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CLINICAL STUDY FOR OXIDATIVE STRESS IN PATIENTS WITH CARDIAC ARRHYTHMIA

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SUMMARY

Cardiac arrhythmia is a disturbance heart rhythm. Most types of arrhythmia are not serious, while there are some that types can be life threatening promptly because it impacts the pumping action of the heart substantially, enough to disrupt blood supply to the body, potentially leading to sudden cardiac death.

The present study is designed to determine the levels of (MDA) (malondialdehyde) as a marker for lipid peroxidation and measurement some parameters of antioxidants including: ceruloplasmin (Cp), transferrin (Tf) and albumin (Alb), in patients of cardiac arrhythmia. The study includes (110) subjects which were divided into five groups: (20) patients of (AF), (17) Patients of (SVT), (20) patients of (VT), (18) patients of (PVCs) and (35) clinically healthy subjects, who were attend blood bank to blood donation as control groups. The patients who were underwent cardiac arrhythmia .The study aims to shed a light on the expected association between arrhythmia and oxidative stress. The results show that there were a significant increases in serum concentrations of each of (MDA) and (CP) in all of patients groups in comparison with control group ($P \leq 0.05$), also there was a significant increase in concentrations of MDA of patients of VT compared with each of AF, SVT, PVCs ($P \leq 0.05$) while there was slightly significant increase between each of AF and SVT compared with PVCs($P \leq 0.05$). In addition, the results showed a significant decreases ($P \leq 0.05$) in concentrations of serum of (Tf and Alb) in all patients groups in comparison with (control), also there were a significant differences($P \leq 0.05$) between each of SVT, AF and VT compared to PVCs($P \leq 0.05$). On the other hand Pearson's correlation coefficient analysis showed a negative correlation between MDA level and each antioxidant parameter (Tf and Alb) and positive correlation between MDA level and (CP). The study suggests that oxidative stress have an important role in the pathogenesismof cardiac arrhythmia and vice versa.

INTRODUCTION

Cardiac arrhythmia is a disturbance heart rhythm result from abnormalities of impulse initiation, impulse conduction, or both.[1] Arrhythmias are classified by rate as either tachycardia (heart rate > 100/min) or bradycardias (heart rate < 60/min).[2] In AL- Hussein Teaching Hospital in Thi Qar province, the number of patients cardiac arrhythmia who were hospitalized in 2015 about 400 patients, among them 223 patients with atrial fibrillation.

The increased level of ROS associated with all major clinical risk factors for arrhythmia is indirect evidence that oxidative stress may be important in the genesis of both atrial and ventricular arrhythmias.[3] Despite the high prevalence and significance of arrhythmias, the mechanisms of Arrhythmogenesis are not fully understood. Some molecular mechanisms known to contribute to arrhythmias include genetic alterations of ion channels leading to electrophysiological dysregulations and structural remodeling of the left ventricle (LV) in hypertrophy and HF.[4, 5,6] Increasing evidence suggests that altered cardiac ion homeostasis and structural remodeling are highly associated with elevated reactive oxygen species (ROS).[7,8]

Oxidative stress can be impair the ability of the endothelium, the inner layer of cells that line the blood vessels, to expand and dilate in response to blood flow, based on this scenario, an accumulation of reactive oxygen species has been linked to cardiac contractile dysfunction, potentially leading to arrhythmia and heart attack.[9] Lipid peroxidation resulting from free radical activation results in the release of products, such as arachidonic acid, into the extracellular space. The involvement of free radicals here is represented by the free radical-induced release of arachidonic acid and subsequent formation of prostaglandins and end peroxides, may be further aggravate the injury process or generate arrhythmias.[10] There is also evidence indicating that arrhythmias of the spontaneously contracting atrium may arise during induction of lipid peroxidation. The decrease in resistance of the atria to the lipid peroxidation inducer associated with an increase in their developed tension can very probably be

explained on the grounds that an increase in the oxygen demand of the contracting muscle leads to activation of lipid peroxidation in it. In turn, activation of lipid peroxidation causes a marked arrhythmogenic effect.[11]

There are data that suggest that sodium channel defect exciteed by lipid peroxidation is a candidate mechanism for ischemia-related conduction abnormalities and arrhythmias.[12] Because cardiac ion channels are embedded in the membrane lipid bilayer, Fukuda et al hypothesized that lipid peroxidation may alter the function of these channel proteins. They reasoned that this could occur either as a result of structural alterations of the lipid bilayer by oxidative modification of membrane lipids or by the abduction of channel proteins by electrophilic short chain aldehydes produced by lipid peroxidation.[12] In this regard, oxidative stress has been found to induce alterations in the function of a number of membrane proteins, including ion channels, enzymes and receptors.[13, 14, 15]

The preventative antioxidants (sequestration of metal ions)- these which prevent the participation of transition metal ions in FRs generation such as transferrin (**Tf**), ferritin, albumin (Alb)[17] and ceruloplasmin–Cu containing has ferroxidase activity "prevents Fe^{2+} from reacting with H_2O_2. The antioxidant property of transferrin is its ability to bind with iron ions and storage it, as a ferritin and prevent the oxidative role of iron which allows generating free radicals by Fenton and Haber-Weiss reactions.[18] Furthermore transferrin, an iron binding protein, transports ferric (Fe^{+3}) ion and stores it as ferritin. Specific cell surface receptors for transferrin facilitate and regulate cellular iron uptake.[19]

Albumin is well known for its ability to bind ions such as copper and iron, they are very effective to generate ROS after a reaction with oxygen. Free Fe(II) and Cu(II) ions can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. the effectiveness of the Cp as an antioxidation depends on the level of Cu^{+2} in protein, because the Cp works to remove $O_2^{\cdot-}$ the root of superoxide negative through reduced copper atom in the protein.

Design of Study

This study is conducted on a group of patients with arrhythmia at AL-Hussein Teaching Hospital in Thi-Qar, at the period between 6/10/2014 to 29/3/2015. They included (110) subjects, control (35) and patients of arrhythmia (75).

Who were divided as the fallowing groups:-

AF groups: 20 patients with atrial fibrillation (AF) [7 males and 13 females] with age range (42—70).

SVT groups: 17 patients with supraventricular tachycardia (SVT) [8 males and 9 females] with age range (35—65).

VT groups: 20 patients with ventricular tachycardia (VT) [9 males and 11 females] with age range (40—70).

PVCs groups: 18 patients with premature ventricular contraction (PVCs) [8 males and 10 females] with age range (40—70).

***Control groups*:** control group, consist of 35 supposed healthy subjects [16 males and 19 females] with no history of systematic illness at age range (40 -65).

Biochemical Parameters Lipid peroxidation Marker (Serum MDA)

Determination of serum MDA level that consider as a lipid peroxidation marker were performed according to the method of Fong.[20] MDA concentrations were calculated, using the molar extinction coefficient of MDA (ε MDA) equal to 1.56 x105 mol-1. Cm-1[21] MDA formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

Serum Cp concentration was measured by the method of[22] which using the extinction coefficient of Cp (ε Cp) equal to (0.68) to calculate it concentration.

The simplest technique is the bromcresol green (BCG) method, colorimetric method. The principle of measurement is based on the relatively high affinity of BCG towards Alb. Albumin in the presence of BCG at a slightly acid pH = 4.2, produces a color change of the indicator from yellow green to green-blue. The intensity of the color formed is proportional to the Alb concentration in the sample.

The iron-binding protein transfesrring, in serum is saturated upon treatment with an axcess of Fe (iii) ions. Unbound (excess) iron is absorbed onto aluminium oxide and precipitated. The trasferrin-bound iron (TIBC) in the supernatant is then determined.[24, 25]

Statistical Analysis

Statistical analysis was done using Microsoft Excel 2010 the results were expressed as mean ± standard deviation (mean ± SD). One way ANOVA was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant. Correlation analysis was calculated using Pearson's correlation coefficient.

RESULT AND DISCUSSION

Lipid Peroxidation Status (Malondialdehyde)

Table (1) show a significant increases in serum MDA levels for each of the (VT), (AF), (SVT) and (PVCs) patients groups' compared with control group ($P \leq 0.05$), also a significant increases in the mentioned parameter levels can be observed in (VT) patients group when they are compared to AF, SVT and PVCs groups ($P \leq 0.05$).

In this study for patients of arrhythmia the level of MDA was significantly higher than those who had no history

with arrhythmia. This hypothesis is comported with.[26,27] It is well known that ischemic heart disease widely accompany with VT.[28] This is clear also in this study, ischemia is characterized by ionic and biochemical alterations capable of initiating and sustaining arrhythmias, in turn VT increases myocardial oxygen demand, which may lead to aggravation of ischemia.[29] Moreover, Probably the most important factor influencing the severity of reperfusion-induced arrhythmias is the duration of the previous period of ischemia whereas extending the duration of ischemia, led to increased incidence of ventricular arrhythmia.[30] lipid peroxidation marker (MDA) is higher in patients with IHD.[31] This may due to the increasing of oxidative stress, may be the main reason to increase the levels of MDA in patients of (VT) compared with all groups of study. It is clear presence of significant increase in each of AF and SVT Compared with PVCs group ($P \leq 0.05$). So far it is not clear whether oxidative stress is a primary pathogenetic event or a consequence of AF. However, there is a possibility that both processes feeding each other leading to a vicious cycle.[32] Other putative pathogenetic mechanisms that may promote oxidative stress in AF include heart failure, it is an important risk factor for AF, development of heart failure, AF can lead to HF and vice versa.[33] It seems logical that oxidative stress represents a common pathophysiologic link between the two conditions.[34] In patients of (SVT), as it is well known, they are not usually associated with structural heart disease[35], also PVCs that thought to be relatively benign in the absence of structural heart disease .Though there is an increase in the level of (MDA), remained significantly in persons without heart disease, this refers to other mechanisms than cardiac structural problems, Oxidative stress may also play critical roles in the initiation and perpetuation of atrial arrhythmias.[36] It is worth mentioning that chronic exposure of the heart to oxidative stress produces a variety of electrophysiological abnormalities, increased susceptibility to cardiac arrhythmia.[37] The study suggests that oxidative stress have an important role in the pathogenesismof cardiac arrhythmia and vice versa.

Table (1): Serum Malondialdehyd levels for Control and Patients groups

Groups	N	MDA (µmol/l)mean ± SD
CONT	35	**1.47^{d} ± 0.21**
AF	20	**3.27^{b} ± 0.48**
SVT	17	**3.22^{b} ± 0.39**
VT	20	**3.70^{a} ± 0.52**
PVCs	18	**2.91^{c} ± 0.23**
LSD		**0.16**

Serum Ceruloplasmin Concentration(Cp)

Table (2) show a significant elevation in serum Cp levels in all patient groups in comparison with control group ($P \leq 0.05$), these result agree with previous study like.[38] While no significant differences in Cp levels that can be observed among (VT), (AF), (SVT) and (PVCs) groups ($P \leq 0.05$). it is well noted increase ceruloplasmin levels with increase (MDA) concentration in all groups of arrhythmia, accordingly, there is a positive correlation between (MDA) and ceruloplasmin levels in each of AF (r=0.5), SVT(r=0.17),VT(r=0.36) and PVCs(r=0.8), these result agree with.[39] The antioxidant activity of CP can be ascribed mainly to its ferroxidase activity, which inhibits ferrous ion-stimulated lipid peroxidation and the formation of hydroxyl radicals in the Fenton reaction, and is also a scavenger of ROS.[40] Thus it prevents the production of MDA.[41] The presence of high concentrations of serum ceruloplasmin for patients of arrhythmia, can lead to suggestion that this protein has the anti-arrhythmia effects, This probably due to that ceruloplasmin molecule can have an antioxidant property against oxidative stress which are associated with arrhythmia, in animal study Atanasiu *et al* suggested that ceruloplasmin act as anti-arrhythmia effects especially against reperfusion-induced arrhythmias. Moreover, they observed that the antiarrhythmic action of cemloplasmin was not related to changes in hemodynamics thus suggesting a direct effect of ceruloplasmin on the myocardium, addition to that, the antiarrhythmic action of ceruloplasmin in oxidative stress conditions is mostly related to its oxygen free radical scavenging ability, directly or acting as a ferroxidase.[41] On the other hand Ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation.[42] Accordingly ceruloplasmin may be considered as an inflammatory molecule. Ceruloplasmin is an inflammation-sensitive protein and an acute phase reactant which rises in inflammatory diseases.[43] It has been suggested that raised levels of ceruloplasmin as inflammatory marker may not be a reflection of the arrhythmia itself but a result of heart disease that associated with it.[44] Therefore could be a possible association between increase of ceruloplasmin level and incidence of arrhythmia.

Table (2):- Serum Ceruloplasmin level for Control and patients groups

Groups	N	Cp Levels (g/l) mean±SD
CONT	35	**3.12[b]±0.52**
AF	20	**4.69[a]±0.78**
SVT	17	**4.67[a]±0.31**
VT	20	**4.82[a]±0.73**
PVCs	18	**4.78[a]±0.74**
LSD		**0.26**

Serum Transferrin Concentration

The results in table (3) show a significant decrease in serum Tf concentration in all patient groups when compared with control groups (P≤0.05). The result also show a significant decrease for each of patient groups (AF), (SVT) and (VT) groups compared with patients of (PVCs) (P≤0.05) and a significant difference in serum Tf concentration between patients group of (VT) and each of patient groups (AF) and (SVT). It is well clear that concentrations of (Tf) are low, when oxidative stress increased. Since the measurement of (MDA) in this study refer to the increased oxidative stress, especially with patients of (VT), followed by patients of (AF), (SVT) and (PVCs). Consequently there is negative correlation between (MDA) and (Tf) levels for each AF(r= -0.42), SVT(r= -0.11), VT(r= -0.51) and PVCs(r= -0.07) these result agree with previous study like.[39] which explained, at low transferrin concentration, free iron does not found transferrin to bind it for transport him, therefore the free iron cause oxidation and generation of more free radicals. Van Campenhout *et al* reported that low levels of Tf can enhance the pro oxidative effects of iron, They argue that these effects are most important causes underlying lipid peroxidation and increase the risk of CVD.[45] However the low plasma transferrin concentration found in humans with increased iron stores may be due to a negative feedback of storage iron levels on transferrin synthesis.[46] Low Tf concentration in patients with arrhythmia may be due to, that Tf is one of protective antioxidants that prevent the formation of free radicals. It binds to iron and prevent it from interacting with the H_2O_2 to form free radicals, as well as Tf works to remove O_{2^-}, the root of superoxide negative. The Tf works to reduce oxidative stress, that occurs in patients with arrhythmia and at fall its level in the blood serum. Transferrin is one of non- enzymatic that limits the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/antioxidative balance shifts to the oxidative side in patients with arrhythmia. One of reasons for increased lipid peroxidation in patients of arrhythmia that found in this study it may be poor non-enzymatic antioxidant defense system including transferrin.

Table (3):- Serum Transferrin level for Control and patients groups

Groups	N	Tf concentration (g/l)Mean ± SD
CONT	35	**3.28[a] ± 0.35**
AF	20	**2.69[c] ± 0.27**
SVT	17	**2.76[c] ± 0.40**
VT	20	**2.53[d] ± 0.41**
PVCs	18	**2.94[b] ± 0.17**
LSD		**0.13**

Serum Albumin Concentrations

The results in table (4) show a significant decrease in serum albumin concentration in each of patient groups when compared with control groups (P≤0.05).the results also showed a significant decrease between each patient group of (AF), (SVT) and (VT) groups with (PVCs) (P≤0.05). in Addition, There are negative correlations between (MDA) and (Alb) levels in each of AF(r=-0.60), SVT(r=-0.58), VT(r=-0.66) and PVCs(r=-0.30). Low levels of albumin for patients of arrhythmia are associated with the increase in the concentration of MDA, this indicates that albumin behaves as antioxidant, This is supported by (47) who suggested that decrease in the levels of this antioxidant accelerate the lipid peroxidation, therefore generating more MDA. Proteins may be damaged by specific interactions of oxidants or free radicals with particularly susceptible amino acids. Low serum albumin has been shown to be associated with a higher risk of total cardiovascular disease.[48] Previous study found a depletion of total antioxidant status in coronary artery disease (CAD) patients.[49]

As it is well known that highly reactive agents cause structural and functional changes of the albumin molecule, leading to the reduction of its antioxidant properties and predisposing to its aggregation and deposition in tissues and organ (50). Moreover increase ROS lead to reduction of albumin ability to bind endogenous and exogenous ions. Modification of its N-terminal amino acids (Asp-Ala- His) leads to decreased transition metal such as (Cu+2) binding capacity of the albumin molecule.[51] Another effect of ROS action is oxidation of sulfhydryl groups (SH) in albumin and their depletion in blood, which subsequently exacerbates

functional and structural disturbances of attacked macromolecules.[52] However, damaged albumin molecules was reported to be rapidly removed from circulation and degraded.[53] Bound to proteins, iron and copper and are generally less susceptible to participate in the Fenton reaction. In plasma, most of the copper is bound to caeruloplasmin, but a high percentage of the metal ion may exist bounded to albumin.[54] ROS are able to react with most biomolecules, but Albumin because of its abundance in the blood is affected to the highest degree.[55]

Table (4):- Serum albumin level for Control and patients groups

Groups	N	Alb (g/dl) Mean ± SD
CONT	35	**4.68[a] ± 0.21**
AF	20	**4.02[c]± 0.48**
SVT	17	**4.04[c] ± 0.27**
VT	20	**4.01[c] ± 0.27**
PVCs	18	**4.22[b]± 0. 12**
LSD		**0.1**

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