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Effect of GSTM1 and GSTT1 Deletions in the Development of Oxidative Stress in Children with Chronic Kidney Disease

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Background: Increased oxidative stress is a hallmark of end-stage renal disease (ESRD). Glutathione S transferases (GST) are involved in the detoxification of xenobiotics and protection from oxidative damage. We hypothesized that genetic polymorphisms in antioxidant enzymes GSTM1 and GSTT1 are more frequent in ESRD and modulate the degree of oxidative stress in these patients. Objectives: The present study was designed to evaluate oxidized LDA (ox-LDL), high-sensitivity C-reactive protein (hs-CRP), and homocysteine (hcy) levels in children with end-stage renal disease (ESRD) treated with maintenance haemodialysis (MHD) or under conservative treatment, to compare these levels with those assayed in control subjects, and to evaluate these levels with different GSTM1 and GSTT1 genotypes. Methods: This case-control study was conducted in 78 children. They were divided into 3 groups: group I (44 on HD), group II (14 on conservative treatment), and group III (20 normal healthy children) served as controls. All enrolled cases and controls were subjected to genotyping for GSTM1 and GSTT1 by polymerase chain reaction (PCR) and determination of ox-LDL, hs-CRP, and hcy levels. Results: Oxidized LDL levels were significantly higher in both the MHD and conservative treatment groups than in controls and levels were higher in the MHD group than in the conservative treatment group (199.48 ± 78.63 µ/l, 182.07 ± 128.77 µ/l, and 88.25 ± 23.02 µ/l, respectively). hs-CRP levels were significantly higher in the MHD group compared to the control group (4.03 ± 4.59 mg/dl and 1.14 ± 0.75 mg/dl). Homocysteine showed significantly higher levels in the MHD group when compared to both conservative treatment and control groups (73.43 ± 35.08, 20.35 ± 32.81 µmol/ml, and 5.9 ± 2.8 µmol/ml, respectively). Dialyzed and conservative-treatment patients had significantly higher frequencies of the GSTM1 and GSTT1 null genotypes when compared to the control group. Conclusion: Patients with GSTM1 or GSTT1 null genotypes are more vulnerable to oxidative stress compared with those who possess normal gene expression in chronic kidney disease. ***J Clin Basic Cardiol 2013; 16 (online): 1–5.***

Key words: *oxidative stress, chronic kidney disease, children, GSTM1, GSTT1, gene polymorphism, homocysteine*

There is a 10–20-fold elevated risk of cardiovascular mortality in patients with end-stage renal disease (ESRD) [1]. Oxidative stress and atherothrombotic vascular disease are associated with high mortality in patients treated with haemodialysis (HD). Alterations of oxidized LDL (ox-LDL) and homocysteine (hcy) levels are important participating agents in the initiation and progression of oxidative and atherogenic events [1]. Several inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP), have been shown to independently predict mortality in ESRD patients [2]. As CRP is so strongly associated with vascular disease, it has been suggested that this hepatic-derived protein is not only a marker but also a mediator of vascular disease [2].

Adverse effects of xenobiotics, which are foreign substances, are excreted via covalent interactions between intermediate metabolites and genetic materials or proteins and their related metabolites [3]. Enzymatic reactions of xenobiotic metabolism are needed to avoid accumulation of lipophilic xenobiotics in cells and tissues. These reactions can be divided into 2 phases during the activation-deactivation sequence, namely phase-I and phase-II enzymatic reactions. The key enzyme systems catalyzing phase-I oxidative metabolism are enzymes of the cytochrome P450 (CYP) superfamily. During these reactions, toxic metabolites are generated which might be processed by phase-II enzymes [3].

Phase-II enzymes complete detoxification by neutralizing reactive electrophiles or by acting as indirect antioxidants [4]. NQO1 and GSTs are both phase-II detoxifying enzymes which play important roles in preventing carcinogen-induced disorders [4].

Glutathione S transferase M1 (GSTM1) and glutathione S transferase T1 (GSTT1) are cancer susceptibility genes because of their ability to regulate the conjugation of carcinogenic compounds to excretable hydrophilic metabolites. Deletion variants lacking in enzyme activity exist for both genes [5]. Individuals with homozygous deletions in the GSTM1 or GSTT1 genes are supposed to have less ability to metabolize carcinogens and may therefore be more susceptible to cancers [5].

There is increasing evidence for the presence of disordered oxidative and glycoxidative chemistry in patients who undergo maintenance haemodialysis (MHD) that may contribute to poor cardiovascular and overall outcome. DNA, in particular, is more susceptible to attack by reactive oxygen species (ROS) than proteins and membrane lipids, which are protected against oxidation by low-molecular-weight antioxidants as well as antioxidant enzymes [6].

Altered GST activity associated with polymorphisms is expected to affect cancer risk through decreased protection against DNA damage from reactive electrophiles. GSTs are expressed and have significant activity in the kidney, but few studies have considered GSTs as a susceptibility to renal carcinoma [7].

The aim of the study is to evaluate ox-LDL, hs-CRP, and hcy levels in children with ESRD treated with maintenance haemodialysis (MHD) or under conservative treatment, to compare these levels with those assayed in control subjects, and to evaluate these levels with different GSTM1 and GSTT1 genotypes.

Material and Methods

58 paediatric patients with advanced chronic renal disease (stages 4 and 5 based on estimated glomerular filtration rate [eGFR] according to the National Kidney Foundation classification) [8] were included in the study. They were divided

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into 2 groups undergoing conservative treatment (n = 14) or MHD (n = 44). MHD children were selected from the haemodialysis unit of the Center of Pediatric Nephrology and Transplantation (CPNT), whereas children undergoing conservative treatment were selected from the paediatric nephrology clinic, Children's Hospital, Cairo University. The study was performed from April 2012 to December 2012. In conservative-treatment patients, the causes of renal failure were renal hypoplasia or dysplasia, obstructive uropathies, neurogenic bladder, unknown, or metabolic. In MHD, the causes of renal failure were hereditary nephropathies, obstructive uropathies, neurogenic bladder, glomerulopathy, renal hypoplasia or dysplasia, and unknown causes.

Inclusion criteria for MHD patients comprised patients with onset of haemodialysis < 16 years with at least 6 months MHD duration. They were treated with haemodialysis for 3–4 h 3× a week with a polysulfone membrane using bicarbonate-buffered dialysate. Duration of haemodialysis was 1.59–2.75 years. MHD patients and conservative-treatment patients were taking antihypertensive drugs.

All control children (n = 20) were healthy with no clinical signs of vascular or renal disease and no family history of renal disease. Informed consent for genetic studies was obtained from parents of all participants. The protocol of the study was read and approved by the Ethics Committee of the National Research Center (NRC) in Egypt. No CKD patient in any group had cardiovascular events on the basis of examination and detailed clinical history. Additional exclusion criteria were liver disease and nephrotic syndrome (defined as daily proteinuria > 3.5 g/1.73 m^2).

All subjects in the study were subjected to medical history-taking, thorough clinical examination, determination of serum levels of hs-CRP, ox-LDL, hcy, and genotyping for GSTT1 and GSTM1. Three ml of venous blood were collected in EDTA vials for the extraction of genomic DNA.

Determination of hs-CRP in serum was performed by solid-phase, chemiluminescent, immunometric assay (Immulite/Immulite 1,000; Siemens Medical Solution Diagnostics, Eschborn, Germany) [9].

Oxidized LDL was measured in plasma samples by means of a commercially available capture ELISA kit (Mercodia, Uppsala, Sweden). The antibody used in the kit was the murine monoclonal antibody, and the assay was based on the direct sandwich technique, in which 2 monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B moiety of LDL. Absorbance values were read spectrophotometrically at 450 nm [10].

Homocysteine, a modified sulfur amino acid known as a potential risk factor for cardiovascular disease that is increased above normal levels in > 90 % of uremic patients, was measured in plasma samples by means of a commercially available ELISA kit (Diazyme Laboratories, Germany) [11].

Detection of GSTT1 and GSTM1 Polymorphisms

Genomic DNA used was extracted from lymphocytes using the QIAamp DNA Mini isolation kit (QIAGEN, # 51304).

Polymorphic deletion of the GSTT1 gene was determined by amplification in a 50-μl reaction containing each of the primers F 46 (5'-GCCCTGGCTAGTTGCTGAAG) and R 137 (5'-GCATCTGATTTGGGGACCACA). The PCR cycle was 94 °C for 4 min; 94 °C for 15 sec, 59 °C for 30 sec, 72 °C for 45 sec for 35 cycles, followed by 72 °C for 7 min. The presence of 1 or 2 GSTT1 alleles identified by the presence of a 112-bp fragment, or complete deletion (null genotype), was visualized by electrophoresis on a 1.5-% agarose gel.

Polymorphic deletion of the GSTM1 gene was determined by amplification in 50-μl reaction volume containing P1 (5'-CGCCATCTTGTGCTACATTGCCCG), P2 (5'-ATCTTCTCCTCTTCTGTCTC), and P3 (5'-TTCTGGA TTGTAGCAGATCA). The PCR cycle was 94 °C for 4 min; 94 °C for 30 sec, 58 °C for 1 min, 72 °C for 1 min for 35 cycles, followed by 72 °C for 7 min. P1 and P3 amplify a 230-bp product that is specific to GSTM1. The presence of one or both GSTM1, identified by a 230-bp fragment, or its complete deletion (null genotype), was visualized by 1.5-% agarose gel electrophoresis [3].

Statistical Analysis

The Statistical Package for Social Science (SPSS, Chicago, IL, USA) programme, version 11.0, was used for data analysis. Data were summarized as mean ± SD, range or percentage. Data were evaluated between the experimental groups by 1-way analysis of variance (ANOVA). Comparison between patients and the control group was performed by t-test (quantitative variables) and Chi-squared test (qualitative variables). $P < 0.05$ was considered significant.

Results

This case-control study included 24 male (54.5 %) and 20 female (45.5 %) patients, their ages ranged from 5–18 yrs (mean 10.41 ± 3.32). Group II included 8 males (57.1 %) and 6 females (42.9 %), their ages ranged from 2–18 yrs (mean 11.21 ± 4.81). Group III included 14 males (70.0 %) and 6 females (30.0 %), their ages ranged from 4–18 yrs (mean 9.1 ± 3.05).

There were no significant differences between groups with respect to age and sex ratios while oxidized LDL levels were significantly higher both in the MHD and conservative treatment groups than in controls and the levels were higher in MHD group than in the conservative treatment group (199.48 ± 78.63 μ/l, 182.07 ± 128.77 μ/l, and 88.25 ± 23.02 μ/l, respectively). hs-CRP levels were significantly higher in the MHD group compared to the control group (4.03 ± 4.59 mg/dl vs 1.14 ± 0.75 mg/dl). Homocysteine showed significantly higher levels in the MHD group when compared to both conservative-treatment and control groups (73.43 ± 35.08 μmol/ml, 20.35 ± 32.81 μmol/ml, and 5.9 ± 2.8 μmol/ml, respectively; Table 1).

Frequencies of GSTM1 and GSTT1 normal and null genotypes among studied groups are shown in Table 2, dialyzed and conservative-treatment patients had significantly higher frequencies of the GSTM1 null genotype when compared to the control group ($p < 0.000$ and $p < 0.000$, respectively) and the frequency of the null genotype of the dialysis group is higher than that of the conservative-treatment group, yet it did not reach significance. For GSTT1 genotypes, there is increased frequency of null genotype among the dialysis and conservative-treatment groups when compared to the control group ($p < 0.000$ and $p < 0.05$, respectively).

Clinical and biochemical characteristics of CKD patients with different GSTM and GSTT genotypes are shown in Table 3. There were no significant differences regarding age, sex, and hs-CRP levels between both normal and null expressions of GSTM1 and GSTT1 genes in both the MHD and conservative treatment groups. Levels of serum-oxidized LDL showed a significant difference between normal and null expression of both GSTM1 and GSTT1 genes: it was higher in both null GSTM1 and GSTT1 expressions ($p < 0.05$ and $p < 0.000$, respectively), while in the conservative treatment group, it showed a highly significant difference between normal and null GSTT1 expression only ($p < 0.000$). Homocysteine levels were significantly higher for null expression of GSTM1 in the MHD group ($p < 0.05$), they were also higher in the null expression of GSTT1 in the MHD group,

Table 1. Clinical and biochemical characteristics of the studied groups (mean ± SD).

	MHD	CT	Control
n	44	14	20
Age (yrs)	10.41 ± 3.32	11.21 ± 4.81	9.1 ± 3.05
Sex (m/f)	24 (54.5 %) 20 (45.5 %)	8 (57.1 %) 6 (42.9 %)	14 (70 %) 6 (30 %)
Ox-LDL (μ/l)	199.48 ± 78.63[ac]	182.07 ± 128.77[b]	88.25 ± 23.02
hs-CRP (mg/dl)	4.03 ± 4.59[a]	2.82 ± 3.32	1.14 ± 0.75
Hcy (μmol/ml)	73.43 ± 35.08[ac]	20.35 ± 32.81	5.9 ± 2.8

Data were evaluated by ANOVA test. Values are presented as mean ± SD or percentage as applicable. MHD: maintenance haemodialysis; CT: conservative treatment; Ox-LDL: oxidized LDL; hs-CRP: high-sensitivity C-reactive protein; hcy: homocysteine. [a] HD vs control; [b] CT vs control $p < 0.000$; [c] HD vs CT $p < 0.000$

Table 2. Frequencies of GSTM1 and GSTT1 normal and null types among studied groups

	MHD	CT	Control
n	44	14	20
GSTM1			
Normal	13 (29.5 %)	5 (35.7 %)	20 (100 %)
Null	31 (70.5 %)[a]	9 (64.3 %)[b]	0 (0 %)
GSTT1			
Normal	24 (54.5 %)	10 (71.4 %)	20 (100 %)
Null	20 (45.5 %)[a]	4 (28.6 %)[b]	0 (0 %)

Data were evaluated by Chi-squared test. Values are presented as percentage. MHD: maintenance haemodialysis; CT: conservative treatment. [a] HD vs control; [b] CT vs control; [c] HD vs CT

Table 3. Age, Ox-LDL, hs-CRP, and homocysteine in chronic kidney disease patients with different GSTM and GSTT genotypes

	MHD				CT			
	GSTM1		GSTT1		GSTM1		GSTT1	
	Normal	Null	Normal	Null	Normal	Null	Normal	Null
n	13	31	24	20	5	9	10	4
Age (ys)	9.92 ± 3.01	10.61 ± 3.47	10.29 ± 3.18	10.55 ± 3.56	12.0 ± 5.47	10.78 ± 4.35	10.9 ± 5.10	12.0 ± 3.55
Ox-LDL (μ/l)	158.7 ± 34.9	216.5 ± 85.7*	161.8 ± 34.8	244.8 ± 92.7*	134.0 ± 68.7	208.7 ± 149.4	116.9 ± 55.4	345.0 ± 114.4*
hs-CRP (mg/dl)	4.51 ± 3.94	3.83 ± 4.88	3.58 ± 3.40	4.58 ± 5.76	4.0 ± 4.93	2.17 ± 2.1	3.82 ± 3.62	0.85 ± 1.05
Homocysteine (μmol/ml)	51.3 ± 29.01	82.7 ± 33.58*	64.87 ± 31.43	83.7 ± 37.23	7.2 ± 1.64	27.68 ± 39.7	17.2 ± 29.92	28.25 ± 43.81

Significance was estimated using the independent t-test. Data are mean ± SD. Ox-LDL: oxidized LDL; hs-CRP: high-sensitivity C-reactive protein. * P significant if < 0.05

and higher in the null expression of both GSTM1 and GSTT1 of the conservative treatment group, yet this did not reach statistical significance. In the control group, there was 100 % normal expression of both GSTM1 and GSTT1 genes.

Discussion

Renal disease is associated with a graded increase in oxidative stress markers even in early CKD. This could be the consequence of an increase in reactive oxygen species as well as a decrease in antioxidant defense. This oxidative stress can accelerate renal injury progression [12]. Genetic variants that affect the capacity to handle oxidative stress may therefore influence the outcome of kidney disease [13].

Increased cardiovascular morbidity and mortality are present across the whole renal dysfunction spectrum even in patients with moderate renal insufficiency. In fact, most patients with CKD die of cardiovascular causes rather than progress to end-stage renal disease in which cardiovascular mortality is 15–30 % higher compared to age-adjusted control groups. This high risk of cardiovascular disease seems to be the consequence of a higher prevalence of risk factors in patients with CKD than in the general population [14].

We examined whether genetic variants of GSTM1 and GSTT1 genes, members of a superfamily of glutathione S transferases, influence the course of kidney disease progression in participating Egyptian children. In addition, investigations in which ox-LDL, hs-CRP, and hcy are considered together as important agents involved in the development of oxidative and atherogenic events in end-stage renal disease are limited.

In this study, oxidized LDL levels were significantly higher in both the MHD and conservative-treatment groups than in controls and levels were higher in the MHD group, hs-CRP levels were significantly higher in the MHD group compared to the control group and homocysteine showed significantly higher levels in the MHD group when compared to both the conservative-treatment and control groups. Mahrooz et al [1] included non-diabetic HD patients in their study and found that ox-LDL levels were significantly increased both before and after HD compared with the control group and homocysteine levels in ESRD patients were higher than control subjects both in pre-dialysis and post-dialysis. There was a significantly positive correlation between ox-LDL and homocysteine in samples obtained before HD.

Osorio et al [15] studied atherogenesis markers in patients with stage-5D chronic kidney disease (CKD-5D) on haemodialysis to determine which parameters are modified and whether their behaviour differs between male and female patients of similar age. They found that CKD-5D patients had significantly lower cholesterol, LDL, and ox-LDL levels and significantly higher hcy levels compared to their respective controls. The reduction in ox-LDL in CKD patients does not imply a lower risk of atherosclerosis. In fact, the risk may be higher due to a greater capture of ox-LDL by macrophage scavenger receptors, which are increased in these patients. Elevated hcy levels may also be a risk factor for atherosclerosis in male and female CKD-5D patients.

Helal et al [16] studied HD patients displaying a marked atherogenic profile, as tested by increased levels of total cholesterol, triglycerides, low-density lipoprotein-cholesterol, apolipoprotein A, CRP, hcy, and lower concentrations of high-

density lipoprotein-cholesterol. The same biological disorders found in HD patients were noted in peritoneal-dialysis patients. The peculiar metabolic changes observed by Helal et al [16] confirm the marked tendency of patients with impaired renal function for developing cardiovascular diseases irrespective of the type of dialysis. Shojaei et al [17] investigated patients with maintenance haemodialysis who were taking atorvastatin or lovastatin, vitamin B_6, and folic acid for at least 6 months. They found that homocysteine levels were 33 % higher on average than the reference value. Lahrach et al [18] found that haemodialysis patients had more frequently atherogenic dyslipidaemia, hyperhomocysteinaemia, and elevated hs-CRP levels when compared to controls.

In this study, GSTM1 null and GSTT1 null genotypes were significantly higher in both haemodialysis and conservative-treatment groups when compared to controls, indicating that these genotypes may be risk factors for ESRD in children with CKD. Such an association was observed earlier by others [19] who determined GSTM1 and GSTT1 genotypes in ESRD patients. Markers of protein and lipid oxidative damage, together with total oxidant status and pro-oxidant-antioxidant balance were determined: they found that individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with the GSTM1 null gene variant having the most pronounced effect. They also found that when patients were stratified according to GSTM1 and GSTT1 the highest oxidant damage was noted in those with the GSTM1 null/GSTT1 null genotype.

Datta et al [20] studied 3 groups of Indian patients (diabetics without CKD, diabetic CKD, and non-diabetic CKD), revealing that GSTM1 and GSTT1 deletions singly or together were associated with lower GST levels and higher oxidative stress in both diabetic and non-diabetic CKD and that GSTT1 deletion appears to be associated with both diabetic and non-diabetic CKD irrespective of the GSTM1 status.

Lin et al [6] found that among MHD patients, GSTM null genotype approximately doubled the risk for all-cause mortality during the mean follow-up of 34 months.

Agrawal et al [21] who assessed GSTT1 and GSTM1 polymorphisms in patients with ESRD from North India found that the GSTM1 null genotype was present in 46.74 % of ESRD patients while the GSTT1 null genotype was present in 58.7 % of ESRD subjects. There was a significant association of null alleles of the GSTM1 and GSTT1 with end-stage renal disease.

Yang et al [22] used multiplex PCR to analyze GSTM1 and GSTT1 polymorphisms. To determine their role in the development of ESRD in diabetic and hypertensive patients they revealed that homozygous deletion of the GSTT1 gene is a risk factor for developing ESRD in diabetic patients but not in hypertensive patients.

In this study, regarding the correlation between oxidative stress markers and the different GSTM1 and GSTT1 polymorphisms in end-stage renal disease patients, ox-LDL levels were significantly higher in the null genotypes of both GSTM1 and GSTT1 genes compared to normal gene expression in the MHD group while this significance was found only in GSTT1 null genotype in the conservative treatment group, also homocysteine levels were significantly higher in the null expression of GSTM1 in the MHD group only, it was also higher in the null expression of GSTT1 of the MHD group, and higher in the null expression of both GSTM1 and GSTT1 of the conservative treatment group, yet it did not reach clinical significance.

Martin et al [23] studied the effect of NQO1, GSTM1, and GSTT1 polymorphisms in coronary heart disease and biomarkers of oxidative stress. They revealed that among the entire population individuals with a GSTM1 null polymorphism have slightly higher hcy levels than those with the wild type but when these data were segregated by case vs control, hcy levels were significantly higher in GSTM1 null subjects in the control group and none of the other biomarkers showed significant variations between GSTM1 or GSTT1 genotypes.

In summary, GSTM1 and GSTT1 null genotypes showed a significantly higher frequency in children with CKD, both on MHD and on conservative treatment. These results suggest that the GST gene polymorphism can serve as a useful genetic marker for evaluation of susceptibility to chronic renal failure. However, the interactions between this genetic predisposition and environmental factors as well haplotype analysis warrant more studies.

Conflict of Interest

The authors declare that they have no competing interests.

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Characteristics and Treatment Pattern of Diabetic Patients with ACS: A Saudi Perspective from the MIDAS Registry

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Background: The Multicenter International Diabetes – Acute coronary Syndromes (MIDAS) study aimed to monitor the adherence to evidence-based therapy among diabetic patients (DM) with unstable angina or non-ST-segment elevation myocardial infarction (ACS) and to describe the in-hospital outcomes of DM in the setting of ACS. We present the data of the Kingdom of Saudi Arabia (KSA) based on the risk profile of the patients in comparison to the international registry. Design: Data of DM patients with ACS who presented at the time of admission to the emergency/coronary care units in 5 hospitals in KSA were extracted from the registry. A total of 3624 patients were enrolled in several countries in Europe, the Middle East, and India. The following variables were extracted: ST deviation ≥0.5 mm, positive troponin, and TIMI risk score. The Z test for 2 proportions was used to compare the KSA values with the international figures. Analysis was done using stata 10. Level of significance was set at 5 %. Results: "Type-2:type-1 DM ratio" in KSA was comparable to the international ratio (93.65 vs 93.83). The high risk factors were significantly less prevalent in KSA in comparison to the international figures (17 % showed significant ST deviation ≥0.5 mm vs 46 % [p = 0.005], 40 % showed positive troponin vs 70 % [p < 0.0001], and 32 % showed a TIMI risk score > 3 in comparison to 62 % [p < 0.0001]). Utilization of GPIIb/IIIa was 18.3 % in KSA vs 37.4 % internationally (p = 0.046). On the contrary, the utilization of clopidogrel/ticlopidine was 96.8 % in KSA vs 74.7 % internationally (p < 0.0001). The percentage of early coronary angiography was 38.9 % in KSA vs 46.1 % internationally (p = 0.292). Among patients who had early coronary angiography, 34.9 % had revascularization (PCI and/or CABG) in KSA in comparison to 54.8 % in the international samples (p = 0.199). Conclusions: Saudi DM patients with ACS had similar demographic data compared to international patients. There was a satisfactory use of evidence-based medicine in the treatment in such a group. ***J Clin Basic Cardiol 2013; 16 (online): 6–9.***

Key words: *diabetics, NSTEMI, ACS, Saudi, GPIIb/IIIa, MIDAS*

The tremendous surge in socioeconomic growth in developing countries like KSA has considerably influenced the lifestyle of the people. Epidemiological studies conducted in KSA have shown a high prevalence of DM with an overall prevalence of NIDDM of 28.82 % and 24.92 % in males and females, respectively, in those over the age of 60 years [1, 2]. On the other hand, in a recent Saudi registry, DM was the most prevalent risk factor for coronary artery disease, present in 56 % of patients, of which 3 % were diagnosed after hospital admission [3].

It is noteworthy that DM was found to be an independent predictor of mortality in the setting of non-ST-segment elevation ACS (NSTEMI-ACS) [4]. Moreover, DM has been associated with worse outcomes following both percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) [5–7].

Despite the preferential benefit observed among high-risk diabetic individuals, previous registry data have shown that these patients are less likely to undergo early invasive strategy and to receive GPIIb/IIIa receptor inhibitors in the setting of ACS [8, 9].

Nevertheless, none of the risk scores has focused on the DM-ACS population, although it may account for 20–30 % of all ACS patients. For instance, the TIMI and GRACE risk scores that allow for prediction of mortality or major morbidity in the setting of ACS remain to be validated in the DM population [10, 11]. This is a crucial step in estimating patient prognosis and optimizing treatment. In addition, it remains to be fully elucidated what is the impact on prognosis of DM-specific parameters such as glucose level, HbA_{1c}, or microalbuminuria in the setting of ACS. These parameters might be a part of future studies.

The MIDAS-ACS study aimed to monitor the adherence to evidence-based antiplatelet therapy among DM with ACS and to assess the impact of DM-specific parameters on early morbidity and mortality in the current era of early invasive strategy and aggressive antiplatelet therapy [12, 13]. In this paper, we present the data of KSA based on the risk profile of the patients in comparison to the international registry.

Design

Data of 142 DM patients presenting with NSTE-ACS at the time of admission to the emergency/coronary care units in 5 hospitals in KSA were extracted from the registry. Unstable angina is defined as ischemic chest pain occurring either at rest (or with minimal exertion) in a crescendo pattern or severely and of new onset. If these symptoms are accompanied by a release of cardiac biomarkers of necrosis (ie, creatine-kinase-MB or troponin), then an NSTEMI has occurred.

Target enrolment was 4000 with a total of 3624 patients finally enrolled. Several countries in Europe (Belgium, Italy, Netherlands, Norway, Spain, and Switzerland) and the Middle East (Israel, Jordan, and KSA) plus India were involved. Primary clinical outcome measure was in-hospital death or ST elevation myocardial infarction (STEMI). The following variables were extracted: utilization of GPIIb/IIIa inhibitors, utilization of clopidogrel/ticlopidine, percentage of early coronary angiography, percentage of PCI procedures, and percentage of patients who had CABG. The Z test for 2 proportions was used to compare the KSA values with the international figures. Analysis was done by stata 10. Level of significance was set at 5 %.

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Results

Baseline characteristics were typical for diabetics presenting with ACS, with a mean age of 67 ± 15 years. Males constituted 36 % of the patients and 94 % of patients were type-2 diabetics.

Evidence of end-organ damage was present in a relatively small percentage (11 % retinopathy, 16 % nephropathy, and 8 % neuropathy). The predominant symptom at presentation was chest pain (occurring in 78 %). 75 % were in class-I Killip classification. 18 % had normal ECG while 47 % had ST depression, 10 % had transient ST elevation, and 17 % had T-wave inversion.

Random glucose at the time of presentation was 203 ± 85 mg/dl, fasting glucose was 155 ± 61 mg/dl, HbA_{1c} was 7.5 ± 1.6 (available for 53 % of patients), and creatinine was 1.3 ± 0.9 mg/dl.

Primary clinical outcome measure was in-hospital death in 3.1 % and STEMI in 4.6 %.

DM medications were mostly applied orally, predominantly metformin and sulfonylureas, while about 1/3 were receiving insulin. "Type-2:type-1 DM ratio" in KSA was comparable to the international ratio (94:6).

The high risk factors were less prevalent in KSA in comparison to the international data: ST ≥ 0.5 mm (17 % vs 46 %; p = 0.005), troponin + ve (40 % vs 70 %; p < 0.0001), TIMI > 3 (32 % vs 62 %; p < 0.0001; Figure 1). The utilization of GPIIb/IIIa inhibitors in the international data of MIDAS was 37.4 %, which was significantly higher than in KSA (18.3 %; p = 0.046; Figure 2).

On the contrary, the utilization of clopidogrel/ticlopidine was higher in KSA (96.8 %, as recommended by the ACC/AHA guidelines), exceeding the international 74.7 % (p < 0.0001; Figure 3).

The overall percentage of early coronary angiography in Saudi Arabia (38.9 %) was lower than in the international samples (46.1 %; p = 0.292; Figure 4).

The percentage of PCI procedures was lower in KSA (24.6 %) in comparison to the international 46.0 % (p = 0.001; Figure 5), while the percentage of patients who had CABG was higher in KSA (10.3 %) in comparison to the international percentage (9 %; p = 0.864; Figure 6). However, the overall percentage of patients who had revascularization (PCI and/or CABG) was lower in KSA (34.9 %) in comparison to the international 54.8 % (p = 0.005; Figure 7).

Discussion

The main goal of the MIDAS registry was to improve the awareness of this potentially deadly combination of DM and ACS and to monitor the application of evidence-based guidelines in this condition. This goal is of great importance since a significant number of patients presenting with NSTE-ACS are diabetic and this number is expected to increase as a result of the global DM epidemic. More importantly, diabetic patients who present with ACS are at high risk for developing subsequent cardiovascular events. At the same time, diabetic patients derive greater benefit than non-diabetic counterparts from aggressive antithrombotic therapy, early coronary angiography, and percutaneous coronary intervention [6].

Despite the evidence of a prothrombotic diabetic state, the exact mechanisms linking DM to the initiation and progression of the atherosclerotic process remain elusive. An angioscopic study performed in ACS patients revealed that plaque ulceration and intracoronary thrombus were more frequent among diabetic patients than among non-diabetics [14]. Similarly, the incidence of thrombus was found to be higher in atherectomy specimens from patients with DM [15]. Moreover, increased levels of procoagulant agents and decreased concentrations of endogenous anticoagulants have been documented in DM [16]. In addition, diabetic individuals have increased platelet activation and aggregation to shear stress and platelet agonists [17] and, more importantly, platelets of diabetic individuals are larger and have an in-

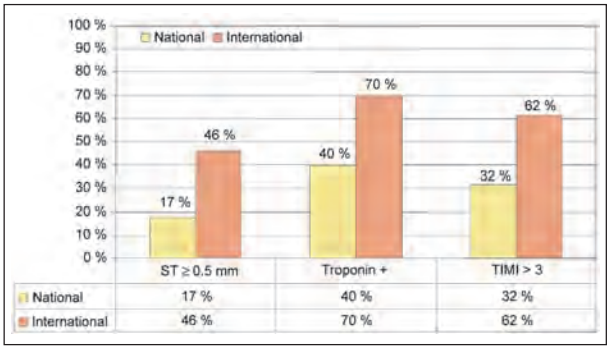

Figure 1. Distribution of risk factor prevalence (%).

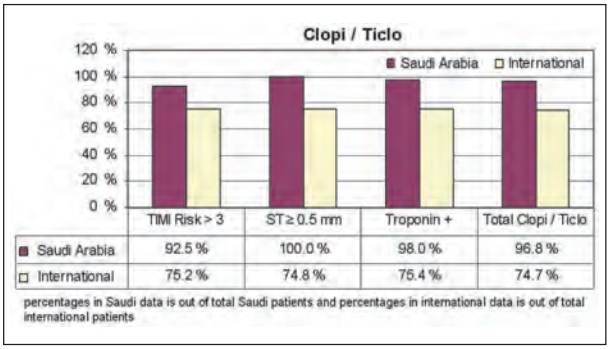

Figure 3. Clopidogrel utilization based on risk factors.

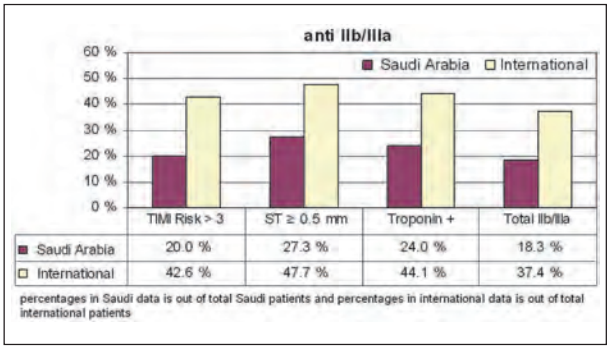

Figure 2. GPIIb/IIIa utilization based on risk factors.

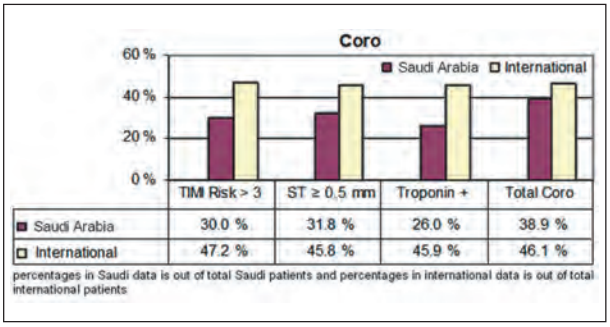

Figure 4. Percentage of early coronary angiography approaches based on risk factors.

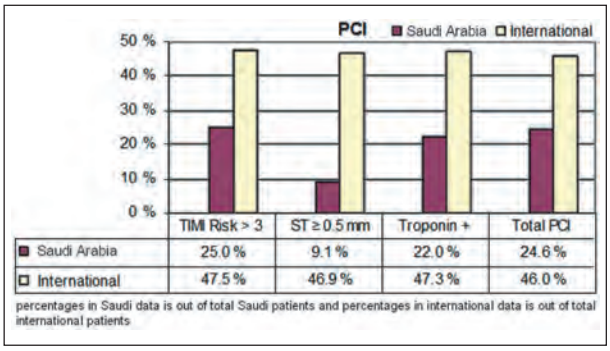

Figure 5. Percentage of PCI based on risk factors.

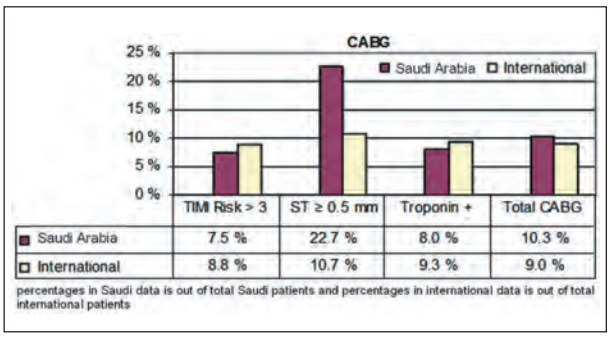

Figure 6. Percentage of CABG based on risk factors.

creased number of glycoprotein (GP) IIb/IIIa receptors per platelet [18].

Data from MIDAS showed, in general, a satisfactory use of evidence-based medicine and acceptable rates of in-hospital death or MI in DM patients who present with ACS. However, there was a marked underutilization of GPIIb/IIIa inhibitors despite recommendations for their use in diabetic patients presenting with ACS. Reviewing the data from KSA, we can easily notice that utilization of GPIIb/IIIa inhibitors, particularly in the elderly population, was significantly less frequent than in the international samples. Seemingly, this is related to concerns about bleeding complications of intensive antiplatelet strategy.

It is also noteworthy that some of the recent reports and guidelines showed more concern about the increased risk of bleeding in this setting, particularly upon using double or triple antiplatelet therapy in combination with heparin [19].

On the contrary, the utilization of clopidogrel/ticlopidine was higher in KSA in comparison to the international figures. This observation was difficult to interpret; however, it might be related to financial issues or the greater interest in use of clopidogrel in patients who are not receiving GPIIb/IIIa inhibitors.

In general, indications for coronary angiography in most cases of the MIDAS registry were predominant because it was a routine strategy at the admitting institution (72 %).

The overall percentage of early coronary angiography in KSA was lower than the international figure. This might be related to the lower number of centers that had catheterization laboratories in the MIDAS registry.

Moreover, the overall percentage of patients who had revascularization (PCI and/or CABG) was lower in KSA in comparison to the international figures, showing a relative tendency towards conservative management in our region. Again, this is probably related to concerns about complications of intervention and a lower number of centers that had catheterization laboratories.

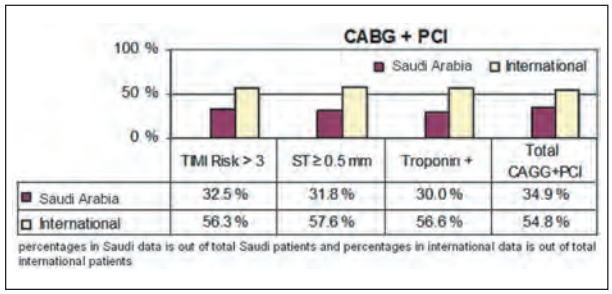

Figure 7. Percentage of CABG + PCI based on risk factors.

However, in another Saudi registry, coronary angiography was performed in 260 of the 435 patients in the entire cohort (60 %); of these, 39 % had percutaneous coronary intervention and coronary artery bypass graft surgery was performed in 9 % of the patients [3].

MIDAS is a registry and accordingly it has some limitations that are common to most registries where no additional diagnostic, monitoring, or therapeutic procedures are applied to the patients. Thorough screening for diabetes mellitus (including glucose tolerance test) was not part of the study and this could have underestimated the real number of patients with diabetes in the study population. Patients were classified into diabetic and non-diabetic according to the local practice.

Conclusions

MIDAS aimed to increase the awareness of the use of evidence-based treatment in the potentially lethal combination of DM and ACS. There was a satisfactory use of evidence-based medicine in KSA in such a patient group. Further studies and clinical trials are required to identify the clinical significance of the differences in the Saudi practice in comparison to the international one.

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Multi-Valve Multi-Organism Infective Endocarditis Associated with Giant Tricuspid Valve Vegetation

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Case Report

A 42-year-old female with a history of intravenous drug use as well as hepatitis B and C presented with septic shock and respiratory distress. Blood cultures grew beta-haemolytic group-B streptococcus species and methicillin-sensitive staphylococcus aureus. She was treated with intravenous antibiotics and was admitted to the intensive care unit for inotrope support and mechanical ventilation. Transthoracic echocardiography demonstrated a giant vegetation on the tricuspid valve measuring 6.6 centimetres in length causing severe tricuspid regurgitation (Figure 1). Additionally, there was a large 2-centimetre vegetation on the aortic valve as well as probable involvement of the posterior leaflet of the mitral valve. While being considered for urgent surgery her pupils became fixed and dilated. Urgent computed tomography scan of her brain demonstrated massive embolic infarcts involving the right anterior and middle cerebral artery territories. The patient passed away shortly after hospital admission.

Discussion

There are only few reports of large tricuspid valve vegetations in the literature. Song et al reported a case of a 33-year-old male presenting with right-sided heart failure who developed a vegetation on the tricuspid valve following catheter ablation [1]. The patient underwent successful tricuspid valve replacement. Furui et al described a case of a 71-year-old male who developed ventricular septal perforation caused by right-sided infective endocarditis and a 1.5-centimetre vegetation on the tricuspid valve [2]. The patient successfully underwent vegetation excision, debridement, ventricular septal perforation patch closure, and tricuspid valve replacement. This patient had only single-organism and single-valve involvement: methicillin-sensitive staphylococcus aureus was identified as the sole organism, involving only the tricuspid valve.

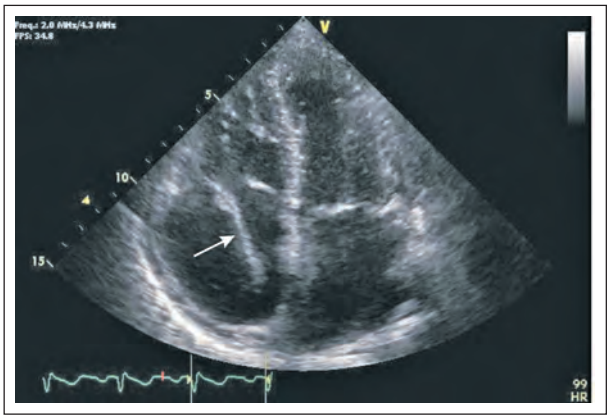

Figure 1. Apical 4-chamber view of transthoracic echocardiogram demonstrating the giant vegetation on the patient's tricuspid valve measuring 6.6 cm in length (arrow), causing severe tricuspid regurgitation.

Our case is unique for 2 reasons. First, the giant tricuspid valve vegetation identified is the largest reported in the literature to date. Second, this case highlights the increased morbidity and mortality of infective endocarditis associated with intravenous drug use, with often multiple valves and multiple organisms being implicated. The other reported cases of tricuspid valve vegetation in the literature occurred in non-intravenous drug users. Those patients were all successfully treated before major multi-organ septic and embolic complications. In comparison, our patient had already developed pulmonary septic emboli at the time of admission, and died shortly after admission from infarcts caused by septic emboli to her brain before any surgical treatment. This demonstrates that even with best available medical care, infective endocarditis in intravenous drug users can have a very high morbidity and mortality rate. We hypothesize that several factors may have contributed to the patient's mortality in this case: (1) The intravenous drug user is less likely to present to medical attention early; (2) due to intravenous drug use and the possibility of needle sharing and recycling, multiple organisms are often involved. In our case, both staphylococcus aureus and streptococcus species were implicated; (3) the involvement of multiple heart valves is more likely in intravenous drug users. Our patient was found to have vegetations on her tricuspid, aortic, and mitral valves. This is very different to the reported cases of single-organism tricuspid valve-only infective endocarditis in non-intravenous drug users.

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