) and the (NO•) nitric oxide (Petrowsky and Clavien, 2015), and can be generated both by the myocytes and other cells in the heart (Digerness et al., 1999). For example, (ROS) reactive oxygen species include hydroxyl (OH•), superoxide anion (O_2•-), hydroperoxyl, alkoxyl (RO^-), alkylperoxyl, ozone (O_3), hydrogen peroxide (H_2O_2), carbon dioxide and carbonate radicals (Sharma et al., 2014), hypochlorous acid (HOCl), peroxyl radical (ROO•), and singlet oxygen ($^1O_2$). And (RNS) reactive nitrogen species (oxidants containing nitrogen), include

peroxynitrite (ONOO−•), nitric oxide (NO•), (Klebanoff, 1980; Bedard and Krause, 2007; Ozcan and Ogun, 2015), and nitrogen dioxide (NO_2) (Pham-Huy et al., 2008). Constancy of these radicals needs electron donation from lipids and membranes, DNA, proteins (Kerksick and Zuhl, 2015), and carbohydrates, (Sharma et al., 2014), which oftentimes cause degradation and damage to these molecules (Kerksick and Zuhl, 2015). Since oxidants and free radicals can be either helpful or harmful to the body, they function a dual role as both beneficial and toxic molecules. At medium or low concentrations, RNS and ROS exert useful effects (Valko et al., 2007), on immune function and cellular responses (Valko et al., 2007; Salman and Ashraf, 2013). They are also important in process of lipids peroxidation, energy generation, nitration, protein and DNA oxidation, catecholamine response and nitrosation or nitrosylation (Rutkowski et al., 2007). It has to be emphasized that RNS and ROS are both generated in a well regulated manner to assist preserve homeostasis at the cellular level in the normal healthy tissues and play a significant role as signaling molecules (Sharma et al., 2014). Contemporary studies identified main sources of RNS and ROS productions: mitochondria, xanthine oxidase, nitric oxide synthases (NOS), and NADPH oxidases (Nox) (nicotinamide adenine dinucleotide phosphate oxidase) (Afanas'ev, 2011). The reactive oxygen-nitrogen species (RONS), are freed from activated immune cells (dendritic, neutrophils, and macrophages cells) in reply to an inflammatory stimulus (Salman and Ashraf, 2013; Ozcan and Ogun, 2015). During this, phagocytic cells release RONS and non-phagocytic cells are catalyzed to generate RONS by pro-inflammatory cytokines (Hussain et al., 2003). RNS and ROS are produced from either exogenous or endogenous sources. Endogenous FRs are produced from inflammation, activation immune cell, ischemia, excessive exercise, aging, cancer, infection, and mental stress. Exogenous ROS/RNS produce from radiation, pollution of water and air, cigarette fume, certain drugs, alcohol, industrial solvents, heavy or transition metals (As, Fe, Pb, Hg, Cd), and cooking (used oil, fat, smoked meat). After breakthrough into our body by various route, these exogenous compounds are decayed or metabolized into FRs (Pham-Huy et al., 2008). Finally, these radicals neutralized by natural antioxidants or enzymatic activity that stop the initial formation of radicals. (Rutkowski et al., 2007).

1.12.1 Peroxynitrite (ONOO−•)

For well over 100 years peroxynitrite (PN), has been known in the chemical literature (Uppu et al., 2007). A reactive nitrogen species, peroxynitrite anion (ONOO−•) Figure 1.10, which is a potent oxidizing agent (Valko et al., 2007), generated from the reaction between superoxide anion (O_2•−) (Ahmad et al., 2015), (O_2•− which is a reactive oxygen species produced in the vascular wall by cellular oxidases (Guzik et al., 2002), and nitric oxide radicals (NO•) (Ahmad et al., 2015), (NO•, which is a free radical generated endogenously by a different set of mammalian cells (Wang and Zweier, 1996), and it able readily penetrate the membranes of the cell and spread outside the cell) (Carballal et al., 2014).

Figure 1. 10 The peroxynitrite anion structure

Therefore, different cell kinds, like endothelial cells, macrophages, and Kuppfer cells, that simultaneously generate and release NO• and O_2•− could generate peroxynitrite with a very quick reaction rate (4 ~ 6.7 * 10^9 M^{-1} S^{-1}) (Yamakura et al., 1998). Peroxynitrite may spontaneously decompose to produce other cytotoxic reactive species, like hydroxyl radical (OH•), nitrogen dioxide (NO_2) (Wang et al., 1998; Ozcan and Ogun, 2015), peroxynitrous acid (ONOOH), etc., (Chen et al., 2014). PN is stable, once protonated it decays quickly (Beckman et al., 1990).

NO• + O_2• ⟶ ONOO− (Barbusıńskı, 2009; Szabó et al., 2012).

$O_2^{\bullet-}$ + NO• ⟶ ONOO− + H^+ ⟷ ONOOH (peroxynitrous acid)

⟶ OH• + $N0_2$• ⟶ NO_3^- + H^+ (Beckman et al., 1990).

PN a cytotoxic molecule (Szabó and Módis, 2010), is known as a strong biological oxidant (Brunner and Wölkart, 2003; Ramdial et al., 2017), and nitrating agent (Carballal et al., 2014), capable to interact with a vast group of biomolecules (Szabó et al., 2007). It able to react immediately with electron-rich groups, like sulfhydryls, iron-sulfur centers, the active site of sulfhydryl in tyrosine phosphatases, and zinc-thiolates. Although peroxynitrite is a potent oxidant, the anion (ONOO−) as well

interacts immediately with nucleophiles, compounds which have a fractional positive charge. The main significance instance is (CO_2) carbon dioxide (Pacher et al., 2007).

PN also able to oxidize transition metal centers of proteins, especially manganese and copper ions, heme and non-heme iron (Carballal et al., 2014). PN reacts at quick rates especially when key targets are available, such as iron, zinc fingers, sulfur centers, and thiol-containing enzymes. These targets are important for respiration and controlling transcription and translation (Wang et al., 1998). The significant high serum peroxynitrite, can use as an index for simultaneous estimation of species of the reactive nitrogen and oxygen **(**Al-Nimer et al., 2010). Downstream oxidative modifications caused by peroxynitrite trigger cell death by an assortment of mechanisms based on the oxidant concentration (Ramdial et al., 2017), Figure 1.11.

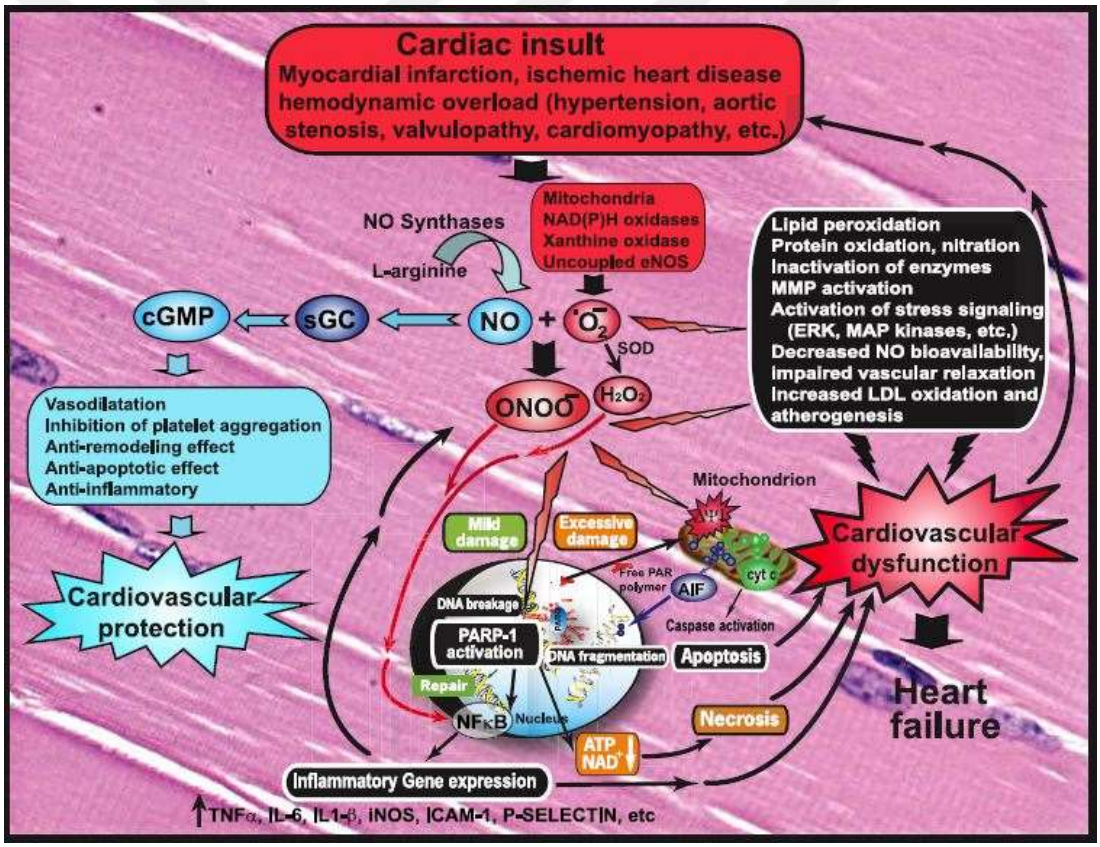

Figure 1.11 The diagram illustrated role of PN and NO in cardiovascular pathophysiology (Pacher et al., 2007).

On the one hand, PN stimulate damage of the cell by lipid peroxidation, deactivation of other proteins and enzymes by nitration and oxidation, and also energizing of stress signaling, matrix metalloproteinases (MMPs) among others. As well as PN

triggers the emission of proapoptotic factors like apoptosis-inducing factor (AIF) and cytochrome c from the mitochondria, which mediate caspase-dependent and -independent apoptotic death pathways. Furthermore, PN, leads DNA stand breaks, and stimulating the nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1), in concert with other oxidants. Mild harm of DNA stimulate the DNA repair machinery. An inverse, once excessive nitrosative and oxidative stress-induced DNA damage appears, such as in different kinds of heart failure and myocardial reperfusion injury, overactivated PARP initiates an energy-consuming cycle by transferring ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to nuclear proteins, resulting in fast depletion of the intracellular NAD^+ and ATP pools, slowing the average of mitochondrial and glycolysis respiration, finally leading to cellular dysfunction and death. Poly (ADP-ribose) glycohydrolase (PARG) degrades poly (ADP-ribose) (PAR) polymers, producing free ADP-ribose and PAR polymer. As well as overactivated PARP smooth's the expression of a different of inflammatory genes cause to increased inflammation and linked oxidative stress, thus simplifying the development of heart failure and cardiovascular dysfunction (Pacher et al., 2007).

So, the possible downstream targets or the biological targets of $ONOO^-$ can attack and damage proteins (Pacher et al., 2005), (nitration of tyrosine residues (Ferdinandy, 2006), oxidation of sulfhydryls), lipids (formation of lipid peroxides), antioxidant reserves depletion (particularly glutathione) (Ronson, et al., 1999; Pacher et al., 2007), and DNA damage by strand breakage (Ferdinandy and Schulz, 2003; Nanetti et al., 2008), or by forming 8-nitroguanine 8-NG) (Kawanishi and Hiraku, 2006; Salman and Ashraf, 2013), followed by the energizing of the nuclear enzyme PARP poly(ADP-ribose) polymerase (Pacher et al., 2007; Szabó et al., 2012). Besides, $ONOO^-$ cause inhibition of mitochondrial respiration (Yamakura et al., 1998), and leading to tissue injury which manifests itself and subsequently cause death of the cell (Liang et al., 2010; Huang et al., 2013), e.g., as a depression in myocardial contractile function (Ónody et al., 2003). $ONOO^-$ seems to be a significant tissue-damaging species produced at the inflammation sites and has been shown to be participatory in some kidney diseases, different neurodegenerative diseases (Halliwell, 2007), cardiovascular disorders (Daiber et al., 2013; Ahmad et al., 2015), and diabetes (Al-Nimer et al., 2012). Key sides of the mechanisms by

which ONOO− modulates cardiac contractile task stay unresolved (Katori et al., 2006). ONOO− can be produced inside the myocyte and in the vasculature (Digerness et al., 1999), yet it still unclear if the production site effects the impact of ONOO− on contractility. In vivo, the properties of the cardiovascular effects of PN still restricted, and the immediate impact of PN on contractility, independent of its vasodilatative characteristic, has not been specified (Katori et al., 2006). Because of its cytotoxicity to bacteria or another invading organism, the reactive chemistry of PN can be count useful at the level of the whole organism (Arteel et al., 1999). Peroxynitrite may play an important role in the proinflammatory responses and modulating vascular injury (Pacher et al., 2007). Whereas in physiologic studies, the beneficial actions of PN may be due to the reaction of PN with red cell glutathione, plasma, or plasma albumin and cysteine, with the production of NO or a NO donor like compound (Nossaman and Kadowitz, 2008). In biological systems, PN also participates in several physiological functions.

Since PN plays varied roles in biological systems, the evolution of techniques for detecting and monitoring PN is so significant. However, it is a challenging function, as it has a high reactivity (Chen et al., 2014), and very short lifetime (Hayashi et al., 2001; Ahmad et al., 2015), (~10 ms in vivo) (Peluffo and Radi, 2007), as well as occasionally PN is existing only in trace quantities (Chen et al., 2014).

1.13 The Antioxidant Defense Systems

Exposure to FRs from an assortment of sources has driven organisms to evolve a chain of defense mechanisms (Cadenas, 1997). Protection mechanisms versus FR-induced oxidative stress include preventative and repair mechanisms, as well as physical and antioxidant defenses (Valko et al., 2007). Antioxidants are molecules which interact with the FRs by donating electron in order to regulate oxidative reactions (Halliwell, 2001). So, the antioxidants are able to neutralize the surplus of FRs (Cadenas and Packer, 1996; Palmieri and Sbendorio, 2007; Salman and Ashraf, 2013), by bind and inactivate them, subsequently they can prevent damage to cells, as well as enhance the immune defense and contribute to disease prevention (Pham-Huy et al., 2008). The concept of antioxidant is: primary and secondary, enzymatic and non-enzymatic, endogenous and exogenous, hydrosoluble and liposoluble, preventative or repair-systems, synthetic or natural. Primary antioxidants are

fundamentally series breakers, by hydrogen donation, it eligible to scavenge the species of the radical. Secondary antioxidants are metal chelators, decomposers of the peroxide, singlet oxygen quenchers, and UV radiation absorbers or oxidative enzyme inhibitors (Pisoschi and Pop, 2015). As well as antioxidants can be either (exogenous antioxidants), which they are outwardly supplied out of supplements and/ or foods or (endogenous antioxidants), that they are generated naturally in situ. These endogenous and exogenous antioxidants work as "scavengers of the FRs" by prohibiting and reforming harms reasoned by RNS and ROS, and subsequently able promote the immune defense and minimize the danger of cancer and degenerative disorders (Pham-Huy et al., 2008). In cells, the endogenous compounds categorized as enzymatic and non-enzymatic antioxidants. The main enzymatic antioxidant defenses immediately participatory in the neutralization of RNS and ROS include (CAT) catalase, (GPx) glutathione peroxidase, (SOD) superoxide dismutase (Singal et al., 1998), and (GRx) glutathione reductase (Pham-Huy et al., 2008). Non-enzymatic antioxidants compounds include: (GSH) glutathione, (vitamin E) α-tocopherol, (vitamin C) ascorbic acid, (Vitamin P) flavonoids, carotenoids (Valko et al., 2007), β-carotene (a precursor of vitamin A), ubiquinol (coenzyme Q-10), urate, lipoic acid, polyamines, polyphenols (García, 2014), Ceruloplasmin, albumin, uric acid (Palmieri and Sbendorio, 2007; Salman and Ashraf, 2013), trace metals (zinc, manganese, and selenium), omega-6 and omega-3 fatty acids (Pham-Huy et al., 2008), bilirubin, melatonin, and other antioxidants (Droge, 2002; Willcox et al., 2004). There is an equilibrium between both the intracellular levels and the activities of these antioxidants under normal situations (Valko et al., 2007). Also, vegetables and fruits consumption including high quantities of antioxidative nutraceuticals have been linked with the equilibrium of the FRs/antioxidants case, which assists to reduce the oxidative stress in the body and minimize the dangers of cardiovascular diseases and cancers (Kaur and Kapoor 2001; Lee, et al., 2004). Subsequently, this equilibrium is fundamental for the organisms' survival and their health.

1.14 Essential and Trace Element

Micronutrients (defined as minerals, trace elements, and vitamins which are fundamental for life (Ekpenyong, 2017), conduct a variety of particular biological roles in the body's structural, regulatory and catalytic functions. They contain trace elements like iodine, zinc, and iron, minerals like magnesium and calcium, and

vitamins. They work as immune modulators, anti-inflammatories, and antioxidants (Xie et al., 2015). Since they don't make endogenously in our body or are made in quantities scanty to meet our needs thus, we can gain them primarily through the diet we eat (Fortmann et al., 2013; Ekpenyong, 2017).

Minerals; (which they are inorganic nutrients (Soetan et al., 2010), and existent in all fluids and tissues of the body and their existence is requisite for keeping of specific physicochemical processes which are major for life) (Malhotra, 1998; Eruvbetine, 2003), it can be categorized as macro (major) or micro (trace) elements, and ultra-trace elements. The macro-minerals involve sodium (Na), calcium (Ca), chloride (Cl^-), and phosphorus (P), while the micro-elements involve zinc (Zn), cobalt (Co), magnesium (Mg), potassium (K), iodine (I), iron (Fe), manganese (Mn), fluoride (F^-), copper (Cu), molybdenum (Mo), selenium (Se), sulfur (S) and chromium (Cr), (Eruvbetine, 2003). As well as ultra-trace elements involve silicon, boron, nickel and arsenic (Albion Research Notes, 1996). All living matter need these minerals or inorganic elements for their normal life processes (Hays and Swenson, 1985; Özcan, 2004).

Trace elements are an essential nutritional component of human's enzymatic systems (Rao and Rao, 1981; Simsek and Aykut, 2007; Bermúdez et al., 2015). So, they are those compounds that need to be available in the human diet to preserve normal physiological functions (Goldhaber, 2003). They are involved in several biochemical pathways (Bermúdez et al., 2015), and are fundamental in the functions, maturation and activation of host defense mechanism (Bendich, 1990). The symptoms of a mineral deficiency be based on the mobility and function of the element (Roberts, 1985). Trace metals are known to play significant roles in the catalytic activities of major antioxidant enzymes (Arinola et al., 2008). For example, zinc, selenium, iron, and copper are integral part of enzymatic anti-oxidants (Ayoglu et al., 2016). Trace elements are needed as essential substances of structural parts of biologically active constituents or of biological enzyme systems (Arinola et al., 2008). Such as all another cells kind, Immune cells, need a sufficient provide of trace elements Fe, Cu and Zn to express and maintain the function and structure of key metalloproteins which take part in housekeeping path like energy generation and to save the cell against highly toxic ROS by scavenging oxygen free radicals (Soetan et al., 2010). Trace elements like Se, Zn, and Cu, are catalytic, regulatory and structural ions for

enzymes, transcription factors, and proteins, thus these elements are critical for varied different homeostatic mechanisms of the body (Fathi et al., 2007). They are also related together against reactive nitrogen and oxygen species in cytosolic defense (Klotz et al., 2003). As well as they are involved in protein synthesis, the immune system and vitamin metabolism of animals (Cortinhas et al., 2010; Cortinhas et al., 2012). Zn, Se, Cu, and Fe exert significant protective or enhancing effects on the development of several diseases (Goldhaber, 2003). Statistically important differences from the normal distributions of these elements have been reported to occur in patients own a different kind of cancer (Zowczak et al., 2001; Kucharzewski et al., 2003; Lin et al., 2006).

Aortic valve sclerosis disease is linked with a clear imbalance in some trace elements of well-known significance for the immune and cardiovascular function such as iron, zinc, selenium, and copper (Nyström-Rosander et al., 2002).

1.14.1 Zinc (Zn)

In the periodic table, zinc (Zn) is the 30th element (Valko et al., 2005), and it's the 2nd most plentiful transition metal after iron in organisms, as well as, it's the single metal that appears in all the categories of the enzymes (Osredkar and Sustar, 2011). Zinc is the 23th most plentiful element in the crust of the earth. Pure zinc is a bluish-white, shiny metal**,** (Kaur et al., 2014), and in nature it's amphoteric (Maret, 2001). Throughout the human body, approximately 2–4 g of zinc is distributed (Ekpenyong, 2017), found in all organs, tissues, fluids, secretions (Valko et al., 2005), bones, and cells (Kaur et al., 2014). Most zinc is found in the bones, skeletal muscle, gastrointestinal tract, kidney, skin, liver, brain, and lung (Valko et al., 2005), with the highest levels in the parts of the eye and the prostate (Osredkar and Sustar, 2011). Zinc popular sources involve beans, red meat [lamb, beef], oysters, liver, and nuts. Other good food sources include cereals, pumpkin seeds, sunflower seeds, seafoods [lobster, and crab], almonds, and whole grains (Ekpenyong, 2017). Likewise, foods possess a high content of the protein are wealthy in zinc content, whilst the diets and foods containing primarily carbohydrate were found to be very lower in zinc content (Kaur et al., 2014). Zinc is existent in the body in all enzyme systems, and it acts in different enzymatic activities as a cofactor (Powell, 2000), and it participates in many aspects of cellular metabolism. Zn is needed for a proper sense of smell and

taste and backing normal outgrowth and evolution through pregnancy, babyhood, and adolescence (Osredkar and Sustar, 2011). Zinc plays an important role in stabilizing biological membranes, in the protection of function of the vascular endothelial, and in safeguarding macromolecules versus ROS (Ekpenyong, 2017), by prevents the production of the hydroxyl (OH•) and superoxide (O_2•−) free radicals through Fenton reaction (Hasan and Al-Shammaree, 2011). It also preserves the cells of the cardiac stem fundamental for a cardiac task (Ekpenyong, 2017). Zinc having antioxidant features, which may keep against quickened aging, and assists quickness up the healing process next an injury (Osredkar and Sustar, 2011). Zinc functions in pathways which are requisite for immunity, growth, and reproductive function (Fayet-Moore et al., 2014). Moreover, Zn has important cardioprotective effects. In vivo and in vitro studies proved that zinc has an inhibitory effect on isoproterenol-induced cardiac oxidative injury (Valko et al., 2005). In biology, Zn tasks are numerous but can be discrete into three major classes: regulatory, structural, and catalytic roles (Osredkar and Sustar, 2011). The liver fundamentally plays a crucial role in preserving systemic Zn homeostasis. Thus, the appearance of chronic liver disorders, like liver cirrhosis, or fatty liver, chronic hepatitis, hepatic encephalopathy, hepatocellular carcinoma, and HCV infection, leads in the impairment of Zn metabolism, and subsequently Zn deficiency (Himoto and Masaki, 2018). Zinc deficiency symptoms, like loss of appetite, growth retardation (Valko et al., 2005), and impaired immune function. In more severe statuses, deficiency of zinc leads to impaired appetite, impotence, hair loss, weight loss, diarrhea, delayed healing of wounds, hypogonadism in males, taste disorder, skin, and eye lesions, delayed sexual maturation, and altered cognition can as well happen (Osredkar and Sustar, 2011). Severe deficiency of Zn is infrequent and usually caused by acquired or genetic circumstances (Valko et al., 2016). When deficiency of Zn does happen, it is usually consequent to insufficient zinc intake or absorption increased requirements for zinc, or increased losses of zinc from the body (Osredkar and Sustar, 2011).

Zn is associated with accelerated aging, immunodeficiency, accelerated progression of HIV infection, increased incidence of abnormal pregnancies, and developmental retardation in children, (Osamu, 2004). In children it leads a rise in infection and diarrhea, causing to the death of around 800,000 children worldwide per year (Osredkar and Sustar, 2011). In pregnancy, a mild deficiency of Zn can lead to

abnormal taste sensation, inefficient labor, increased maternal morbidity, prolonged gestation, a raise danger to the fetus, and atonic bleeding. This deficiency of Zn in premature infants could be consequent to high fecal losses of Zn, an increase in Zn requirement for rapid growth, and low body concentration of Zn at birth (Kaur et al., 2014). Deficiency of Zn resulting from long-term decreased intake of zinc has been detected in both laboratory animals and humans and is associated with increased scores of oxidative damage, like DNA and protein oxidation, and lipids peroxidation (Valko et al., 2016). Although Zn is a fundamental requirement for good health, surplus zinc may be harmful. Excessive absorption of zinc suppresses absorption of copper and iron. Acute adverse impacts of high intake of zinc involve loss of appetite, headaches, nausea, diarrhea, vomiting, and abdominal cramps (Osredkar and Sustar, 2011). In adolescents, excess zinc enhances obesity and linked disorders (Singh and Taneja, 2009), and is as well linked to occurrence of severe anemia (Kaur et al., 2014). When the zinc intake is high, enzymatic activity of pancreas increases and so does mucin production in the intestine. Excess of Zn is not only associated with deficiency of copper but also cytopenias which usually resolve with the elimination of surplus zinc sources (Fong et al., 2007). Zn^{2+} is not an antioxidant, because it does not participate in redox reactions. It is considered a proantioxidant. This term describes many antioxidant impacts that zinc has, without immediately taking part in redox biochemistry. It can act through stabilization of cell membranes and sulfhydryls or as a structural component of antioxidant enzymes, like copper/zinc superoxide dismutase (Al-Dohan et al., 2015).

1.14.2 Copper (Cu)

In the periodic table, the 29^{th} element is called copper (Cu), (cuprum) (Valko et al., 2005). Cu is a basic component required in low amounts for normal physiology (Ou et al., 2017). Despite the fact that Cu is the 3rd most plentiful follow metal in the body (after Fe and Zn), 75-100 mg is the aggregate sum of copper in the body. Cu is available in each body tissues but is stored mostly in the liver, with fewer ratios available in the kidney, muscles, heart, and brain. Cu is an essential dietary supplement, which absorbed in the gut then bound to albumin when transported to the liver. Through the plasma protein called Ceruloplasmin, Cu enters the bloodstream, where its digestion is controlled and is excreted in bile. beef and organ meats (especially liver), nuts and sunflower seeds, oysters and other sea food,

enriched cereals, green olives, dark green leafy vegetables, chocolate, black pepper, avocados, cocoa, and dried legumes are copper rich sources (Osredkar and Sustar, 2011). Cu is a basic micro-nutrient supplement important for the neurologic and hematologic systems. Likewise, it is important in the nervous systems for the production (creation) of myelin sheaths, assists in the integration of Fe in hemoglobin, as well as helps in the transfer of Fe from tissues to the plasma, and in the gastrointestinal tract (GIT), it aids the absorption of iron (Soetan et al., 2010). It is a catalytic element for several enzymes and is additionally a basic part of other imperative proteins, (Al-Dohan et al., 2015). Our metabolism needs copper as an essential role player in this process, generally in light of the fact that it enables numerous critical enzymes to work appropriately. Cu is fundamental for keeping up the quality of the skin, connective tissue, epithelial tissue, and blood vessels throughout the body. It likewise keeps thyroid organ working typically (Osredkar and Sustar, 2011). Copper neutralize free radicals when it works as an antioxidant and may diminish or help keep a portion of the harm they cause (Xu et al., 2013).

Since copper is associated with numerous jobs of the body, lack of copper can create a broad scope of side effects. lack of Cu can result in aneurysms, hernias, iron deficiency anemia, blood vessel breakage showing as wounding or nosebleeds, brain disturbances, weakness, abnormalities in cholesterol and glucose metabolism, poor thyroid function, joint and osteoporosis problems, skin sores, loss of pigment, low body temperature, fatigue, irregular heartbeat, and due to poor immune function [neutropenia] it can cause an increased susceptibility to infections (Osredkar and Sustar, 2011). Other disorders related to Cu insufficiencies incorporate neonatal ataxia, bone disease, abnormal growth and depigmentation of hair, gastrointestinal disorders, and impaired growth (Soetan et al., 2010), furthermore, Menke's disorder (Bedwal and Bahuguna, 1994; Osamu, 2004), As well as several cardiovascular problems (Ekpenyong, 2017). In the event that copper is essential in the structure of cellular membrane, at that point a Cu insufficiency could seriously change the motion of nutrients through the cell walls (Osredkar and Sustar, 2011). Lack of Copper leads to altered the enzymes of the antioxidants, lipid peroxidation, increased oxidative stress and free radicals (Ekpenyong, 2017). Likewise, it leads to disorder in the skeletal, nervous, integumentary, hemopoietic, reproductive systems, and immune systems (Bedwal and Bahuguna, 1994). Finally, the biometals iron, copper,

and manganese, have been linked to Parkinsonism and Parkinson's disorder (Osredkar and Sustar, 2011). Since surplus Cu is secreted through bile, Cu toxicity is most probably to happen in people with liver disorders or other medical cases in which the secretion of bile is compromised (Osredkar and Sustar, 2011). For instance, Wilson's disease (Bedwal and Bahuguna, 1994).

1.14.3 Selenium (Se)

Selenium (Se) is a fundamental trace element, which can exist in many various chemical forms in biological materials either as organic Se compounds, like dimethylselenide and selenomethionine, and inorganic selenites and selenates. Se is mainly existing as selenomethionine in foods, which is a significant source of dietary Se in humans, and also as a chemical form that is commonly utilized for Se supplements in clinical trials (Tinggi, 2008). Selenium is a trace mineral available in water, soil, meat, vegetables (grains, garlic, soybean, and onion), yeast, liver, and sea food（Willcox et al., 2004). Food grains and livestock grown in the plains states are notably wealthy selenium sources. Other certain foods like ocean fish and nuts include especially plentiful quantities of Se（Cooper et al., 2007). In plasma, the Se is fundamentally found in the albumin portion, the suitable concentration in plasma selenium is ranged from 51 to 85 μg/L (Mehdi and Dufrasne, 2016). In inverse to other bio-elements, Se is characterized by a tight domain among its toxic and therapeutic doses. For this causes it is termed (the most toxic element necessary for life) (Andrzejak et al., 1996).

Because Se is utilized in industry, its compounds can push through to the environment and constitute a risk to health and life (Wietecha et al., 2002). In recent years Se has aroused increasing attention. It has become the topic of specific interest for persons joined with different branches of industry and environmental protection, and also persons correlated with toxicology, biochemistry, and medicine. For several years Se was taken into consideration just as an element that was carcinogenic and toxic for animals and people. Today, it is recognized to be requisite for the normal functioning of different living organisms: people, plants, bacteria, and animals (Barceloux, 1999), in spite of its toxicity (Wietecha et al., 2002).

Selenium supplementation increases enzymatic antioxidant activity (Ou et al., 2017), such as glutathione peroxidase (GSH-Px) (Fayet-Moore et al., 2014; Wacewicz et al.,

2017), thioredoxin reductase (TrxR), selenoprotein P, and some isoforms of methionine sulfoxide reductases (Msrs) (Casaril et al., 2017), to safeguard cells from harm caused by FRs (Clausen et al., 1989; Rayman, 2000), and it also decreases lipid peroxidation (Flores-Mateo et al., 2006). Selenium, a constituent of selenoproteins as selenocysteine (Sec), has important antioxidant properties (Burk et al., 2001; Thomson, 2004). Because of its antioxidant properties, it has long been hypothesized that selenium may prevent cardiovascular and other chronic diseases (Flores-Mateo et al., 2006). Se is occupying center phase as a possible anticancer agent (Clark et al., 1996), by enhancing formation of white blood cells which ruins the cells of cancer. Additionally, it enhances the immune system (Girodon et al., 1999), by raising the number and activity of white blood cells and prevents inflammatory diseases, early aging, rheumatoid arthritis, cataracts, degenerative disorders, and stroke. Likewise, Se needful for normal thyroid functions (Dejneka et al., 2007). As well as, it takes part in safeguard versus ultraviolet induced oxidative DNA damage, apoptosis in cultured skin cells, and death of the cell (Wacewicz et al., 2017). So we could conclude that Se behaves both as an anti-inflammatory and antioxidant agent. This is because Se able minimize phospholipid hydroperoxides, hydrogen peroxide, subsequently, inhibiting the spread of ROS and FRs (Kosar et al., 2005). Se is a fundamental micronutrient at low level but toxic at elevated level with a comparatively little variation between these concentrations (Cuparigova and Stafilov, 2011). Deficiency of Se may happen in gastrointestinal disease patients and in patients on total parenteral nutrition (TPN) (Pham-Huy et al., 2008).

Se can be a factor for essential hypertension (Babalola et al., 2007), as well as it may predispose to inflammatory skin disorders (Wacewicz et al., 2017). Deficiency can occur with inadequate dietary intake (Ou et al., 2017). Deficiency of Se is more of trouble geographically than is selenium toxicity (Mehdi and Dufrasne, 2016). The optimum daily dietary intake of Se is 70 μg/day for men and 55 μg/day for women (Klapec et al., 1998). Overriding the acceptable upper intake concentration of 400 μg Se/day may lead to selenosis (Pham-Huy et al., 2008), which is a selenium poisoning characterized by loss of nail and hair, and gastrointestinal diseases (Gasmi et al., 1997), cirrhosis, pulmonary edema and death (Pham-Huy et al., 2008), However, surplus Se intakes through supplementation and its possibility misuse as health

treatment could also pose a danger of adverse health impacts if its utility is not duly regulated (Tinggi, 2008).

1.14.4 Iron (Fe)

In the periodic table, the essential trace element Iron (Fe) is the 26th element (Valko et al., 2005). It is the 2nd most plentiful metal in the crust of the earth (Muñoz-Bravo et al., 2013). The rate adult body contains around 4 g of iron, approximately 1 g in storage, and 3 g in functional or active form (Oladiji, 2003). Skeletal muscle accounts for 10–15% of the total body iron, and the system controlling iron metabolism is existing there (Jankowska et al., 2012). In the blood, Fe exists principally as transferrin in the plasma, and as hemoglobin in the erythrocytes. It is transported as transferrin; stored as haemosiderin or ferritin and it is lost in sloughed cells and by bleeding (Soetan et al., 2010). Iron is wanted for optimal hematopoiesis. The majority part of it is taken up by reticulocytes and erythroblasts for synthesis of hemoglobin (Andrews, 1999; Nemeth, 2008; Camaschella and Pagani, 2011). The main source of Fe is provided by the macrophages which recycle Fe from senescent red blood cells (Polin et al., 2013). The iron regulatory hormone is Hepcidin which produced in the liver (Fitzsimons and Doughty, 2015). Absorption of iron increases when stores of the iron in the body are low and decreases when they are adequate (Drozd et al., 2017). There are two forms of absorbable Fe: ferric (Fe^{3+}), and ferrous (Fe^{2+}) (Polin et al., 2013). Ferric iron is less bioavailable than ferrous iron, and this is due to the lower solubility (Johnson-Wimbley and Graham, 2011). Fe main sources involve liver, fish, dark green leafy vegetables, nuts, red meat, molasses, egg yolk, legumes, spleen, kidney, and heart (Soetan et al., 2010). The electronic structure of Fe and its ability to lead one-electron reactions predetermines Fe as a main component in the generation and metabolism of FRs in biological systems (Valko et al., 2005).

Iron is an active micronutrient and arguably the body's most important biological catalyst (Okonko et al., 2011), which is fundamental for the synthesis of myoglobin (Okonko et al., 2011; Fitzsimons and Doughty, 2015), and haemoglobin molecule (Hb) (one molecule of Hb contains four atoms of the iron) (Polin et al., 2013), as also, it is an important co-factor for enzymatic reactions (Kell, 2009; Hower et al., 2009; Jankowska et al., 2012), required for oxidative metabolism, including those

occurring in the myocardium (Okonko et al., 2011; Fitzsimons and Doughty, 2015). Iron is a micronutrient fundamental for the effective metabolism (Oladiji, 2003), and cellular energy, necessary for maintaining body homoeostasis (Jankowska et al., 2012), it plays a vital role in the storage of oxygen (myoglobin component), transport of oxygen (haemoglobin component), metabolism of the cardiac and skeletal muscle (component of oxidative enzymes and respiratory chain proteins), disintegration and synthesis of lipids, ribonucleic acids, proteins (Andrews, 1999; Hower et al., 2009; Kell, 2009), and mitochondrial function (Jankowska et al., 2012). Iron deficiency (ID) is the commonest nutritional deficiency worldwide, affecting most than one-third of the population. (Andrews, 1999; Zimmermann and Hurrell, 2007; Kell, 2009; Milman, 2011). ID has been defined as a ferritin level <100 mg/L or normal ferritin (100–300 µg/L) with a transferrin saturation (Tsat), <20% (Klip et al., 2013; Ponikowski et al., 2014; Fitzsimons and Doughty, 2015). ID leads in resistance to haematopoietic growth factors (e.g. erythropoietin) and weakens the maturation and differentiation of all haematopoietic cells types (Van der Putten et al., 2008; Elliott et al., 2009). Deficiencies of the micronutrient which are of utmost public health importance are ID, causing varying grades of weakness in cognitive performance, minimized capacity of the work, complications of the pregnancy, and lowered immunity to infections (Soetan et al., 2010). The ID consequences may extend from anemia to mental retardation in growing children (Valko et al., 2005). Deficiency of Fe adversely influences the function and limits the survival of living organisms at every complexity level (Andrews, 1999; Haas and Brownlie, 2001), Figure 1.12.

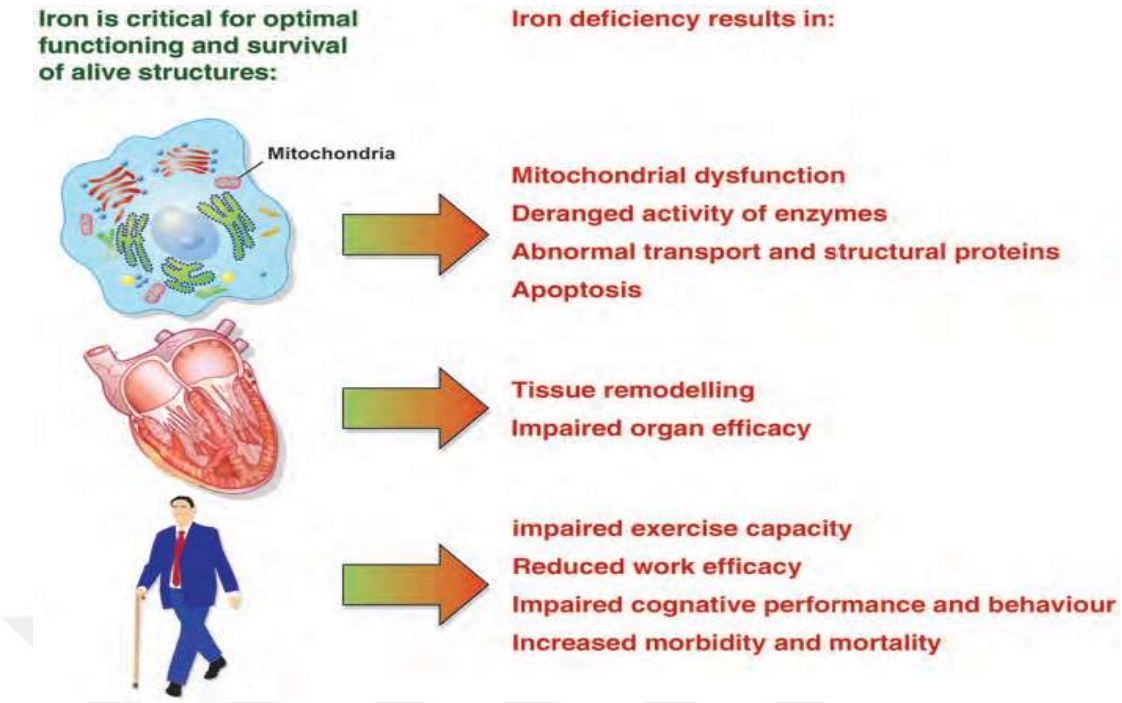

Figure 1.12 The photo shows the significance of Fe for functioning and survival across all levels of complexity of living structures (Jankowska et al., 2012).

Deficiency of Fe causes to a reduction of the hemoglobin molecule synthesis (Polin et al., 2013). The first ID reports in cardiovascular disorder were promulgated before 50 years (Somers, 1959**;** Duke and Abelmann, 1969; Jankowska et al., 2012). Increased Fe loss is linked with some gastrointestinal diseases (Fe malabsorption or digestive blood loss (Polin et al., 2013), gastritis, peptic ulceration, duodenitis, and esophagitis), recurrent blood sampling, and loss of the menstrual blood (Drozd et al., 2017). However, Fe surplus can be poisonous because it has the capacity to take and donate electrons by exchanging among ferric and ferrous forms. This exchanging may produce ROS through the reactions of Haber-Weiss and Fenton, causing oxidation of the organic biomolecule and oxidative stress (Muñoz-Bravo et al., 2013). Free Fe can be deposited in different organs involving the heart, liver, joints, pancreas, and other tissues. The cumulative quantity of Fe in body of the human may reach as much as 20 g. Next some years, patients develop particular symptoms (fatigue, and weakness) followed by more dangerous situations like liver cancer, arthritis, and cirrhosis (Valko et al., 2016), neurodegenerative disorders, heart and liver disorder, diabetes, cancer, abnormalities of the immune system, and hormonal abnormalities (Valko et al., 2005).

1.15 Aim of Study

The importance of the study for human health; so the main objectives of the present study were i) to investigate the association among aortic valve sclerosis disease and serum of the nitrosative stress of peroxynitrite, and the essential trace elements zinc, selenium, copper and iron in the serum of 80 subjects for Turkish population/ Gaziantep, and ii) To explore, the clinical significance of serum human peroxynitrite, selenium, zinc, iron, and copper levels as potential new biomarkers for the AVSc patients, and to clarify pathophysiological role in individuals at risk for developing AVSc.

CHAPTER 2

LITERATURE REVIEW

For the past decades, aortic valve calcification (AVC) was recognized and described in detail by Monckeberg (Akat et al., 2009; Sathyamurthy and Alex, 2015).

In the 1970s, it was recognized by the work of Roberts and Pomerance that a third etiological group existed, namely a degenerative, wear-related form of aortic stenosis AS, which affects preferably the older part of the population (Wallby, 2008).

By the measurement of plasma nitrotyrosine in coronary sinus effluent (CS) and arterial blood (Ao), Hayashi and his colleagues Hayashi et al. (2001) found clinically for the first time that $ONOO^-$ was generated from human myocardium after ischemia-reperfusion during the open heart operation.

Branchetti et al. (2013) were shown that the tissue of aortic valve from the patients with pathological dysfunctions of the valve (both severe aortic stenosis and aortic sclerosis), around the calcified regions in the patients of AVSc appeared an accumulation of nitrotyrosine, oxidative injuries, as well as side-specific accumulation in the fibrosa layers. They used whole cell extracts, from AVSc- AVS- and AVC- derived patients' tissues and proved that peroxynitrite concentrations were higher in noncalcified sclerotic and calcified-stenotic aortic valve as compared with healthy valves.

Furthermore, in earlier study of Nyström-Rosander et al. (2004) they recorded in the serum and tissue of sclerosis heart valves patients who operated on for replacement aortic stenosis because of advanced aortic sclerosis a statistically significant effect of sera Cu and Se levels in the patient's blood in comparison with healthy donors' plasma.

Whereas, they found a significant decrease in sera Zn values and no changes in sera Fe levels between the groups. On the other hand, they found the opposite results in the tissue of the sclerosis valve patients when a significant increase in both Fe and Zn value and a significant decrease in the values of Cu and Se were detected among the patients tissue in compared with normal valves plasma.

Morever serum samples and aortic valve tissue were taken from sclerotic aortic valves patients undergoing open-heart surgery for the replacement of stenotic aortic valves due to aortic sclerosis in the study of (Nyström-Rosander et al., 2003), half of those patients suffered from chlamydia pneumonia, a significant increase in the levels of Zn, and Fe in the patient's tissues were observed. Furthermore, a significant effect of sera copper values and copper/zinc ratio as well as, statistically significant decrease of the sera Zn were found in the patient's serum as compared with the plasma of healthy group.

The study of Nyström-Rosander et al. (2002) showed that the tissue concentrations of Cu, and Se moderately changed in the aortic valves from patients undergoing surgical aortic valve replacement because of aortic stenosis, when they record a decrease in their levels as compared with the healthy donors. However, there were clear increases in the levels of Zn, and Fe in the patients valves than the normal valves.

Uetani et al. (2005) were they found that tissue concentrations of Fe, Zn, Se, and Cu in heart of the patients which exposed to cadmium environmental showed no changes, whereas Cu and Zn level tended to decrease significantly in their aorta as compared with control subjects.

Ilyas and Shah (2017) reported that there were high average concentrations of Fe, and Zn in the blood of valvular heart disease (VHD) patients as compared with healthy valves, whereas Cu recorded almost comparable levels in the blood of both donor groups.

Lis et al. (2014) showed a significantly high concentration of Cu tissue in early lesions of calcified human aortic valves, while the further accumulation of copper was not associated with late lesions as compared to normal areas of the valves. In the case of Fe there was a decrease in iron concentration in late lesions. As well as Zn

tissue concentration showed significantly high content in late lesions of the valves disorder than early lesions.

Tohno et al. (2000) aimed in their study to examine whether there was a relationship in the contents of Zn, and Fe among the four cardiac valves. They were found that there were no significant correlations in both the contents of zinc and iron between the aortic and mitral valves, the pulmonary and aortic valves, and among the tricuspid and aortic valves.

Ohnishi et al. (2003) stated that a significant correlations between age and either Fe or Zn content in all of the cardiac valves of Thai was not found.

In both the pulmonary and aortic valves of the pig, Tohno et al. (2007) found that Zn and Fe levels decreased significantly with development, but they did not decrease significantly in the tricuspid and mitral valves of the pig with development.

Menetti et al. (2005) conducted on cardiac valves of Rhesus and Japanese monkeys. They found in the infant monkey, that Zn concentration was high in all of the four cardiac valves, especially high in the pulmonary and aortic valves, but decreased quickly in all of the four cardiac valves of the monkey with development.

Roeser and Powell (1970) showed that aortic valve disease is more particularly linked with urinary iron loss and hemolysis than either mitral valve disorder or mixed mitral and aortic disorder.

CHAPTER 3

PATIENTS AND METHODS

3.1 Subjects

This study included 80 subjects consisted of two groups, 40 patients group (30 females and 10 males) between 18-65 years old with aortic valve sclerosis (AVSc) and 40 healthy volunteers as a control group with similar gender ratio. The diagnosis of AVSc in a Turkish population (Gaziantep) was done based on the echocardiography scans by the cardiologist (Dr. Murat Sucu). Likely, Echocardiographical evaluation was carried out for the healthy individuals who had no cardiac pathology as the control group. Control donors were with normal aortic valve cusps and did not have any cardiovascular disorder or other diseases, (e.g., disorders of liver, kidney disorder, diabetes, hypertension, and cancers). Pregnant patients, and patients with severe diseases such as (renal dysfunction, lung disease, blood diseases, rheumatoid arthritis patients, cancer, liver disease, congenital heart diseases, and other heart diseases were also excluded from our study to prevent the impact on serum biochemical parameters. The clinical evaluation of AVSc was performed during the visiting at a single cardiac center (Gaziantep University General Hospital, Gaziantep, Turkey) between February 2018 and April 2018. The protocol of the present study was approved by the Clinical Research Ethics Committee of Gaziantep University on the date of 26.12.2017 numbered 2017/429. Besides, AVSc and healthy subjects provided their written, informed approval to share in the present study.

3.2 Clinical Measurements

Clinical patients' information was gathered from all topics involving essential demographics: gender, age, diabetes, hypertension, weight, height, body mass index (BMI), smoking, alcohol, and drugs. Detailed clinical checks, including laboratory

parameters, and echocardiography scans were recorded and joined with a physical examination (the specialist cardiologists). The standard for diagnosing AVSc was depend on the hemodynamic and morphologic findings in the echocardiographic study in the aortic valve by the wall thickness of a given hump of (2-6 mm at minimum one abnormal leaflet per valve), with a transaortic flow rate <2.5 m/s. Anthropometric measurements were recorded for the body height and weight by patients standing barefoot and taking into account wearing light clothing by utilizing a digital instrument (Davi & Cia Weighing Equipment, Barcelona, Spain). Furthermore, the equation of BMI = weight/height^2 (kg/m^2), was used to determine the Body Mass Index (BMI). The systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg were taken as the hypertension criteria. Hypertension of the subjects was measured by Bedside Monitor instrument Figure 3.1.

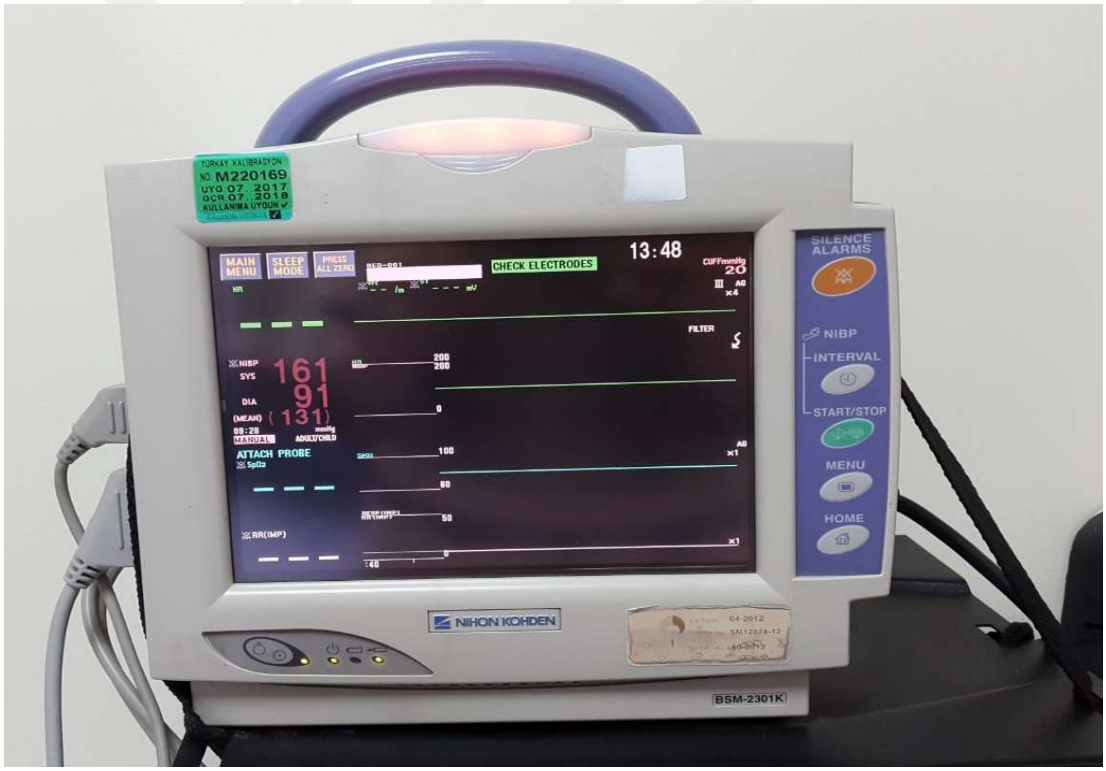

Figure 3.1 Described hypertension instrument (Bedside Monitor, Nihon Kohden Corporation, Model BSM-2301K, Tokyo, Japan, 2003).

3.3 Collection of Blood Sample

Drawing blood and collection process from the subjects were performed in the Venesection Unity of Gaziantep University Medical Faculty Hospital. Subjects in the sitting position, the antecubital area of the forearm was taken after the sterilization by wiping with alcoholic cotton. Then 5ml of blood venous were drawing by utilizing a BD Vacutainer needle Figure 3.2, then transferred and gathered into the tubes (VACUETTE® Tube, 8 ml, U.S, Z Serum Separator Clot Activator containing microscopic silica particles stimulates coagulating) Figure 3.2. Then blood samples were allowed to coagulate for 10 to 15 min at the room temperature. Afterward, by utilizing centrifuge the specimens were centrifuged for 10 min at 4000 rpm Figure 3.3. The serum was separated and kept into a micro Eppendorf tube and stored at -80 ºC. The trace elements, zinc, and copper levels of serum specimens were measured by use of an atomic absorption spectroscopy (Shimadzu, AA-6800 Spectrometer), selenium by utilize of inductively coupled plasma mass spectrometry (NexION® 350 ICP-MS spectrometer) device, and iron by use of (Beckman Coulter® Model Au5800, Japan, 2007) Figure 3.4. Peroxynitrite levels were measured by the spectrophotometric (Spectrophotometry UV- 1601, SHIMADZU, Japan) method (van Uffelen et al., 1998) Figure 3.5. These analyzes were carried out at Gaziantep University Medical Faculty Hospital Central Laboratory and Gaziantep University Medical Biochemistry Department.

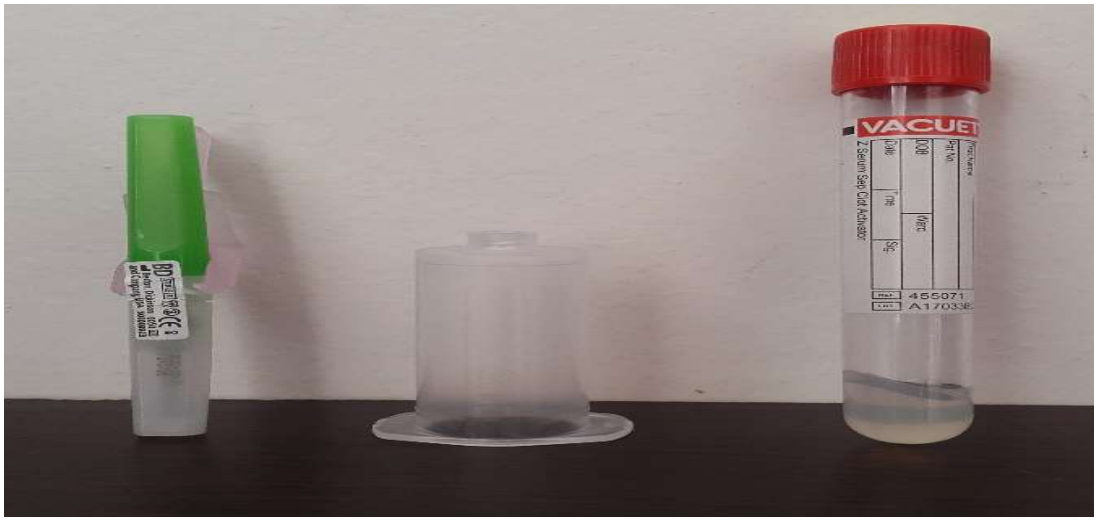

Figure 3.2 BD Vacutainer needle and tubes.

Figure 3.3 Illustrated Centrifuge instrument (Nuve NF800R, Ankara, Turkey).

Figure 3.4 Illustrated Fe measurement device (Beckman Coulter® Model Au5800, Japan, 2007).

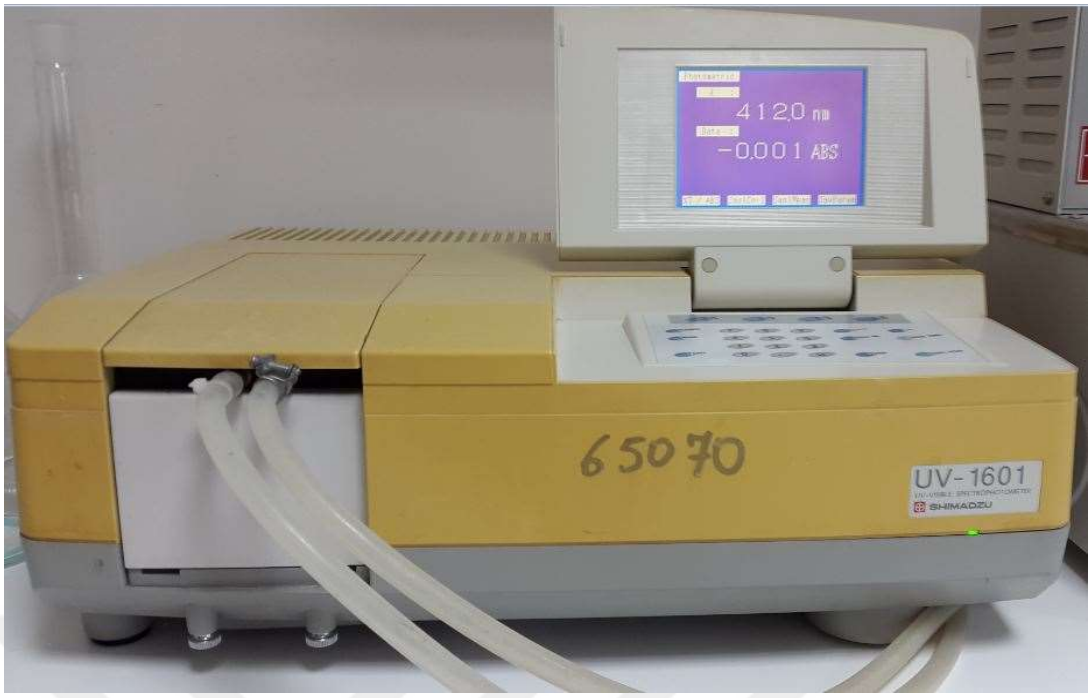

Figure 3.5 Illustrated spectrophotometry device (UV- 1601, SHIMADZU, Japan).

3.4 Biochemical Laboratory Analysis

3.4.1 Measurements of Serum Human Peroxynitrite (ONOO•–) Levels

Estimate of nitrosative stress status in AVSc patients was carried out by measurement of ONOO•. The principle for serum peroxynitrite levels was assessed by spectrophotometric method procedure utilizing clinical chemistry laboratory device (Spectrophotometry UV- 1601, SHIMADZU, Japan), Figure 3.5. Levels of sera peroxynitrite were an assessment as described by (Van Uffelen et al., 1998). In which the free radicals of peroxynitrite (ONOO•–) mediated nitration of phenol to nitrophenol was estimated spectrophotometrically at 412 nm. The nitrophenol amount in the serum is linked to the peroxynitrite concentration. The peroxynitite methodology was made by Spectrophotometry UV- 1601, SHIMADZU System. Were in a glass test tube 10 µL of sample sera was added to 5 mM phenol in 50 mM sodium phosphate buffer (pH 7.4) to get a final volume of 2 mL and after the 2 h incubation in dark place at 37°C, 15 µL of 0.1M NaOH was added and the absorbance of the samples at a wavelength of 412 nm were directly registered. The yield of nitrophenol was calculated from ε = 4400/M/cm.

3.4.2 Measurements of Serum Human Iron (Fe)

Serum Fe was measured based on the photometric color test method procedure by utilizing automatic clinical chemistry laboratory analyzer system (BECKMAN COULTER® Model Au5800, Japan, 2007) analysers, Figure 3.4. Measured serum iron concentration is principally the Fe (III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. The normal reference ranges of serum Fe for adults was (100 – 250 µg/dL).

The Fe methodology was utilised TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen. In an acidic medium, transferrin-bound iron dissociates into free ferric ions and apo-transferrin. Hydrochloric acid and sodium ascorbate reduce the ferric ions to the ferrous state. The ferrous ions then react with TPTZ to form a blue colored complex which can be measured bichromatically at 600/800 nm. The increase in absorbance is directly proportional to the amount of iron present.

Chemical reaction scheme:

$$\text{Transferrin 2 } (Fe^{3+}) \xrightarrow{\text{Buffer}} 2\ (Fe^{3+}) + \text{Apo-transferrin}$$

$$2\ Fe^{3+} + \text{Ascorbic Acid} + 2\ H_2O \longrightarrow 2\ Fe^{2+} + \text{Dehydroascorbic Acid} + 2\ H_3O^+$$

$$Fe^{2+} + \text{TPTZ} \longrightarrow \text{Iron-complex}^{2+} \text{ (blue coloured complex).}$$

Final concentration of reactive ingredients:

Glycine buffer (pH 1.7)	215 mmol/L
L-ascorbic acid	4.7 mmol/L
2,4,6-Tri(2-pyridyl)-5-triazine	0.5 mmol/L
Preservative	

Calibration Fe procedure was performed by utilizing of the chemistry calibrator system Cat. No. 66300.

3.4.3 Measurements of Serum Zinc (Zn)

Analysis methods principle: Serum Zn analysis is performed by use of flame Atomic Absorption Spectrometry (Shimadzu, AA-6800 Spectrometer). The basic principle of the analysis is the decomposition of the zinc atoms in the serum Zn sample by heat energy and the fact that these atoms retain part of the light released from the serum Zn lamp. The decrease in intensity of light is directly proportional to the concentration of zinc in the sample. The unit of this decrease in the intensity of light is absorbance. Accordingly, the increase in concentration increases the absorbance in direct proportion. For both gender adults the normal sera Zn range from (65 - 140) µg/dL.

Materials:

Reagents:

Nitric acid solution: Take 5.34 mL of concentrated nitric acid and make up to 500 mL with distilled water so that 1% (v/v) nitric acid solution is prepared. This solution is used to dilute standards.

Distilled water: Pure water from Bome is used.

ZINC Lamp: The hollow cathode lamp used in ZINC analysis. The service life is 5000 mA /h.

10 mL autosampler sample containers: Calibrators used for drawing the calibration curve are transferred to 10 mL autosampler sample containers or prepared in these containers.

Procedure steps:

*Calibration: High-purity 100 2-g/mL ZINC standard:

1- Liquid and stored at 2-8 ºC. A 100 ppm zinc standard is diluted 100 fold using 1% nitric acid solution and a 10 ppm intermediate standard solution is prepared. Working standards of 0.1–0.2-0.4 ppm are then prepared using 1% nitric acid solution.

2- 0.1 ppm zinc r standard: 0.1 mL from 10 ppm intermediate standard and complete with distilled water to 10 mL.

3- 0.2 ppm zinc standard: 0.2 mL from 10 ppm intermediate standard and complete with distilled water to 10 mL.

4- 0.4 ppm zinc standard: 10 mL from 10 ppm intermediate standard and 10 mL with distilled water.

*SERONORM Trace Elements Serum level 1 and 2 (10x5 mL): Store unopened until expiry date, after opening (-18ºC and below) for 30 days, (2 - 8) ºC for 7 days. Take 500 ul of the test process and complete with distilled water to 5 ml.

*Performing the calibration: In each study, 3 known standards are used. It is automatically evaluated in the calibration curve with the absorbance readings against the concentrations of the standards.

*Testing process

Performing the calibration: In each study, 3 known standards are used. It is automatically evaluated in the calibration curve with the absorbance readings against the concentrations of the standards.

*Calculation: Results are entered manually in the automatic LIS.

3.4.4 Measurements of Serum Copper

Analysis methods principle: Serum copper analysis is performed by using a flame Atomic Absorption Spectrometer (Shimadzu, AA-6800 Spectrometer). The basic principle of the analysis is the decomposition of the copper into the atoms of the serum and these atoms hold a portion of the light released from the copper lamp. The decrease in intensity of light is directly proportional to the concentration of copper in the sample. The unit of this decrease in the intensity of light is Absorbance. Accordingly, the increase in concentration increases the absorbance in direct proportion. The normal serum Cu reference ranged from (20 - 70) µg/dL.

Materials:

Reagents:

Distilled water: Pure water from Bome is used.
Copper (Cu) Lamp: The hollow cathode lamp used in the copper analysis. The service life is 5000 mA/hour.

10 mL autosampler sample containers: Calibrators used for drawing the calibration curve are transferred to 10 mL autosampler sample containers or prepared in these containers.

Procedure steps:

*Calibration: High-purity 100 2-g/mL copper standard:

1- It is in the liquid state and stored at 2-8 ºC. 100 ppm copper standard is diluted 100-fold using 1% nitric acid solution and 10 ppm intermediate standard solution is prepared. Working standards of 0.5–1-2 ppm are then prepared using 1% nitric acid solution.

2- 0.5 ppm copper standard: 0.5 mL is taken from the 10 ppm intermediate standard and is completed with distilled water to 10 mL.

3- 1 ppm copper standard: 1 mL of 10 ppm intermediate standard is taken and finished with distilled water to 10 mL.

4- 2 ppm copper standard: 2 mL is taken from 10 ppm intermediate standard and finished with distilled water to 10 mL.

*SERONORM Trace Elements Serum level 1 and 2 (10x5 mL): Store unopened until expiry date, after opening (-18ºC and below) for 30 days, (2 - 8) ºC for 7 days. Take 500 ul of the test process and complete with distilled water to 5 ml.

*Performing the calibration: In each study, 3 known standards are used. It is automatically evaluated in the calibration curve with the absorbance readings against the concentrations of the standards.

*Calculation: Results are entered manually in the automatic LIS.

3.4.5 Measurements of Serum Human Selenium (Se)

Analysis methods principle: Serum selenium analysis is performed by inductively coupled plasma mass spectrometry (NexION® 350 ICP-MS spectrometer).

The basic working principle: ICP-MS is a technique that combines two technologies into one: 1-A high-temperature ICP source, which is in a range between 6000 and 10,000 K. The high-temperature ICP source converts the atoms of the elements in the

sample to ions. 2-These ions are then separated and detected by a mass spectrometer. The ions formed by the ICP discharge are typically positive ions, M^+ or M^{2+}.

After 80 blood samples were collected from the volunteers, the specimens were centrifuged at 4000 rpm. Then the serum was stored at −80°C until analysis. After that, the serum specimens were diluted 1 to 10 with 10% acetic acid with 0.1% Triton X-100, and rhodium and germanium were added as internal standards to correct the matrix-induced ion signal fluctuations and instrumental drifts (20 µg/L). Finally, by used inductively coupled plasma mass spectrometry (ICP-MS) device, we measured the concentration of selenium. The reference range of sera Se is (16 – 71) µg/L.

3.5 Statistical Analysis

The normality of distribution of continuous variables was tested by Shaphiro Wilk test. Student's t-test and Mann-Whitney U test were used to compare two independent groups of variables with a normal distribution and a non-normal distribution, respectively. Chi-square test was used to assess the relation between categorical variables. Statistical analysis was performed with SPSS for Windows version 22.0 and a P value < 0.05 was accepted as statistically significant.

3.6 Approval of Ethics Committee

The protocol of our study was approved by the Clinical Research Ethics Committee of Gaziantep University on the date of 26.12.2017 numbered 2017/429. As well as all AVSc patients and control subjects gave their written, informed approval to share in our study to collect the blood samples in order to estimate the serum peroxynitrite, zinc, selenium, copper, and iron.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Descriptive Statistics

Results of descriptive analyses are given in Table 4.1. Ages in the patient group ranged from 39 to 65, whereas the control group changed between 42 and 65. High BMI (~23 – 55 kg/m^2) was found in the patient group compared with the control group (~21 – 49 kg/m^2). Peroxynitrite (mean 4.33 µmol/L) varied between 0.68 and 8.86 µmol/L. Besides, the subjects had different heavy metals values in their sera.

Table 4.1 Descriptive analyses (mean±SD standard deviation) of subjects, BMI is body mass Index and N is subjects' **number.**

	N	Min	Max	Mean	Std. Deviation
Age	80	39.0	65.0	56.6	7.2
BMI (kg/m^2)	80	21.1	54.69	31.12	6.08
Systolic blood pressure (mmHg):	80	108.0	168.00	132.13	16.83
Diastolic blood pressure (mmHg):	80	54.0	99.00	69.26	10.51
Peroxynitrite (µmol/L)	80	0.68	8.86	4.33	2.26
Iron (µg/dL)	80	11.00	131.00	64.63	30.61
Copper (µg/dL)	80	35.60	167.10	90.38	24.63
Selenium (µg/L)	80	41.70	92.90	61.17	11.41
Zinc (µg/dL)	80	60.10	146.00	86.87	23.16

Baseline clinical characteristics of the: gender, diabetes, alcohols, and smoking for both patients and healthy valves were described in Table 4.2. Among the 40 AVSc subjects there were 10 males (25%), and 30 females (75%) with the same number and sex for the control donors. We observed from the table that there was no significant difference among the gender and both of (patients and the healthy subjects) *(p*>0.050). Morever there were 17 (~42%) patients among the patient group had diabetes

.

However, diabetes was significantly higher in the AVSc group compared to the control groups (p=0.001). In addition, there were 10 persons in the AVSc group (~2%) drink alcohol, and 5 patients (~12%) smoke cigarettes, but there was no significant link among (alcohol and smoking) and the groups when (p=0.424, p=0.411) sequentially.

Table 4.2 Comparisons of categorical variables.

		Group				
		Patient		Control		
		Count	%	Count	%	p
Gender	Male	10	25	10	25	1.000
	Female	30	75	30	75	
Diabetes	Yes	17	42.5	0	0.0	0.001*
	No	23	57.50	40	100.00	
Alcohol	Yes	10	2.50	0	0.00	0.424
	Quit	2	5.00	1	2.50	
	No	37	92.50	39	97.50	
Smoking	Yes	5	12.50	2	5.00	0.411
	Quit	4	10.00	3	7.50	
	No	31	77.50	35	87.50	

Abbreviations: %, percentage, *p<0.05, students t-test, *: p<0.05 is statistically significiant level.

Descriptive statistics of the AVSc patients' drugs are shown in Table 4.3. In this table, we conducted a comparison among AVSc patients according to their visit to the hospital as a newly patients and their exposure to the echocardiogram as the first time or not. As well as comparing them in terms of taking of medicines or not. Generally, our 40 AVSc patients group involved 25 patients were they take drugs, whereas, 15 patients didn't utilize medicines. However, 55% of them visited the hospital to check their heart by echocardiogram for the first time while 45% of them visited the hospital and they underwent an echocardiogram previously. Approximately half of the AVSc patients 45% visited the hospital and they underwent an echocardiogram previously and they also take drugs since about (2-10) years Table 4.3. In contrast, 37.5% of the patients visited the hospital to examine their heart by echocardiogram for the first time and they did not use any medication until those moments.

Furthermore, only 7 patients which they visited the hospital to check their heart by echocardiogram for the first time and they were taking medicines for other purposes, not for their heart problem purposes.

Table 4.3 Baseline clinical characteristics of our 40 AVSc patients.

Characteristics		Count	%
Drugs status	No drugs- first time heart check	15	37.5
	Take drugs- first time heart check	7	17.5
	Take drugs- not first time	18	45.0
Drug usage	Yes	25	62.5
	No	15	37.5
Drugs	First time	22	55.0
	Not first time	18	45.0

Mean categorical variables of subjects are given in Table 4.4. No significant difference in the age between AVSc patients and the healthy subjects (p>0.05) was observed. Further, significant higher BMI was found in female of the patient group (34.37 kg/m^2) compared with the female of control group (29.47 kg/m^2) (p=0.003), but not found in the male gender. Higher blood pressures were observed in both genders of the AVSc patient group, compared with the healthy group (p<0.05). On the other hand, the AVSc patients group had 42% diabetes, whereas not present in the control donors. Also, there was no significant difference in the number diabetes between gender of the AVSc patients (p>0.05). The AVSc patient group had significantly higher peroxynitrite for both genders, compared the healthy group (p<0.01). Box plot of peroxynitrite between AVSc patients and healthy subjects are shown in Figure 4.1.

Table 4.4 Comparisons of numerical variables for patients and controls. Data are shown as the mean ± standard deviation (mean±SD.).

		Patient	Control	
	Gender	(mean+SD)	(mean+SD)	*P*
Age	Female	56.63±7.66^{a,A}	57.43±7.07^{a,A}	0.676
	Male	56.80±6.21^{a,A}	53.40±7.58^{a,A}	0.287
	Total	56.67±7.25^{a}	56.42±7.32^{a}	0.878
BMI	Female	34.37±6.51^{a,A}	29.47±5.86^{b,A}	0.003
	Male	28.71±2.95^{a,B}	28.73±3.40^{a,A}	0.992
	Total	32.96±6.30^{a}	29.28±5.32^{b}	0.006
Systolic blood pressure (mmHg)	Female	145.06±15.91^{a,A}	121.73±9.02^{b,A}	0.000
	Male	136.70±15.36^{a,A}	120.00±8.28^{b,A}	0.007
	Total	142.97±16.00^{a}	121.30±8.77^{b}	0.000
Diastolic blood pressure (mmHg)	Female	74.56±11.71^{a,A}	64.76±7.80^{b,A}	0.000
	Male	72.30±9.60^{a,A}	63.80±5.92^{b,A}	0.028
	Total	74.00±11.14^{a}	64.52±7.32^{b}	0.000
Diabetes	Female	1.43±0.50^{a,A}	1.00±0.00^{b,A}	0.000
	Male	1.40±0.51^{a,A}	1.00±0.00^{b,A}	0.025
	Total	1.42±0.50^{a}	1.00±0.00^{b}	0.000
Peroxynitrite (μmol/L)	Female	6.11±1.70^{a,A}	2.78±0.98^{b,A}	0.000
	Male	5.88±2.28^{a,A}	2.11±0.80^{b,A}	0.000
	Total	6.05±1.83^{a}	2.61±0.97^{b}	0.000
Iron (Fe) (μg/dL)	Female	56.60±24.02^{a,A}	67.80±34.97^{a,A}	0.154
	Male	62.90±32.18^{a,A}	81.00±28.99^{a,A}	0.203
	Total	58.17±26.00^{a}	71.10±33.71^{a}	0.059
Copper (Cu) (μg/dL)	Female	96.88±27.51^{a,A}	85.19±21.48^{a,A}	0.072
	Male	84.08±25.14^{a,A}	92.77±22.10^{a,A}	0.423
	Total	93.68±27.21^{a}	87.08±21.60^{a}	0.233
Selenium (Se) (μg/L)	Female	61.71±11.36^{a,A}	59.76±11.33^{a,A}	0.509
	Male	58.30±12.68^{a,A}	66.66±10.18^{a,A}	0.122
	Total	60.85±11.63^{a}	61.48±11.33^{a}	0.807
Zinc (Zn) (μg/dL)	Female	72.91±9.04^{a,A}	101.23±24.17^{b,A}	0.000
	Male	73.71±14.70^{a,A}	98.86±25.61^{b,A}	0.015
	Total	73.11±10.52^{a}	100.64±24.23^{b}	0.000

Abbreviations: Different capital letters indicate a statistical difference at p=0.05 level in each column. Different lower-case letters indicate statistical difference at p=0.05 level between patient and control. The same letters indicate no significant difference.

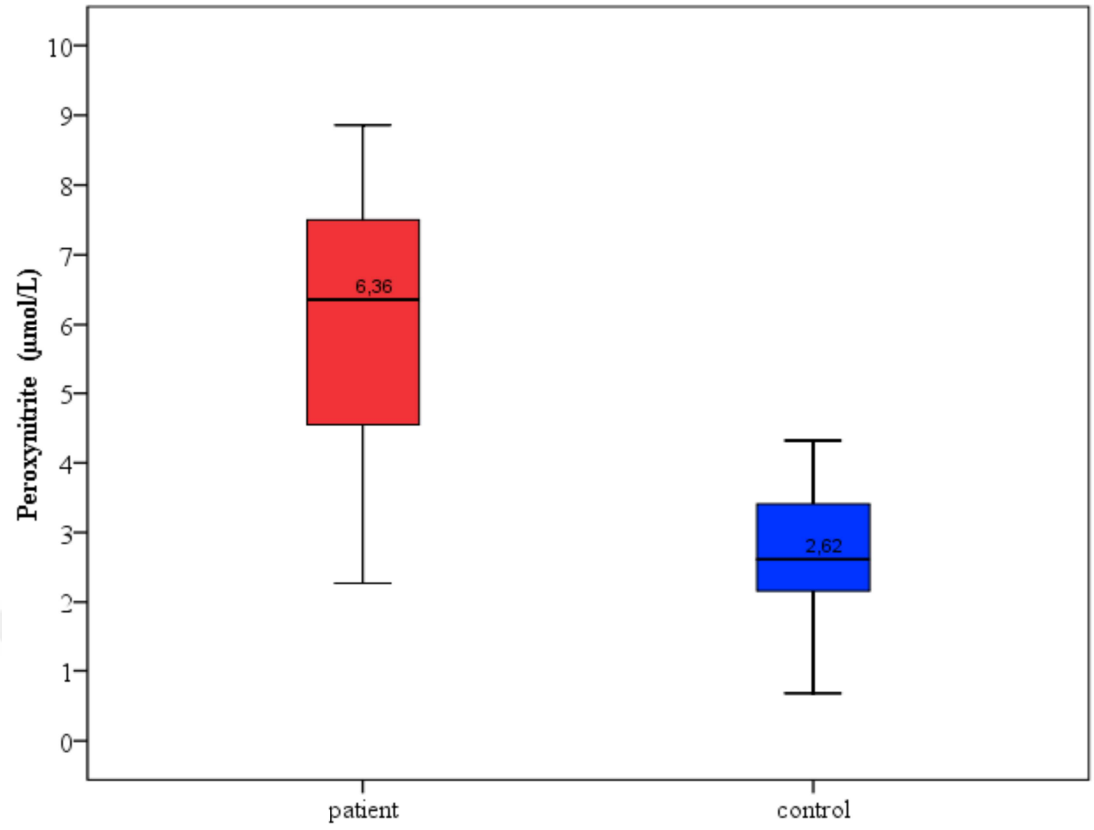

Figure 4.1 Box plot of serum peroxynitrite was significantly higher in AVSc patients than in the control group.

The studied elements values of the sera were found in the normal elements serum ranges (100 – 250 µg/dL Fe; 16 – 71 µg/L Se; 20 - 70 µg/dL Cu; and 65 - 140 µg/dL Zn). AVSc group had lower in Fe and Se concentrations of sera than those of the healthy valves group. However, there was no significant difference in values of Fe and Se between the patient and the control groups (p>0.05) as shown in Figure 4.2, and Figure 4.3, sequentially.

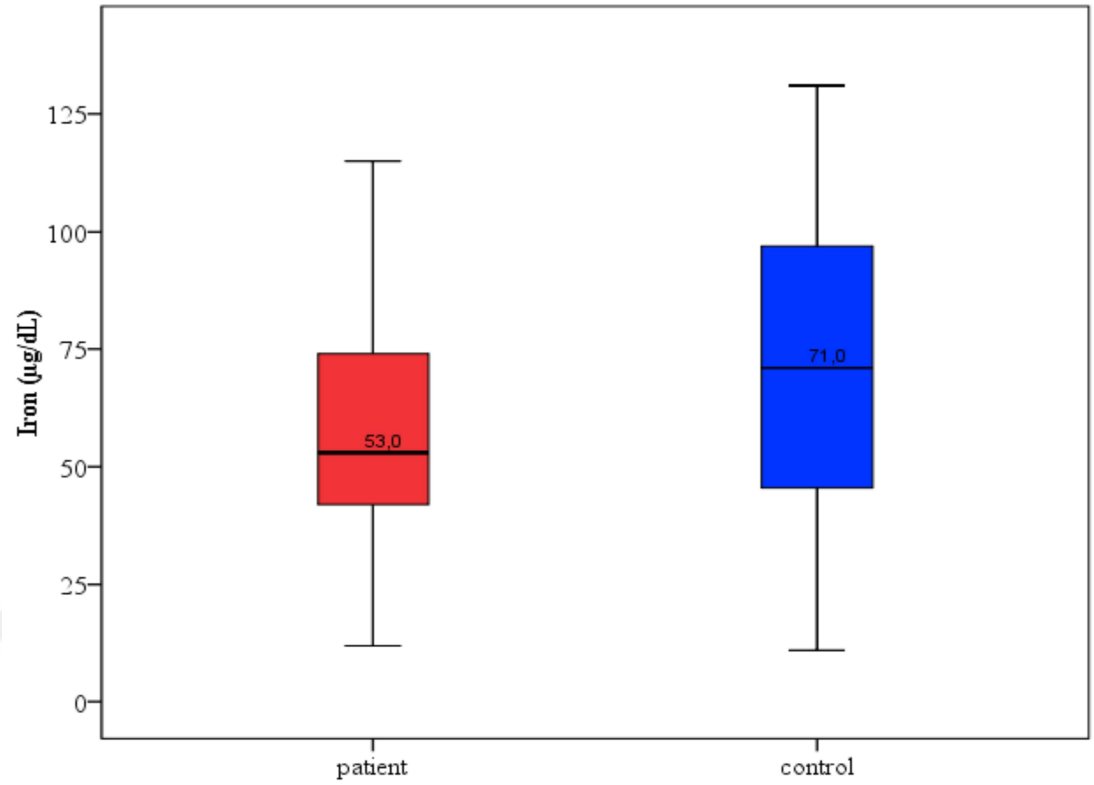

Figure 4.2 Box plot of the serum Fe values was decreased within the AVSc patients in comparison to the control subjects.

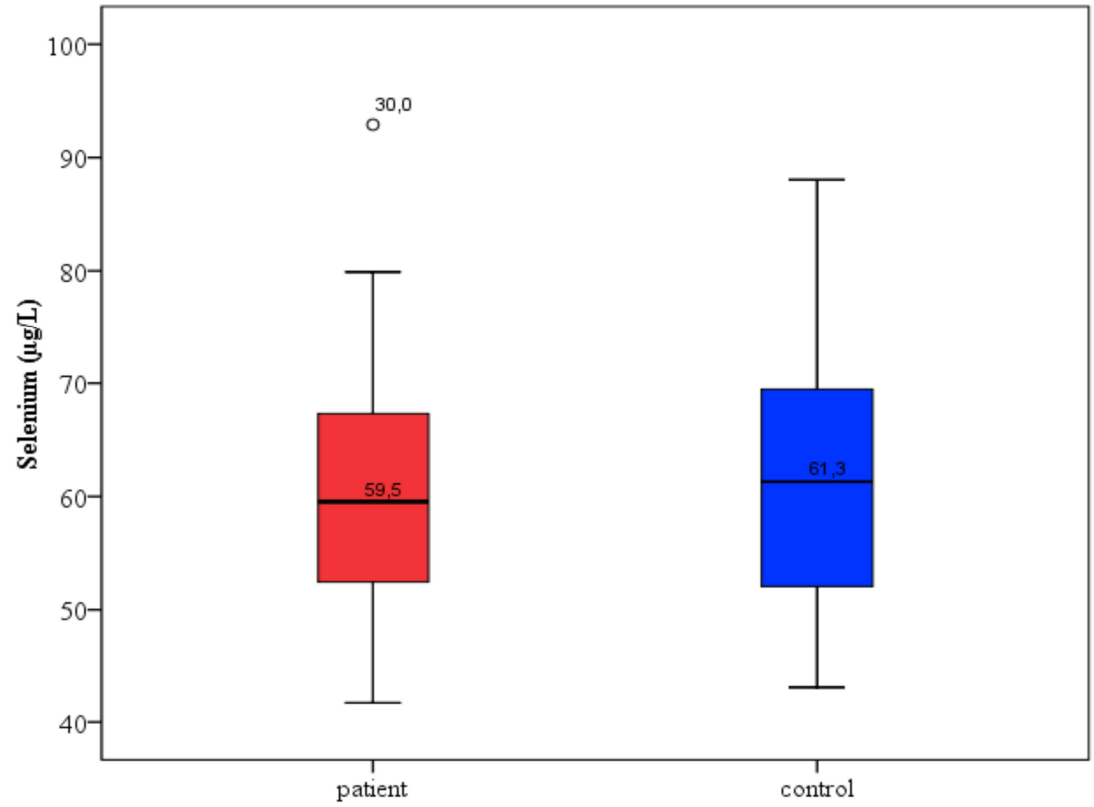

Figure 4.3 Box plot of serum Se was lower in AVSc patients than in the healthy group.

Whereas, the AVSc patient's female had high Se levels (61.71±11.36) in comparison with healthy females group (59.76±11.33) with no static association between them (p=0.509). Also, there was an increase in sera Cu concentrations in the females and total abnormal valves group compared with the healthy valves donors (96.88±27.51, 93.68±27.21) and (85.19±21.48, 87.08±21.60) respectively, but there was no significantly connection among them (p>0.05) as illustrated in Figure 4.4.

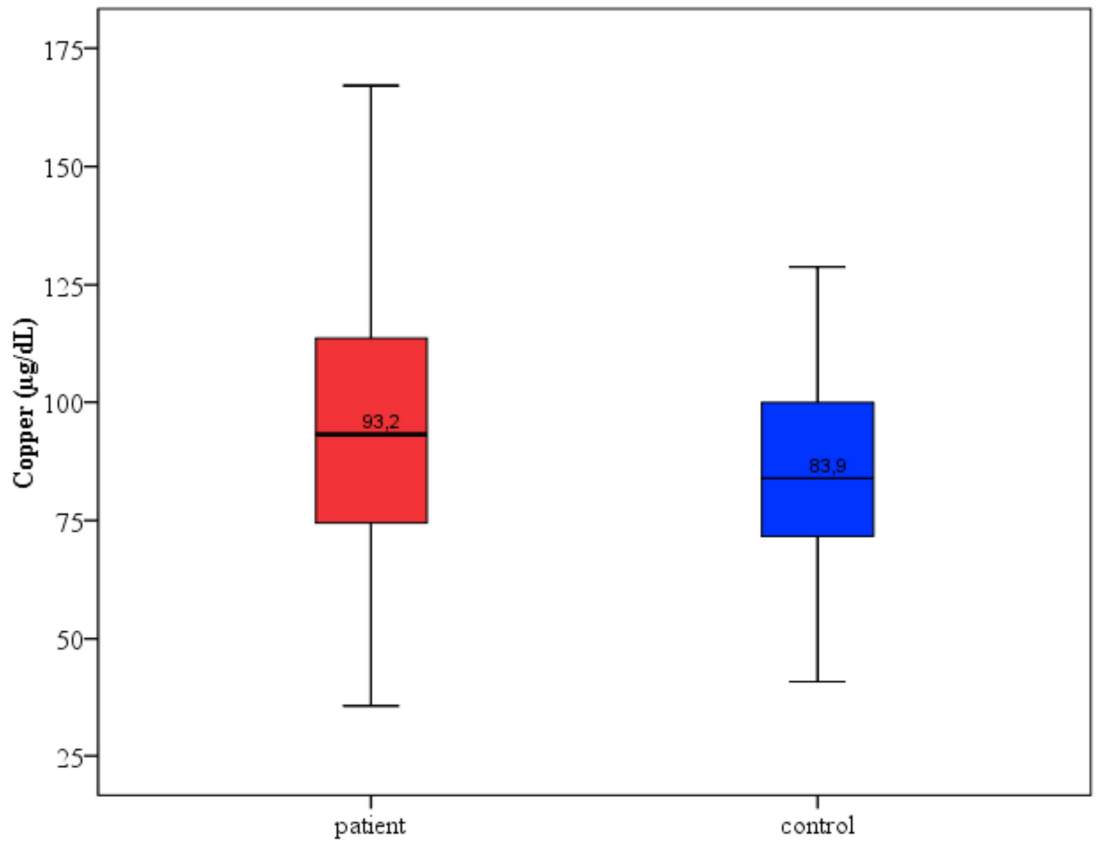

Figure 4.4 Box plot of serum Cu was higher in AVSc patients than in the control group.

A significant decrease was found in the serum zinc levels in the patients group when the compared with the control group (p<0.01), as shown in Figure 4.5.

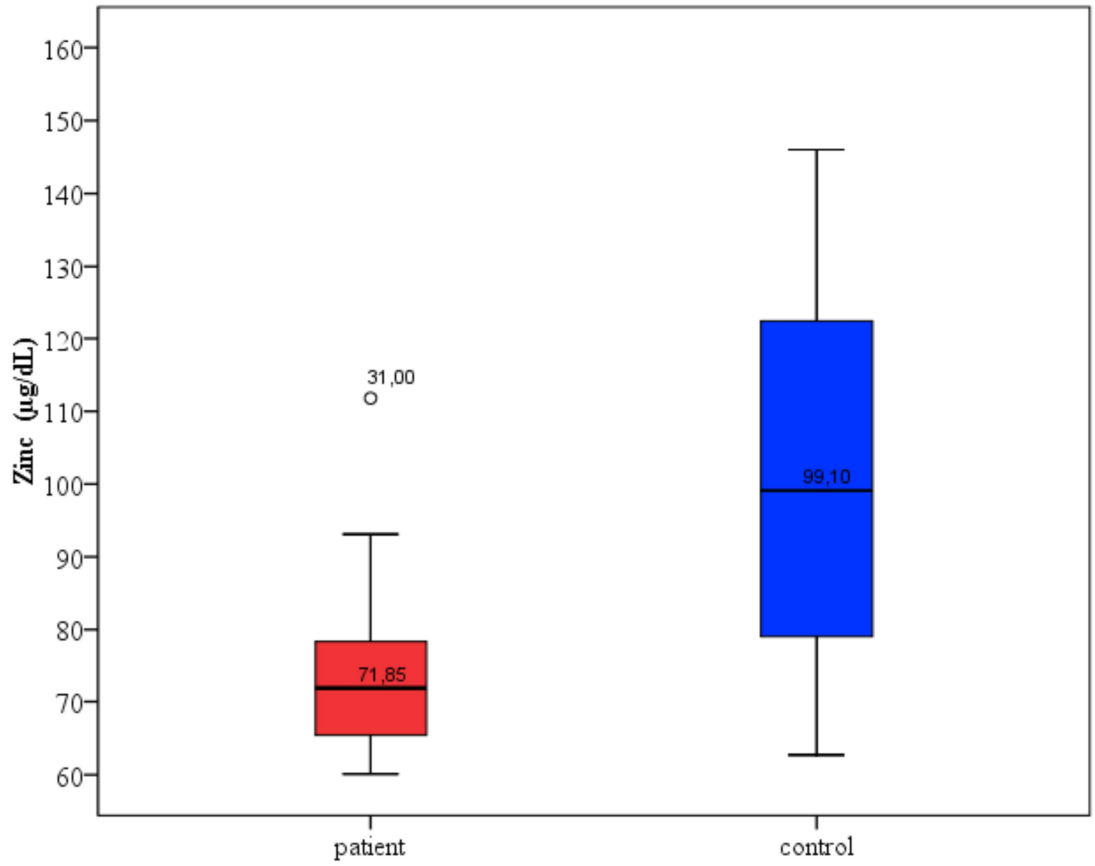

Figure 4.5 Box plot of serum Zn was significantly lower in AVSc patients in comparison to the healthy subjects.

Comparisons of numerical variables among patients group are given in Table 4.5. As it is clear from the table there were two classes among the AVSc patients group according to the history disease or the time were they affected by AVSc disease and/ or they examined their heart for the first time or not.

Table 4.5 Comparisons of categorical variables among the AVSc group.

Variables	First time (n=22)	Not first time (n=18)	T
Peroxynitrite (µmol/L)	5.37±1.77	6.89±1.59	0.007*
Iron (µg/dL)	66.55±29.03	47.94±17.55	0.017*
Copper (µg/dL)	91.23±29.92	96.68±24	0.535
Selenium (µg/L)	63.43±13.29	57.72±8.56	0.109
Zinc (µg/dL)	74.59±11.9	71.32±8.55	0.411

Abbreviations: *p<0.05, students t-test, *: p<0.05 is statistically significiant level.

Generally, 22 patients were affected newly by AVSc disease and they check their heart by echocardiogram for the first time. So among this group there were an increase in sera Se and Zn values (63.43±13.29, 74.59±11.9), respectively in compared with the patients who had a history with AVSc disease since about (2-10) years (57.72±8.56, 71.32±8.55), sequentially, with no significant link between them (p>0.05). Moreover, there were a significant increase of sera Fe levels, and in contrast, a significant decrease of sera peroxynitrite concentrations between the newly patients who examined their heart for the first time in comparison with the patients who affected by the disease from long time (p=0.017, p=0.007), respectively. Table 4.5. In addition, no statistically change in sera Cu values (p=0.535) were observed among the newly patients in compared with those patients who affected approximately since about (2-10) years.

4.2 The Correlation Analysis of the AVSc Group

The correlations among the studied variables in the patients group are given in Table 4.6. Systolic blood pressure had significant positive correlations with age (r=0.399, p<0.05), BMI (r=0.442, p<0.01), and diastolic blood pressure (r=0.517, p<0.01). Any significant correlation was not found between znic and diabetes values with any parameter. As well as, Fe positively correlated with Se (r=0.374, p<0.05), and negatively correlated with Cu values (r=-0.423, p<0.01)**.** Moreover, PN positively correlated with Cu (r=0.080, p<0.05), and negatively correlated with Fe (r=-0.165, p<0.05).

Table 4.6 Correlation between serum human (peroxynitrite, Fe, Cu, Se, and Zn and various clinical parameters in AVSc patients. Sbp., systolic blood pressure, Dbp., diastolic blood pressure, Dia., **diabetes, PN., peroxynitrite. * and ** correlations are significant at the 0.05 level and 0.01 level (2-tailed), respectively.**

	Age	BMI	Sbp.	Dbp.	Dia.	PN	Fe	Cu	Se	Zn
Age										
BMI	0.036									
Sbp.	0.399*	0.442**								
Dbp.	0.079	-0.002	0.517**							
Dia.	0.235	0.070	-0.151	-0.274						
PN	0.121	-0.086	0.066	-0.129	0.002					
Fe	0.080	0.142	0.043	0.186	-0.114	-0.165				
Cu	-0.159	0.154	-0.027	-0.253	-0.167	0.080	-0.423**			
Se	-0.042	0.167	0.106	0.216	-0.169	-0.002	0.374*	0.089		
Zn	0.233	0.204	-0.026	-0.083	0.167	0.114	0.180	0.019	0.303	

4.3 The Correlation Analysis of the Control Group

Correlation analysis among all of the characteristic in the control group are shown in Table 4.7. Age had significantly correlated with each of peroxynitrite (r=0.468, p<0.01), zinc (r=0.597, p<0.01), diabetes (r=0.544, p<0.01), and systolic blood pressure (r=0.819, p<0.01). In addition, PN negatively correlated with Se (r= -0.333, p<0.05), and positively correlated with Sbp (r=0.446, p<0.01), and zinc (r=0.452, p<0.01). However, our results showed that there were a strong positive significant association between Sbp and Dbp (r=0.710, p<0.01). Furthermore, Zn were present a positive significant link with Fe (r=0.478, p<0.01), and Sbp (r=0.479, p<0.01). Lastly, Fe, Cu, and BMI values showed no significant correlation with any one of the parameters.

Table 4.7 Correlation between serum human peroxynitrite, Fe, Cu, Se, and Zn and various clinical parameters in the control group.

	Age	BMI	Sbp.	Dbp.	PN	Fe	Cu	Se	Zn
age									
BMI	-0.252								
Sbp.	0.819**	-0.188							
Dbp.	0.544**	-0.038	0.710**						
PN	0.468**	0.167	0.446**	0.304					
Fe	0.167	-0.028	0.246	-0.059	0.264				
Cu	0.006	0.041	0.115	0.222	0.022	-0.188			
Se	-0.293	0.207	-0.174	-0.114	-0.333*	-0.063	-0.139		
Zn	0.597**	0.068	0.479**	0.249	0.452**	0.478**	-0.018	-0.055	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.8 The previous studies in sera zinc, copper, selenium, iron and peroxynitrite levels in different diseases states and countries. The concentrations are shown as (mean±SD.) or median. PN is peroxynitrite.

	Country	**Disease**	**Sera levels**	***p*-value**	**References No.**
PN	France	Acute coronary syndrome (ACS)	-	$p<0.01$	Gheddouchi et al 2015
	Turkey	Aortic valve sclerosis (AVSc)	6.05±1.83 µmol/L	$p=0.000$	This study
Copper (Cu)	Sweden	Sclerotic heart valves	1258±412 ng /ml	$P<0.05$	Nyström-Rosander et al., 2004
	Turkey	Rheumatic heart disease (RHD)	1.93±0.59 µg/l	$p<0.001$	Kosar et al., 2005
	Iraq	Obstructive coronary artery disease	171.27±28.87 µg/dl	0.0001	Al-Dohan et al., 2015
	Turkey	Heart failure (HF)	880±185 µg/l	0.000	Kosar et al., 2006
	Netherlands	Cardiovascular death	1.32±0.31 mg/L	$p>0.05$	Kok et al., 1988
	Turkey	Aortic valve sclerosis (AVSc)	93.68 ± 27.21 µg/dL	$p=0.233$	This study
Iron (Fe)	Sweden	Sclerotic heart valves	1357±481 ng /ml	$p>0.05$	Nyström-Rosander et al., 2004
	Iraq	Obstructive coronary artery disease	113.33±24.15 µg/dl	0.115	Al-Dohan et al., 2015
	Poland	heart failure (HF)	79±44 µg/dL	-	Tkaczyszyn et al., 2018
	Turkey	Aortic valve sclerosis (AVSc)	58.17±26.00 µg/dL	$p=0.059$	**This study**
Selenium (Se)	Sweden	Sclerotic heart valves	99.9±13.8 ng /ml	$p<0.001$	Nyström-Rosander et al., 2004
	Turkey	Rheumatic heart disease	136±11 µg/l	$p<0.05$	Kosar et al., 2005
	Finland	Coronary heart disease	51.8±13.82 µg/L	-	Salonen et al., 1982
	Turkey	Heart failure (HF)	121±5 µg/l	0.000	Kosar et al., 2006
	France	Cardiomyopathy	69±2 µg/L	-	Auzepy et al. 1987
	Finland	Acute Myocardial Infarction (AMI)	48±12 µg/L	-	Westermarck, 1977
	Turkey	Aortic valve sclerosis (AVSc)	60.85±11.63 µg/L	$p=0.807$	**This study**
Zinc (Zn)	Sweden	Sclerotic heart valves	752±216 ng /ml	$p<0.05$	Nyström-Rosander et al., 2004
	Turkey	Rheumatic heart disease (RHD)	0.41±0.16 µg/l	$p<0.001$	Kosar et al., 2005
	Iraq	Obstructive coronary artery disease	56.60±11.68 µg/dl	0.0001	Al-Dohan et al., 2015
	Turkey	Heart failure (HF)	555±104 µg/l	<0.01	Kosar et al., 2006
	Netherlands	Cardiovascular death	0.71±0.19 mg/L	$p>0.05$	Kok et al., 1988
	Turkey	Aortic valve sclerosis (AVSc)	73.11±10.52 µg/dL	$p=0.000$	**This study**

4.4 Discussion

AVSc is a form of aortic valve disorder (Milin et al., 2014), and it's the most popular valve disorder in the advanced nations (Markus et al., 2015). AVSc is defined as thickening and calcification of the aortic valve leaflets **(**Agmon et al., 2001), in the absence of obstruction of ventricular outflow (Khilla et al., 2018). The present study is the first study attempt to evaluate serum peroxynitrite levels and serum trace elements (Zn, Fe, Cu, and Se) in the patients of AVSc compared with the control group. The main findings of the present study indicated that peroxynitrite concentrations were higher in the AVSc, compared with the control group Table 4.4. Similar results were also repeated by Gheddouchi et al., (2015) in which statistically significant effect of peroxynitrite value in patients with ACS acute coronary syndrome (France) than these of non ACS. Among the AVSc patients group, females had higher levels of peroxynitriate than that of male's counterparts. Also, among our patients there were 22 patients were affected newly by AVSc disease, a statistically significant decrease of sera peroxynitrite values was detected between the newly patients who examined their heart for the first time in compared with the patients who had a history with AVSc disease since about (2-10) years Table 4.5. Besides, among the healthy subjects PN had significant negative correlation with Se (p<0.05), and in contrast, PN had significant positive relationship with Sbp (p<0.01) Table 4.7. Under several circumstances, high ONOO− levels demonstrate anti−microbial, anti−parasitic activities, and anti−viral, while low ONOO− levels catalyze protective mechanisms in the respiratory systems, nervous, and cardiovascular, as well as serves as a potential player in the organizing of growth of the cells (Chen et al., 2014). With regard gender, alcohol and smoking, there were no significant difference in the groups (p>0.05) Table 4.2.

With respect to the patients group correlation analysis, no significant correlation between age and the parameters were found except for systolic blood pressure values were had significant positive correlation with age**.** Moreover, a significant positive correlation between patients BMI values and systolic blood pressure values were observed (p<0.01) Table 4.6. In addition, this study indicated that there were significant differences between blood pressure, and diabetes of the valves patients group in comparison with the healthy valves group (p<0.05) Table 4.4. The results of both studies of (Bonapace et al., 2014; Naseem and Samir, 2015), were similar to our

results were they found a statistically significant prevalence of hypertension in the patients with AVSc than those without AVSc. As well as, in a previous study of Al-Dohan et al., (2015), identical values were detected in which significantly higher prevalence of hypertension and diabetes mellitus in CAD obstructive coronary artery disease patients than non CAD patients group (p<0.05).

Deficiencies of trace elements and minerals are widespread and common among the populations (Liu et al., 2016), and lead to nutritional problems (Goldhaber, 2003; Osamu, 2004), also they are in noticeable relation with adverse cardiovascular endpoints (Ekpenyong, 2017). Their presence in excess can lead to obesity (Osamu, 2004), and the resulting toxicity (Goldhaber, 2003).

In the present study we also found among the Turkish population, imbalance in some of the trace elements. For instance, high accumulation of Cu was found in the AVSc subjects, when compared to the normal serum ranges. This increase in serum Cu levels more likely reflects increased or ongoing inflammation process in this disease. Cu values in AVSc was found to be higher in the present study than those of Cu levels in RHD rheumatic heart disease patients (Turkey) (Kosar et al., 2005), and in HF heart failure patients (Turkey) (Kosar et al., 2006), and lower than that of CAD obstructive coronary artery disease patients (Iraq) (Al-Dohan et al., 2015), and in sclerotic heart valves patients (Uppsala-Sweden) (Nyström-Rosander et al., 2004), Table 4.8. The micro-nutrient copper has physiological capacities linked with bone development, heart function, cellular respiration, the processes of keratinization and pigmentation, and myelination of the spinal line (Cortinhas et al., 2012). Excess aggregation of Cu might impact other trace elements metabolisms, such as Fe and Zn (Himoto and Masaki, 2018). Also excessive copper can cause headache, nausea, diarrhea, dizziness, a metallic taste in the mouth, weakness, vomiting, cramps and abdominal pain (Osredkar and Sustar, 2011). Furthermore, our study detected among the patients group a significant inverse correlation between sera Cu concentrations and sera Fe concentration (p<0.01, r=-0.423) Table 4.6. Besides, among the healthy subjects no significant correlation was recorded between the levels of Cu, Fe, and BMI with the parameters Table 4.7.

Our study also recorded low in Fe values in the AVSc group compared with the control subjects but not statically significant (p>0.05) Table 4.4. Fe levels in AVSc

was found to be lower in the present study than those of Fe values in the sclerotic heart valves patients (Uppsala-Sweden) (Nyström-Rosander et al., 2004), in HF heart failure patients (Poland) (Tkaczyszyn et al., 2018), and in CAD obstructive coronary artery disease patients (Iraq) (Al-Dohan et al., 2015), sequentially Table 4.8. In spite of being existent in trace quantities (Muñoz-Bravo et al., 2013), the essential trace element iron required for growth and survival of roughly all organism (Valko et al., 2005), and plays a role in the host defense mechanisms in our bodies (Ganz, 2003). Iron deficiency (ID) leads to a complication of chronic disorders (e.g. Parkinson's disease, inflammatory bowel disorder, chronic renal failure, rheumatoid disease), irrespective of concomitant anemia (Kell, 2009; Baker and Ghio, 2009; Jankowska et al., 2012). ID is familiar to be linked with some CVDs involving heart failure, CAD, and pulmonary arterial hypertension. Following iron supplementation, betterment in these disorders has been registered, proving the hypothesis that ID is a popular trouble in cardiovascular patients (Ekpenyong, 2017). Further, a statistically significant elevation in serum Fe concentrations was observed between patients with a recent history of AVSc disease and others with AVSc disease history since about (2-10) Table 4.5. In addition, a significant positive correlation was found between Fe and Se among the patients group Table 4.6.

Moreover, the present study reported lower levels in sera of Se between the patients group as compared with healthy group (p>0.05) Table 4.4. A similar pattern of decrease was found in the studies results of (Westermarck, 1977; Salonen et al., 1982; Auzepy et al. 1987), were they observed lower sera Se values among AMI acute myocardial infarction patients (Finland), CHD coronary heart disease patients (Finland), and cardiomyopathy patients (France) respectively, in comparison with normal subjects. Whereas, the results of (Nyström-Rosander et al., 2004), were not agreement with our data were they demonstrated a significant increase in sera Se values in the patient's blood of sclerosis heart valves (Uppsala-Sweden) as compared with normal valves plasma Table 4.8. Se is a trace mineral with both enzymic and structural roles that are fundamental for normal physiology (Cooper et al., 2007). Se deficiency has been linked with thyroid disease, infertility and adverse reproductive outcomes (Fayet-Moore et al., 2014), cancer and different heart diseases (Tinggi, 2008), including Chagas (Nyström-Rosander et al., 2004), and Keshan disease

(Chen, 2012), (a cardiomyopathy), as well as it can lead to Kaschin-Beck disease, an osteoarthropathy (Brown, 1994).

The present study also indicated that there was a statistically significant decrease (p<0.05) in sera Zn concentrations in AVSc patients than those of the control group Table 4.4. This agreed with results of the studies of (Nyström-Rosander et al., 2004; Kosar et al., 2005; Kosar et al., 2006; Al-Dohan et al., 2015), in which sera Zn concentrations was significant decreased in the sclerotic heart valves patients (Uppsala- Sweden), in RHD rheumatic heart disease patients (Turkey), in HF heart failure patients (Turkey), and in the CAD coronary artery disease patients (Iraq) respectively, compared to healthy donors Table 4.8. This can indicate the association of Zn deficiency with AVSc disease. And zinc deficiency can be linked with acrodermatitis enteropathica, malabsorption, malignancy, sickle cell disorder, chronic renal disorder (Osredkar and Sustar, 2011), type 2 diabetes mellitus (DM), chronic liver disorder (Himoto and Masaki, 2018), cardiovascular disorder (Ekpenyong, 2017), and Parkinson's and Alzheimer's disease patients (Brewer et al., 2010). Zn is a fundamental trace element that desired for the catalytic activity of more than 200 enzymes, also it plays pivotal roles in wound healing, immune function, synthesis of DNA and protein (Osredkar and Sustar, 2011), as well as division of the cell (Wessels et al., 2017). Additionally, our study demonstrated in the control group correlation analysis, that Zn had significant positive correlation with Fe, Sbp, and PN Table 4.7. Changes in blood Zn and Cu values might be indicative of a low-grade infectious/ inflammatory process, as well as any condition linked with increased oxidative stress or inflammation may be expected to decrease Zn and Se values. Because AVSc is an inflammatory state, it is not surprising to observe low Zn and Se values in our patients. In other words, we can speculate that the alterations in the trace element levels of these patients might be the result of either the ongoing inflammatory process or the inadequate dietary intake of trace element. Trace elements and peroxynitrire concentrations in various diseases are given in Table 4.8. Respecting both of sera Cu and Zn concentrations in death case with cardiovascular disease patients, the study of (Kok et al., 1988), in (Netherlands) showed slightly increase in these parameters in death case with cardiovascular disease patients in comparison with their matched controls.

CHAPTER 5

CONCLUSION

The present study indicated first time that patients with AVSc had two fold peroxynitrite levels in their sera as compared with the healthy donors. Accumulation in trace elements in sera was evaluated. Patients with AVSc had high prevalence of obesity, diabetes, and hypertension. So we recommend the patients with AVSc to loss their weight by a daily sport and consume a healthy food to arrange their diabetes and hypertension levels in order to prevent their disease progress. Also, regularly measuring serum peroxynitrite, Fe, Cu, Se, and Zn of AVSc patients will be beneficial to prevent any risk. According to results of our study AVSc patients may take iron, selenium, and zinc rich foods such as sea foods [crab and lobster], red meat [beef, lamb], liver, and nuts daily which will benefit to provide recommended intakes of Fe, Se, and Zn and preserve normal storage levels of these minerals. Furthermore, patients who need supplementation must be considered. In contrast, AVSc patients must reduce the foods which are rich of Cu such as sunflower seeds, dark green leafy vegetables, chocolate, and dried legumes. This is the first attempt that indicating that serum peroxynitrite levels were markedly evaluated in patients with AVSc and is a helpful diagnostic indicator.

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