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Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Journal of Medical Laboratory and Diagnosis

Full Length Research Paper

Prevalence of zinc-α 2-glycoprotein binding peptide among Omani blood donors

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Zinc alpha-2-glycoprotien (ZAG) binding peptide is a multi-functional protein, which is structurally similar to a major histocompatibility complex class I. It has been discovered as a novel adipokine enhancing lipolysis and influencing other physiological processes such as sperm mobility and melanin production. Furthermore, ZAG level has been correlated to a variety of diseases such as atherosclerosis and diabetes type II with a potential use as a tumor biomarker in future. In this study, we aim to investigate the prevalence of ZAG among healthy blood donors attending to the Sultan Qaboos University Hospital blood bank and correlate it with their age and sex. The ZAG levels analysis of the sera from 106 (49 females and 57 males) apparently healthy donors from different regions was carried out using a competitive type of enzyme-linked immunosorbent assay (ELISA) (Abnova GmbH-Germany). Analysis was mainly based on two parameters; age and sex. Out of the 106 subjects, 78% of blood donors have high ZAG levels (>35 ng/ml), 13% have a normal level (20 to 35 ng/ml) while 9% have a level lower than 20 ng/ml. A significant association was found between ZAG level and sex (*P* = 0.012) with males showing low levels. Although high ZAG level was correlated between age and ZAG levels in the female group, higher levels were also found in donors below and above 22 years old (*P* = 0.0099). The prevalence of ZAG levels in blood donors was found to be high, especially in those between 20 to 30 years old. This emphasizes the measurement of ZAG level prior to blood transfusion to patient(s) who are clinical under weight. Gender and age significantly influences the plasma level of ZAG.

Key words: Zinc alpha-2-glycoprotien (ZAG), Oman, Sultan Qaboos University Hospital (SQUH), blood donation.

INTRODUCTION

Zinc-α-2-glycoprotein (ZAG) binding peptide is a 43 kDa soluble protein which plays multiple roles in human (Stejskal et al., 2008). The ZAG binding peptide normally produced by the epithelial cells in several tissues including the liver, adipose tissue, sweat glands, breast and the gastrointestinal tract, so that it can be found in various body fluids such as the plasma, semen, sweat, milk and the cerebrospinal fluid (Hassan et al., 2008a). The plasma concentration of ZAG binding peptide is affected by several factors including the body weight and the health status but a range between 20 to 35 ng/ml is considered normal.

Its structure has been found to be similar to the major histocompatibility complex (MHC) Class I (Stejskal et al., 2008). Both molecules share the basic three alpha chains (α1, α2, α3) structure in the same arrangement with the binding groove formed between α1 and α2. However, α3 chain in ZAG binding peptide molecule does not bind to

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an additional β2 microglobulin (β2M) like in the case of MHC class I. It does instead bind to a zinc molecule in the same way of metalloprotease enzyme proteins. The second major difference is the presence of a hydrophobic molecule - polyethylene glycol in the binding groove of ZAG binding peptide protein (Hassan et al., 2008b). While MHC class I can only bind to peptides, this specific feature gives ZAG binding peptide the ability to bind to different types of ligands including fatty acids.

ZAG binding peptide was first discovered by Burgi and Schmid in 1961 (Zhu et al., 2012). Yet, its function remained unclear. Recent studies showed that ZAG binding peptide has multi-functions due to its high distribution and can be used as a marker for several diseases.

The discovery of ZAG binding peptide as a novel adipokine has a major role in understanding the effect of this protein in the normal and pathological health conditions. Researchers linked the high levels of ZAG binding peptide in the plasma to high rates of lipolysis (Hassan et al., 2008b). It was found that the level of ZAG binding peptide is directly proportional to the level of cholesterol and fatty acids both in the human body and animal models (Gong et al., 2010). Therefore, ZAG binding peptide is considered to be a key player in obesity and obesity-linked diseases especially type 2 diabetes (Olofsson et al., 2010). It is suggested that it will be used as a future treatment of obesity (Choi et al., 2012). On the other hand, ZAG binding peptide level has a high correlation to the fat and muscle wasting condition termed cachexia which cancer patient suffer from (Rolli et al., 2007).

The exact mechanism by which ZAG plays its function through is still unclear. Researchers suggest that it works through β3- adrenoceptors leading to an increase in the concentration of cyclic adenosine monophosphate (cAMP), which enhances lipolysis (Russell and Tisdale, 2012). Moreover, ZAG binding peptide expression is found to be primarily controlled by another adipokine, which is the tumor necrosis factor (TNF-α) (Bao et al., 2005). In addition to that the semen level of ZAG binding peptide, it is reported to be 6 folds higher than the plasma level (Hassan et al., 2008b). The effect of ZAG binding peptide in fertilization is focused in the role it plays in sperm motility, as sperms are independent on the metabolism of lipid to initiate and maintain the motility of their flagella. Moreover, ZAG binding peptide has showed an ability to bind to the sperm membrane and significantly increasing the cAMP concentration (Fei Qu, 2007). The cAMP-dependent phosphorylation is counted as one of the most important pathways for regulating sperm motility.

This protein, ZAG binding peptide, is normally produced by keratinocytes in the epidermis layer of the skin. A very low level of ZAG binding peptide is required for the production of melanin (Hassan et al., 2008b). However, researchers found that ZAG binding peptide generally has an inhibitory effect in melanin production. The primarily mechanism is through decreasing the level of tyrosinase enzyme which plays a key role in the process of melanin production (Hale, 2002). Other studies suggest indirect mechanisms through TNF-α, which is also known to decrease this process (Hassan et al., 2008b).

One of the recent discoveries of ZAG binding peptide properties indicates its potential usage as an early biomarker for more than one type of cancer. Although studies showed that ZAG can suppress the proliferation of malignant cells by activating apoptosis (Hassan et al., 2008b), researches showed that a number of tumors especially, breast and prostate cancer produce ZAG in the early stages. About 40 to 50% of breast cancers show high levels of ZAG binding peptide while up to 73% of prostate cancers are ZAG-producing tumors (Bondar et al. 2007). However, in both types the production of ZAG binding peptide is inversely related to the differentiation of the tumor, as poorly differentiated tumors showed very low levels or absence of ZAG production (Hale et al., 2001).

The multi-functions of ZAG binding peptide are still under study worldwide. The high levels of ZAG and its links to different diseases and conditions such as metabolic syndromes (Stejskal et al., 2008), chronic hemodialysis (Philipp et al. 2011; Leal et al., 2012), insulin resistance (Ceperuelo-Mallafré et al., 2009) and cardiovascular diseases (Tedeschi et al., 2012), suggested that ZAG binding peptide level will be a good candidate to be involved in a variety of diagnostic procedures and as well as it may be considered also to be used therapeutically.

Despite the global interest in this unique and significantly useful protein, no research has been published neither in Oman nor in the Middle East region. To the best of our knowledge, this study is possibly the first in the region to investigate the prevalence of ZAG binding peptide in Omani blood donors. For the first time, this study aims to correlate the levels of ZAG binding peptide to the gender and age.

MATERIALS AND METHODS

The study was carried out at the Immunology Unit, Department of Microbiology and Immunology, Sultan Qaboos University (SQU), College of Medicine and Health Sciences. Subjects of this research included 106 healthy blood donors who attended to the Sultan Qaboos University Hospital (SQUH) blood bank from different regions in Oman. The group comprised of 98 males and 49 females and 57 male with an age range of 18 to 57 years. The majority (68%) of subjects' age was between 20 to 30 years old (median 22 years). Analysis of ZAG binding peptide levels was carried out using a competitive enzyme-linked immunosorbent assay (ELISA) (Abnova GmbH-Heidelberg, Germany). The Kit (KA1689 kit detects ZAG 278aa) is an *in vitro* assay for detecting ZAG binding peptide based on the principle of competitive enzyme immunoassay. The micro-plate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-ZAG antibody, both biotinylated ZAG peptide and peptide standard or targeted peptide in sera samples interacts competitively with the

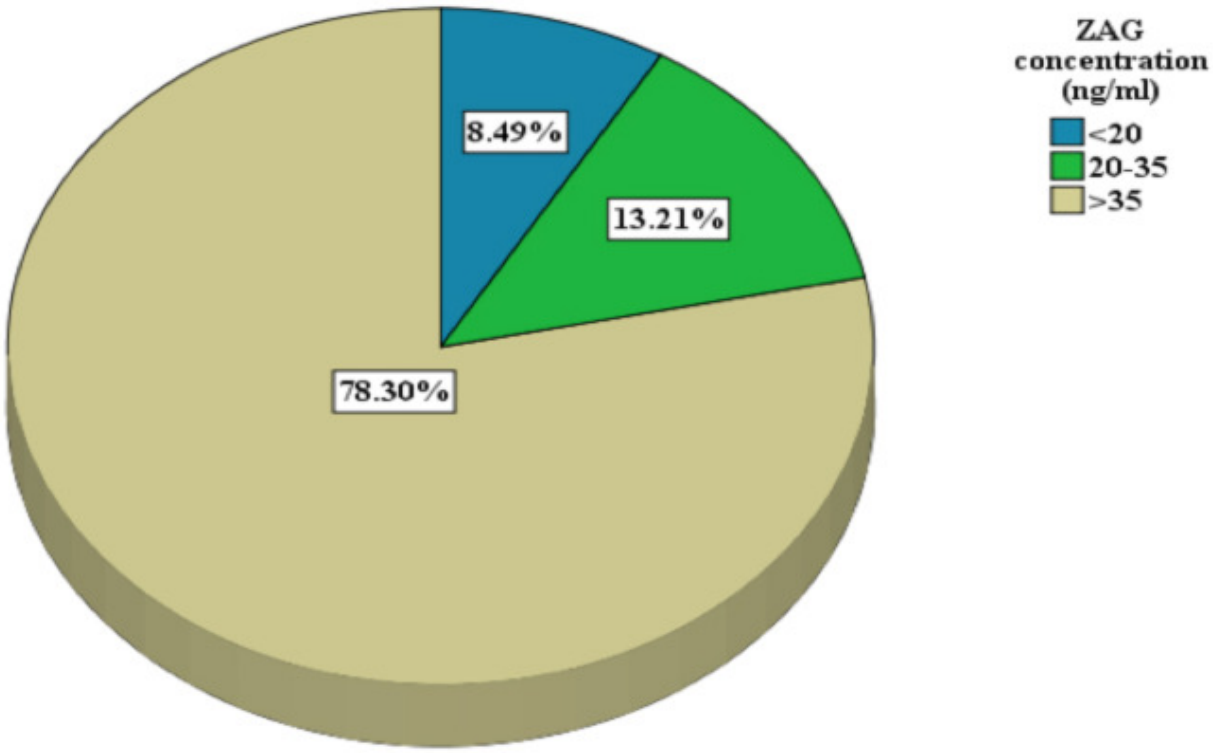

Figure 1. The distribution of ZAG level among 106 Omani blood donors attending SQUH, classified into low (<20 ng/ml), normal (20 to 35 ng/ml) and high (>35 ng/ml).

ZAG antibody.

Uncompeted (bound) biotinylated ZAG peptide then interacts with streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SAHRP complex and inversely proportional to the amount of ZAG peptide in the standard or samples. This is due to the competitive binding to ZAG antibody between biotinylated ZAG peptide and peptides in standard or samples. The concentration of ZAG peptide in the samples was calculated accordingly using a standard curve of known concentration of ZAG binding peptide. Chi-square test was used to test for statistically significant associations between the level of ZAG binding peptide and the subjects' age and sex. The presence of the significant correlations was assessed using Spearman test. The results data analyzed here was determined using IBM statistical package for social sciences (SPSS) Statistics software.

RESULTS

The general distribution of ZAG binding peptide levels based on normal, low or high, among the 106 Omani blood donors included in this study is demonstrated in Figure 1. Surprisingly, it clearly states that the majority of donors (78%) were found to have high ZAG binding peptide levels, that is > 35 ng/ml (mean 177.53 ng/ml), while only a percentage of 13% showed ZAG binding peptide levels within the normal range of 20 to 35 ng/ml mean 29.35 SD ± 4.75), which leaves around 9% who had ZAG binding peptide level of less than 20 ng/ml (mean 9.11 SD± 4.56).

In order to associate the ZAG binding peptide level to the gender of the donors additional subdivisions are illustrated in Figure 2. A significant correlation between the age and the levels of ZAG binding peptide was found only in female (ρ = 0.5; p = *0.0003*) as illustrated in Figure 3. Although both gender showed a close percentage of high ZAG binding peptide level, a significant association was found between the normal and low ZAG binding peptide level with the gender. Out of the 106 donors, 20.4% of female donors were within the normal range of ZAG binding peptide level, while this percentage fell to 7% in male participants. On the other hand, the female percentage of low ZAG binding peptide level was only 2% in comparison to male where it raised to 14%. Chi-square test showed a significant association between ZAG level and sex (p = 0.012) with 99.9% level of confidence. Although ZAG binding peptide level was correlated with age in the female group as shown in Figure 3, higher levels was also found in donors below and above 22 years old (p = 0.0099) (Figure 4). As stated before, the majority of donors lie in the category of high ZAG binding peptide level (>35 ng/ml). Furthermore, the histogram (Figure 5) clearly shows that in this category more than half of the donors (~56%) are between 20 to 30 years old. Yet, correlation was statistically not significant (p = 0.08).

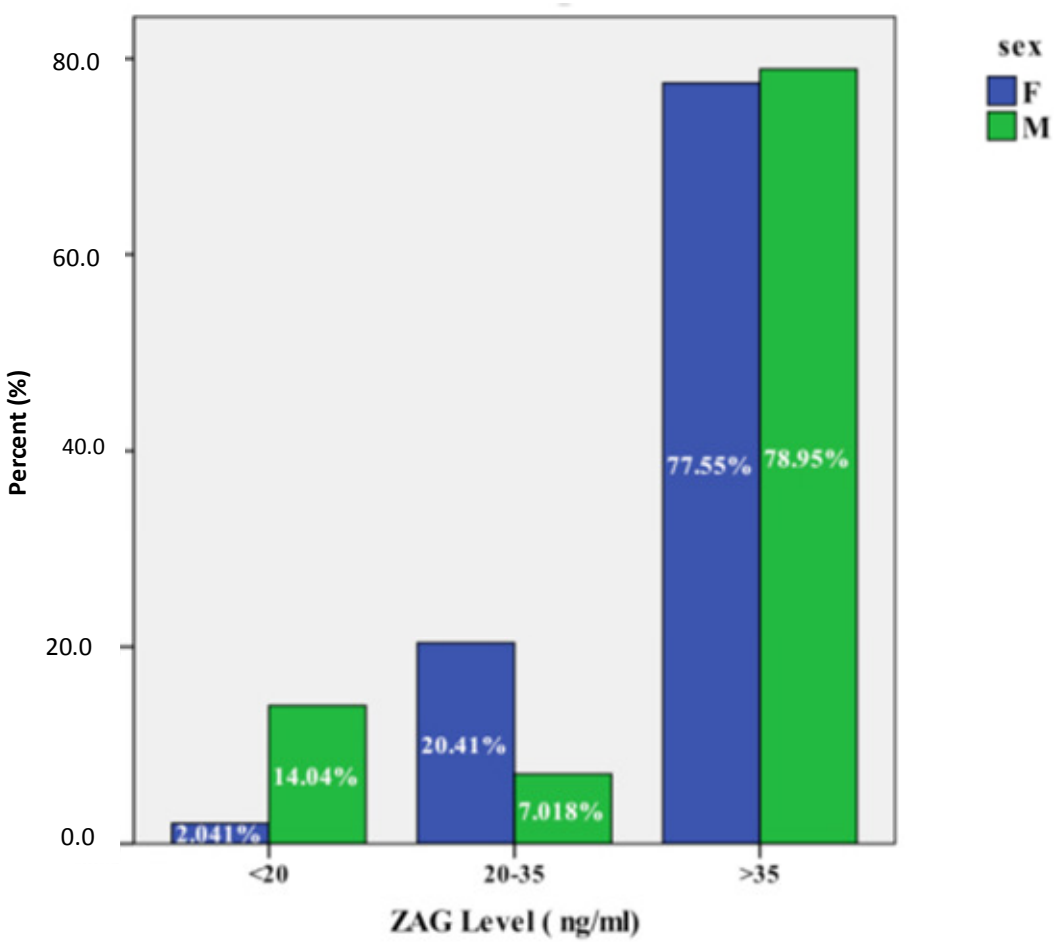

Figure 2. The distribution of ZAG level between 106 Omani males and females into low (<20 ng/ml), normal (20-35 ng/ml) and high (>35 ng/ml).

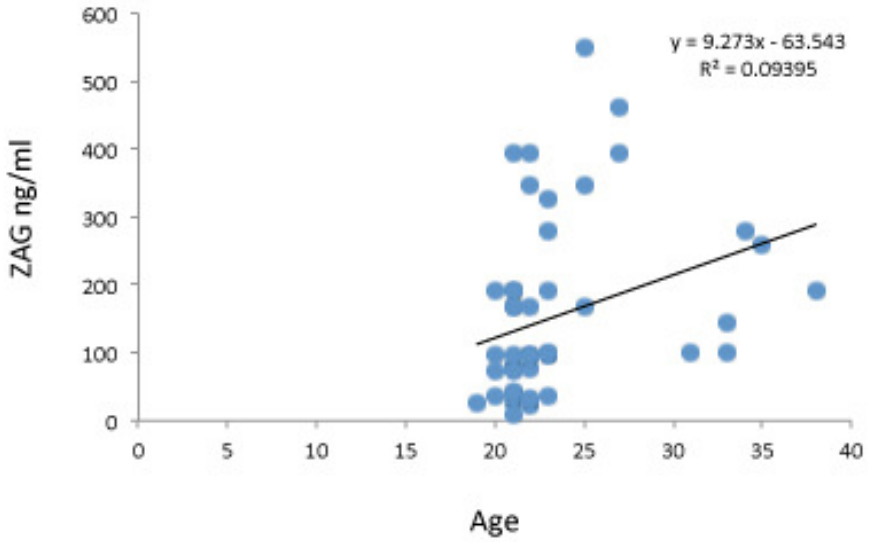

Figure 3. Showed a correlation of the levels of ZAG level among the female group with different ages. A significant association was found between the normal and low ZAG binding peptide level within the female group (R^2 = 0.09395).

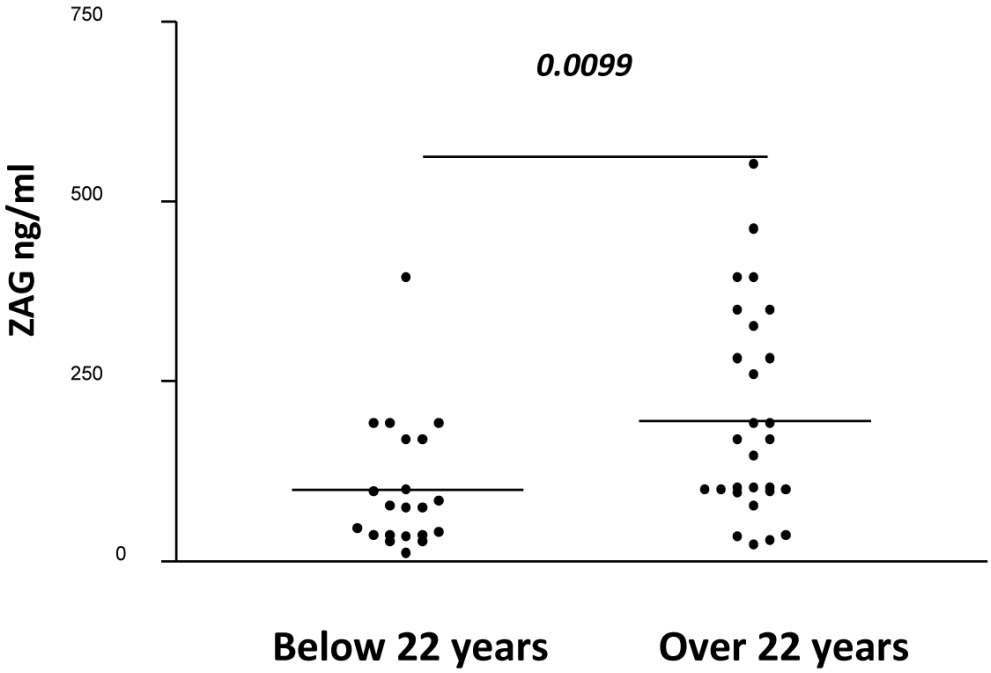

Figure 4. The prevalence of high, normal and low ZAG levels in relation to age among 106 Omani blood donors. The histogram has shown a significant association between the level of ZAG binding peptide and the donors' sex as both sex groups showed a very close percentage of high ZAG level with a significant variation within the low and normal levels of ZAG binding peptide.

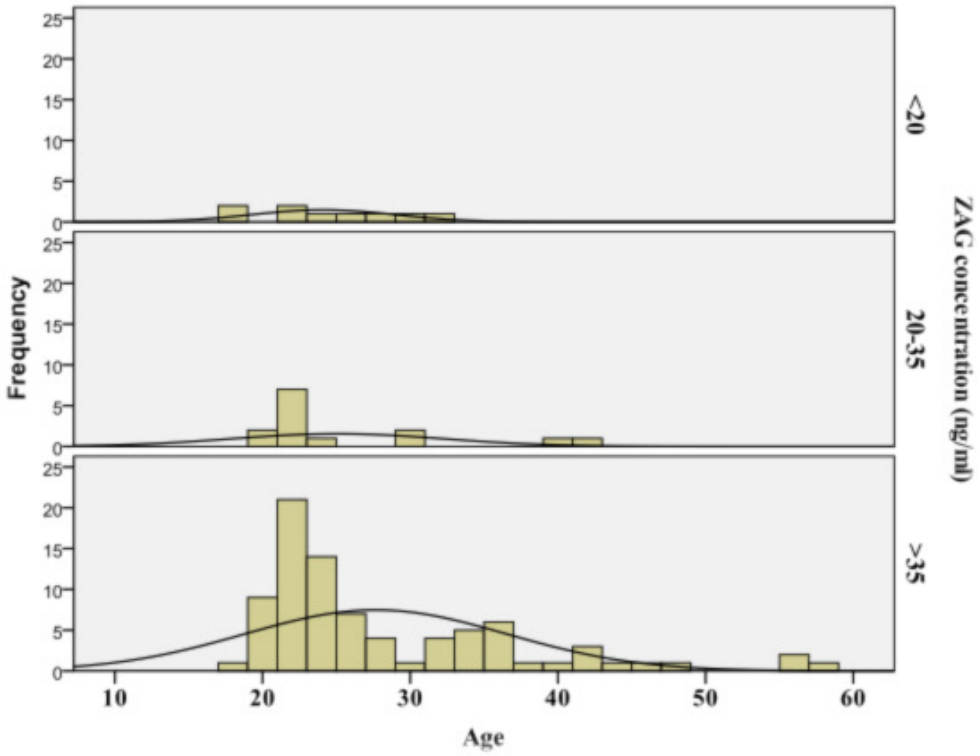

Figure 5. Showed higher levels of ZAG were found in blood donors within the age group below and above 22 years old (p = 0.0099).

DISCUSSION

In this study, the majority of blood donors attending to the SQUH blood bank were found to have high ZAG levels. This has a significant implication not only in the aim to study the pattern of ZAG binding peptide level among healthy Omani individuals, but also in the process of donating blood to those in need which will indeed be affected by such high levels, as some of the recipient are clinically under weight. The out come of this study raised two major points worth to be discussed. First is to find an explanation of the high ZAG binding peptide level expressed by 78% of the donors. As ZAG binding peptide is highly correlated to the metabolism of fat, it is estimated that its high level in the blood of the donors is directly related to their body weight, that is, increase fat metabolism. Other factors which may have influenced the ZAG binding peptide level include chronic diseases, cardiovascular or kidney diseases. It may also suggest an early development of cancer or it can be related to the genetic variation within the population. Hence, it is worthwhile to have a medical history of the blood donors which can explain such threshold of this protein.

The second point to be raised is the impact of this high level in patients receiving the blood. It is important to keep in mind that patients who have physiological abnormalities and/or clinical complications such as patients who are already losing weight due to a chronic disease especially hematological diseases, as they require frequent transfusion of blood and patients with atherosclerosis and cachectic cancer patients receiving such transfusion of blood with high level of ZAG binding peptide, can deteriorate their heath status. Therefore, due to such significant findings, we suggest that ZAG binding peptide should be assigned as a routine investigation in blood donation to assure and implement the safety issues regarding whether or not to accept or reject blood donation based on ZAG binding peptide level.

Interestingly, the ZAG binding peptide level was correlated with age in the female group as shown in Figure 3. Moreover, higher levels were also found in donors below and above 22 years old. Results have also shown a significant association between the level of ZAG binding peptide and the donors' sex. Interestingly, both sex groups showed a very close percentage of high ZAG level with a significant variation within the low and normal levels of ZAG binding peptide. The high percentage level with the male group in comparison to that shown by a low level in females can be explained by the study done by Hong et al. (2009) on mice where the male mice have higher tendency to gain weight than female. Furthermore, the study suggested that this "obesity-protection" is caused by the ovarian hormones as ovariectomized female mice showed an equal tendency to gain weight to males (Hong et al., 2009). The anti-atherosclerotic affect of estrogen, an ovarian hormone, may also explain the relatively high percentage of female with normal ZAG binding peptide level (Marsh et al., 1999).

Moreover, an association between the age of the donors and their ZAG binding peptide level showed that participants between 20 to 30 years old form of more than half the cases of high ZAG binding peptide level. Such result is accepted if we consider the fact that the metabolic rate including lipid metabolism in people, between age group of 20 to 40 years old, is higher than those older/younger (Shock, 1955). Finally, it would be an advantage if the sera samples were analyzed using an automated analysis in parallel with the manual ELISA kit protocol. This may improve the interlaboratory variability of both the assay and/or the results obtained.

Conclusion

More than 70% of blood donors included in this study were found to have high sera ZAG binding peptide level suggesting that its measurement should be tested routinely. Furthermore, our study emphasizes the measurement of ZAG binding peptide level prior to blood transfusion to patient(s) who are clinically under weight. Gender and age significantly influences the plasma level of ZAG binding peptide.

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Role of oxidative stress in aggravating kidney dysfunction in coronary artery disease patients- A laboratory finding

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Acute cardiac decompensation results in activation of hemodynamic and neurohormonal factors that lead to an acute drop in glomerular filtration rate (GFR) resulting in acute kidney injury. This relationship is referred to as cardiorenal syndrome type 1 (CRS), which usually goes unnoticed. The present study was designed to evaluate the occurrence of kidney dysfunction in coronary artery disease (CAD) patients visiting the clinical biochemistry laboratory. Ninety percent (90%) of CAD patients were observed to have stage 3 chronic kidney disease (CKD) on the basis of their GFR. They had significantly raised ($p < 0.05$) blood urea and serum creatinine levels as compared to those without kidney dysfunction and healthy controls. Renal dysfunction was more pronounced in CAD patients suffering from congestive heart failure along with hypertension. All these patients were advised serum uric acid estimations by the clinician. A close look at serum uric acid levels interestingly showed that levels were significantly low ($p < 0.05$) in CAD patients having kidney dysfunction as compared to those without kidney disease and healthy controls. Uric acid is an important antioxidant molecule in the body. CAD patients with stage 3 CKD had relatively increased oxidative stress as revealed from their low serum superoxide dismutase (SOD) and catalase activity which might lead to quenching of uric acid resulting in its low concentrations. It was proposed that the reduced free radical scavenging capacity of the body may be responsible for inflammatory conditions prevailing in the body in response to the injury to the cell membrane and hence causing organ dysfunction which could be the involvement of kidneys in CAD patients leading to CRS type1. Hence it is very important to check the pro-oxidant-antioxidant balance at the very initial stages.

Key words: Cardio-renal syndrome, creatinine, urea, coronary artery disease, oxidative stress

INTRODUCTION

Heart and kidney share responsibility for maintaining hemodynamic stability through a tight-knit relationship that controls cardiac output, volume status and vascular tone. The reactive oxygen species initiate a cascade of events leading to inflammatory response and hence compromised cellular function. Primary disorders of either heart or kidneys often results in secondary dysfunction or injury to the other. Such interaction represents the patho-physiological basis for a clinical entity called cardiorenal syndrome (CRS). There are evidences of risk and occurrence of kidney dysfunction along with coronary artery disease which at times remains undiagnosed and unattended.

In CRS type 1, acute cardiac decompensation results in activation of hemodynamic and neurohormonal factors that lead to an acute drop in glomerular filtration rate

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(GFR) and hence the development of acute kidney injury. Approximately 25% of patients with chronic heart failure have been found to have reduced GFR (Hillege et al., 2006). In one of the studies, it has been reported that 21% of patients with heart failure had their serum creatinine concentrations at more than 2 mg/dl and 9% had more than 3 mg/dl (Adams et al., 2005). The reduction in kidney function has significant impact on both morbidity and mortality (Wencker, 2007).

A study by Dammon and colleagues showed that congestive heart failure is associated with increased markers of tubulointerstitial damage such as N-acteyl-beta-D-glucosamine (NAG), kidney injury molecule-1(KIM) and neutrophil gelatinase associated lipocalin (NGAL) (Damman et al., 2007). Acute CRS type1 is more frequent in patients suffering from acute decompensated heart failure (Eren et al., 2012). The cornerstone of CRS therapy is the early identification of worsening kidney function. Various workers recommended the use of certain potential biomarkers such as cystatin-C, brain natriuretic peptide (BNP), IL-18 and fatty acid binding protein (Haase et al., 2009). These parameters might provide significant information of the tubulointerstitial damage in dysfunction in coronary artery disease (CAD) patients. The novelty of new biomarkers of kidney disease cannot be questioned but a critical role of oxidative stress in the initiation and progression of any disease could also not be ignored. Hence, checking the major culprit that is, oxidative stress at initial stages may be more beneficial in controlling the kidney dysfunction in CAD patients and vice versa.

In addition to other antioxidant molecules, the role of uric acid as an antioxidant has come into picture. In humans, it contributes as much as 2/3 of all the free radical scavenging capacity in plasma (Squadrito et al., 2000). It seems that too much lowering of uric acid in the body due to any reason could create prooxidant-antioxidant imbalance and probably contribute in the organ dysfunction. The aim of the present study was to evaluate the occurrence of renal dysfunction in CAD patients (North West Punjabi population) visiting the Clinical Biochemistry Laboratory for their routine investigations. It is pertinent to mention that these patients were confirmed cases of CAD and no diagnosis of their kidney dysfunction was made earlier.

MATERIALS AND METHODS

The present study was conducted in the Clinical Biochemistry Laboratory of Guru Nanak Dev Hospital (attached hospital of Government Medical College) Amritsar, India. A total of hundred (n = 100) CAD patients visiting the clinical biochemistry laboratory were selected. The diagnosis of CAD was done by the clinician on the basis of clinical symptoms, electrocardiography (ECG) changes and treadmill test (TMT) wherever required. The data of 50 normal healthy subjects (from our previous studies) serving as control group was included for reference (Sharma et al., 2006). CAD patients with diagnosed diabetes mellitus, thyroid disease, gout or any acute infection were excluded. All the patients were screened for serum total lipid profile, plasma serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood glucose and serum uric acid levels along with blood urea and serum creatinine to evaluate their kidney functions. Their demographic data was obtained from their case history files. GFR was calculated by modification of diet in renal disease (MDRD) formula and patients were considered for different clinical stage of chronic kidney disease (CKD) as per kidney disease outcomes quality initiative guidelines (NKF-KDOQI, 2000). Written informed consent was obtained from all the patients as per The Ethical Committee of the Institute. Serum super oxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund (1988). Serum catalase activity was assayed by the method of Aebi (1984). Routine biochemical investigations were done with commercially available kits (Alpha-Chem, Harayana, India) on semi-auto-analyzer (Tran-Asia) and were validated against the reference sera. Student's *t*-test was applied to check the significance at level $p < 0.05$.

RESULTS

Table 1 shows the percentage of CAD patients suffering from kidney dysfunction. Out of 100 CAD patients visiting the clinical biochemistry laboratory, 90% were observed to have kidney dysfunction which was revealed from their blood urea and serum creatinine concentrations. In these patients, mean blood urea levels were 88 ± 5.2 mg/dl and those of creatinine were 7 ± 2.0 mg/dl. Both these levels were significantly raised ($p < 0.05$) as compared to controls and CAD patients without kidney dysfunction. The data clearly indicates the increased risk of kidney dysfunction in patients suffering from CAD. GFR of all the CAD patients with and without renal dysfunction was calculated with MDRD formula (ref) in order to evaluate the clinical stage of CKD. Further, CAD patients were screened for various biochemical parameters and their demographic data was obtained from the records (Table 2). Body mass index (BMI) of CAD patients with or without chronic kidney disease was not significantly different from controls. However, GFR of CAD patients with chronic kidney disease was in the range of 30 to 59 ml/min, hence these patients were considered as stage 3 CKD as per Kidney Disease Outcomes Quality Initiative guidelines (2000). Majority of the CAD patients (n = 60) were on cardiac and antihypertensive treatment. Total lipid profile that is, levels of serum total cholesterol, triglycerides, very-low-density lipoprotein (VLDL-C), low-density lipoprotein (LDL-C) and serum high-density lipoprotein (HDL) cholesterol levels of these patients were not significantly different ($p > 0.05$) in comparison to controls (Table 2). Similar results were observed in case of SGOT, SGPT, alkaline phosphatase and fasting blood glucose levels

Table 3 showed antioxidant profile of CAD patients with stage 3 CKD. These patients had significantly low ($p < 0.05$) levels of serum uric acid, SOD and catalase activity in comparison to normal subjects (controls) and CAD patients without kidney dysfunction. This data clearly

Table 1. Percentage of CAD patients having kidney dysfunction.

Subject	Parameter	Blood urea (mean±SD)	Serum creatinine (mean±SD)
Controls (n=50)	No kidney dysfunction	24±3.0	0.5±0.9
CAD patients (n=100)	10% Kidney dysfunction	(-)28±1.2	0.6±0.08
	90% Kidney dysfunction (Stage 3 CKD)	(+)88±5.2*	7±2.0*

Results were expressed as Mean±SD mg/dl; *p < 0.05: significantly increased blood urea and serum creatinine levels in CAD patients with kidney dysfunction (Stage 3 CKD) as compared to those without kidney dysfunction and control group comprising healthy subjects.

indicate the low antioxidant potential in CAD patients with severe kidney dysfunction and increased risk of further complications.

CAD patients with stage 3 CKD were further classified into three groups on the basis of their differential diagnosis to have a better idea of their renal functions as well as their antioxidant status (Table 4). In Group 3 patients (CHF+HTN), blood urea and serum creatinine levels were significantly ($p < 0.05$) elevated as compared to the patients in other two groups, suggesting that group 3 patients suffering from congestive heart failure along with hypertension had more severe kidney dysfunction as compared to the patients in other two groups. On the same lines, serum uric acid levels were also significantly low ($p < 0.05$) in these patients in comparison to the other two groups. However, serum SOD and catalase activities did not show significant difference ($p > 0.05$) among the three patient groups. However, it is pertinent to mention here that overall, all the CAD patients with stage 3 CKD were having significantly low ($p < 0.05$) serum SOD and catalase activity as compared to controls and patients without kidney dysfunction as mentioned in Table 3. It may be noticed that these activities of antioxidant enzymes were compared with that of normal healthy subjects in studies done previously in our own laboratory (Table 3) (Sharma et al., 2006).

DISCUSSION

A strong communication exists between heart and kidneys through a variety of pathways. The mediators of these pathways include the sympathetic nervous system, the rennin-angiotensin-aldosterone axis and arterial natriuretic peptide. In the setting of underlying heart disease or chronic kidney disease, the capacity of each organ to respond to perturbation caused by the other may become compromised. This has led to the characterization of the cardiorenal syndrome (CRS). The present study was an observational approach on the blood samples of CAD patients received in the clinical biochemistry laboratory for analysis. It may be noticed that these patients were confirmed cases of CAD and their their renal status was unknown as no diagnosis of any CKD was mentioned on their outpatient department (OPD)/ward medical cards. All these patients were from various wards and outpatient departments of the Department of Medicine who were under treatment by the physician. It was observed that in all of these diagnosed and treated cases of CAD, lipid profile and the enzymatic estimations were in a normal reference range whereas the disturbances in the routine biochemical investigations such as blood urea and serum creatinine were quite prominent, hence bringing to attention the current developments in literature providing evidences for occurrence of renal failure in these CAD patients.

Approximately 90% of CAD patients were having significantly raised blood urea and serum creatinine levels when compared to the that of healthy controls and it was observed to be more prominent when the values were compared to that of rest 10% patients of coronary artery disease patients who were having normal kidney functions as suggested by their normal levels of blood urea and serum creatinine. These 90% CAD patients were considered as stage 3 CKD on the basis of their estimated GFR calculated with MDRD formula and as per Kidney Disease Outcomes Quality Initiative guidelines (2000). Besides, renal dysfunction was observed to be more severe in patients having congestive heart failure along with hypertension. Heart failure is a common chronic condition affecting 2% of the adult population (Mcmurray et al., 2005). The decompensated heart failure results in reduced effective arterial filling volume (Schrier and Abraham, 1999). The CHF patients in the present study were decompensated and this information was revealed from their Medical case history files.

All the patients were advised serum uric acid estimations by the clinician. A close look at the values of serum uric acid interestingly revealed that the uric acid levels were significantly low in CAD patients having disturbed renal function than those having relatively better renal profile as well as the controls. All these findings are suggestive of the fact that probably oxidative stress is playing a significant role in causing injury to the cellular membrane (reaction to injury hypothesis), hence the inflammatory response in the initial phases of coronary

Table 2. Demographic data and Biochemical profile of CAD patients (with and without kidney dysfunction) and controls

Parameter	Age (years)	Male:female	BMI (kg/m²)	GFR (ml/min)	Blood pressure (mm/Hg)	Treatment	Serum total cholesterol	Serum TG	Serum VLDLC	Serum LDL-C	Serum HDL-C	SGOT	SGPT	ALP	Glucose
Control	42±5	22:28	24±4	80-125	<140/90	-	200±5.0	130±6.0	27±3.0	128±5.0	45±3.0	28±4.2	30±5.0	80±4.8	70±6.0
CAD patients with stage 3 CKD	45±5	32:58	25±5	30-59	>140/90	+	**180**±4.2	124±3.0	24±2.8	115±5.0	41±1.6	27±3.2	28±4.1	73±3.5	80±4.0
CAD patients without CKD	43±6	4:6	26±2*	75-100	>140/90	+	210±5.0*	130±5.2*	28±3.0*	140±4.0*	42±3.0*	30±3.8*	24±5.3*	82±4.0*	84±2.0*

*$p > 0.05$ Insignificant difference in different parameters among the three groups; CAD patients were on cardiac and antihypertensive treatment.

artery disease. This was supported from the fact that serum uric acid levels, SOD and catalase activities were significantly lower in CAD patients with stage 3 CKD as compared to the normal healthy subjects enrolled in our previous reported studies (Sharma et al., 2006) and patients without kidney dysfunction (Table 3).

The role of oxidative stress leads to reaction to injury hypothesis and has already been reported by number of workers in CAD (Puddu et al., 2012). It was quite evident from the literature that the oxidation of LDL by free radicals initiates a cascade of events leading to increased inflammation and injury to the cardiac membrane (Manzano-Leon et al., 2013). Similar events of inflammation could produce injury to the membrane of the kidneys also. Acute kidney injury may complicate one-third of the admissions and a 22% higher mortality rate through adversely affecting cardiac performance through electrolyte dysequilibration, volume overload, and negative inotropy (Wencker, 2007). Hence the involvement of kidneys in CAD and vice versa cannot be ruled out.

Mahajan et al. (2009) reported an important role of uric acid as free radical scavenger, better than vitamin C in patients suffering from rheumatoid arthritis. Urates possess preventive antioxidant property in addition to the chain breaking anti-oxidant activity (Waring et al., 2001). Since uric acid concentrations in plasma of humans are much more than that of plasma ascorbic acid, uric acid contributes more to the scavenging action of free radicals than ascorbic acid. In the extra-cellular environment, uric acid behaves as a powerful antioxidant, particularly it scavenges peroxynitrite radicals and it is the nature of host environment which decides its role as pro-oxidant or antioxidant (Kuzkaya et al., 2005). Oxidative stress causing injury to the glomerular membrane might be responsible for compromised glomerular functions, and decreased glomerular perfusion or decreased cardiac output activates the rennin-angiotensin system, nitric oxide, adenosine and prostaglandin production to prevent dramatic changes in kidney function (Tang and Mullens, 2010).

Renin angitensin system activation results in increased AII which stimulates nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Griendling et al., 1994). The resulting NADPH/NADH suppresses superoxide dismutase and increases reactive oxygen species. In the present study too, a significant decrease in serum SOD and catalase activity was observed in CAD patients irrespective of whether they were having renal dysfunction or not. Compromised antioxidant functions result in the well known cascade of hypoxic ischemic injury, inflammation, apoptosis and cell death (Griendling et al., 1994). Moreover, it has been suggested that there is defective regulation of monocyte apoptosis in patients suffering from CRS type 1 leading to increased inflammation and oxidative stress (Virzi et al., 2012).

The early identification of worsening kidney function is highly essential. Various biomarkers such as NGAL, NAG and KIM-1 have been implicated in the tubulointerstitial damage and have been used to identify acute kidney injury (Haase et al., 2009). Serum cystatin C is a marker of reduced glomerular filtration while urinary Cystatin C is a marker of tubular dysfunction (Herget-Rosenthal et al., 2007). In addition to these novel biomarkers, we suggest that the need of the hour appears to bring a check to the disturbed antioxidant status in CAD patients so that the multiorgan involvement especially that of kidneys can be controlled at the earliest when CAD is diagnosed. Uric acid could act as promising parameter in this respect. The evaluation of the trend of uric acid levels with the course of either CAD or renal disease might provide significant information of the underlying oxidative stress and hence inflammation.

Ninety cases out of hundred represent only a fraction of patient population suffering from CRS type 1 and the number may be many folds more in the actual conditions.

Table 3. Antioxidant profile of CAD patients with stage 3 CKD

Antioxidant parameter	Control	CAD patients with stage 3 CKD	CAD patients without kidney dysfunction
Serum uric acid (mg/dl)	5.8±1.2	3.8±1.0*	5.2±0.8
Serum SOD activity (IU/ml)	6.4±1.3	2.1±0.4*	4.5±1.0
Serum Catalase activity	7.8±2.0	4.0±1.1*	6.2±0.6

*p < 0.05: Significantly low uric acid levels, SOD and catalase activity in CAD patients with stage 3 CKD as compared to controls and patients without kidney dysfunction.

Table 4. Increased oxidative stress and renal dysfunction in patients suffering from coronary artery disease (CAD patients with stage 3 CKD).

Parameter	CAD patients with renal dysfunction		
	Group1 (CAD cases) (n=30) [Range; M±SD]	Group 2 (CAD+HTN) (n=25) [Range; M±SD]	Group3 (CHF+HTN) (n=35) [Range; M±SD]
Serum uric acid (mg/dl)^	3.5-5.5; 4.5±1.0	3-5.0; 4.2±1.2	2.5-4.2; 3.5±0.98*
Serum SOD activity	1.5-3.5; 2.4±0.59	2-3.0; 2.5±0.7	1.8-3.2; 2.4±0.82**
Serum catalase activity	2-5.6; 4.6±1.2	2.5-5.7; 4.1±1.5	2.5-4.8; 3.5±1.6**
Blood urea (mg/dl)^	55-88; 75±5.1	56-98; 82±3.4	67-120; 109±5.2*
Serum creatinine (mg/dl)^	1.3-4.0; 2.8±1.3	1.6-6.8; 5.0±1.8	3.2-10.5; 8±2.0*

*p<0.05 significantly raised levels of serum uric acid, blood urea and serum creatinine in Group 3 patients as compared to other two groups whereas serum SOD and catalase activities showed insignificant difference in this respect (**p>0.05). HTN: Hypertensin; CHF: congestive heart failure.

Secondly, in this observational study only 10% of CAD cases were observed to have normal renal functions in comparison to 90% who were having disturbed kidney function, hence less number of cases in the former could be the limitation of the study, however, indirectly this ratio suggest the increased occurrence of renal dysfunction in CAD. The observation of normal cardiac profile as well as other enzyme activities could be the result of the treatment given to these patients. An important point to discuss over here is that blood urea and serum creatinine levels were not normal, rather they were significantly elevated, this clearly indicates that CRS at times remains undiagnosed and unattended to (quite evident from the present study) which could add complexities in the disease management.

Since it was an observational study and not a comparative one, hence the robustness of blood urea, serum creatinine and uric acid over other new biomarkers cannot be questioned. The results obtained from the present study is only a small representation of the population actually suffering from CRS, hence more and more data is required to evaluate the role of oxidative stress in diagnosis and management of cardio-renal syndrome. A follow-up study aiming at investigating the uric acid levels in a healthy population would be beneficial to have a better idea of its variations during a course of acquired CAD or CKD. Further, no direct marker of inflammation has been evaluated because of the observational nature of the present study but certainly on the basis of these findings, a case-control study would be planned taking into account the novel markers of oxidative stress and inflammation in evaluating CRS.

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