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GRACE score of myocardial infarction patients correlates with oxidative stress index, hsCRP and inflammation

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ABSTRACT

Background: Etiopathogenesis of myocardial infarction (MI) is contributed by oxidative injury and inflammatory response. The interplay of these processes determines outcomes in MI patients. However, studies showing the relationship of oxidative stress and inflammatory cytokines with prognosis and severity of MI are lacking.
Objective: The present study was designed to assess the degree of oxidative stress and inflammation in correlation with GRACE (Global Registry of Acute Coronary Events) risk score in patients of MI.
Methods: MI patients were segregated according to GRACE risk score and age. Blood samples of the patients were used for determination of level of total peroxide, Total Antioxidant Status (TAS), Oxidative Stress Index (OSI), pro-inflammatory molecules such as high sensitive C-reactive protein (hsCRP), Tumor Necrosis Factor α (TNFα), interleukin 1 β (IL 1β), interleukin 6 (IL 6), anti-inflammatory cytokine interleukin 10 (IL 10), and TNFα/IL 10 cytokine ratio.
Results: We found significant elevation in concentration of total peroxide, TAS and OSI in all MI patients than healthy volunteers, this elevation showed pronouncement with higher GRACE score (GS) and age. Alteration in pro-inflammatory and anti-inflammatory cytokines was seen in MI patients than control group, and this alteration displayed polarization with GS and age.
Conclusion: MI patients with higher GS and age have greater degree of OSI and inflammation, and these biochemical parameters were significantly correlated with GS and thus disease severity.

1. Introduction

Ischemic heart disease remains at the zenith of responsible factors for majority of the cardiovascular events and heart failure worldwide. Myocardial infarction is also an ischemic heart disease, occurring due to occlusion of coronary artery. Oxidative stress and inflammation play a pivotal role in the etiopathogenesis of plaque formation, induction of its instability and subsequent formation of the thrombus (Kobayashi et al., 2003). Local inflammatory cells like neutrophils and macrophages along with cells of the vessels produce variety of chemokines, cytokines, and adhesion molecules instigating the biosynthesis of many other pro-inflammatory molecules, involving both innate and adaptive immune responses (Businaro, 2013). These inflammatory infiltrates also produces reactive oxygen species (ROS) and promote endothelial dysfunction by oxidation of various cellular signaling proteins and lipid bilayer. Superoxide radical among ROS reacts with anti-atherogenic component, nitric oxide and forms more intoxicating pro-atherogenic peroxynitrite (Leeuwenburg et al., 1997). However, damage caused by these reactive intermediates depend upon amount of antioxidants present for limiting them. Hence, measurement of total antioxidant status (TAS) is imperative, and studies on TAS in MI are very less.

Furthermore, enlarged production of high sensitive C-reactive protein (hsCRP) and interleukin 6 (IL6), a pro-inflammatory cytokine serving as the major determinant of formation of acute phase proteins have been found in patients of MI (Liuzzo et al., 1994; Andreotti et al., 1990). hsCRP serve as a risk predictor for recurrent events in patients of MI. But the triggers responsible for thrombotic obstruction of coronary artery in MI may not be restricted to hsCRP, IL 6 and other related cytokines like Tumor Necrosis Factor α (TNFα), interleukin 1β (IL 1β), there may be multiple factors involved including growth factors like vascular endothelial growth factors (VEGF), the key molecule promoting angiogenesis. They regulate function, growth, permeability, dilation and homeostasis of blood vessels and thus, are important mediators of angiogenesis in ischemic myocardial tissue and are also regarded as potential agents for treatment of MI and other peripheral vascular disease (Vuorio et al., 2012).

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Various risk classification systems are quiet often used for assessment of risk and prognosis in MI patients, one such scoring system is Global Registry of Acute Coronary Events (GRACE) risk score (Granger et al., 2003). In our study, we divided MI patients on the basis of GRACE score (GS) into two groups, one having GS less than or equal to hundred (GS ≤ 100), while the other having GS greater than hundred (GS > 100). These patients were also segregated into two groups on the basis of age, patients having age less than or equal to 45 years (MI ≤ 45 years) and patients having age greater than 45 years (MI > 45 years). Similarly, the healthy volunteers were also divided in these two age groups. Although, GS is associated with early and late mortality prediction in patients of Acute Coronary Syndrome (ACS), but linkage of the score with inflammatory markers remains unknown. So, the present study was undertaken to explicate the level of pro-inflammatory (TNF α, IL 1β, IL 6), anti-inflammatory (IL 10) cytokines, pro-inflammatory/ anti-inflammatory cytokine ratio (TNFα/IL 10), hsCRP, and VEGF in MI patients with low and high GS. In addition to this, we also estimated the degree of plasma total peroxide, TAS, and oxidative stress index (OSI) in different study groups. We further elucidated the relatedness of these biochemical parameters with GS.

2. Materials and methods

2.1. Study population

One hundred twenty five patients diagnosed with MI Thygesen et al. (2007) were enrolled for the study. Fifty seven healthy individuals served as control group in our study. Informed consent in written form was obtained from healthy individuals and relatives of the admitted patients. The study was approved by Institutional Ethics and Research Advisory Committee of the hospital. The duration of study period was 15 months from January 2016 to March 2017 and patients were inhabitants of north Indian region. Pre-hospital treatment of hypertensive patients was angiotensin converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blockers; diabetic patients were taking oral antidiabetic agents (metformin, sulphonylurea) whereas hyperlipidemics were on lipid lowering drugs (statins).

Patients having arterial fibrillation, nonspecific electrocardiogram changes, left bundle branch block, heart failure, neoplastic disease and age above seventy five years were not included in the study.

Blood samples were collected from the patients at the time of admission and from healthy individuals during their routine medical checkup in two tubes for separation of plasma and serum.

2.2. Calculation of GS

For each patient, GS was calculated using GRACE 2.0 calculator at the time of admission GRACE ACS Risk Score (2016) by using following variables: age, systolic blood pressure, heart rate, creatinine, cardiac arrest at admission, ST-segment deviation in electrocardiogram, abnormal cardiac markers and Killip class.

2.3. Estimation of plasma level of total peroxide

Total peroxides concentration in plasma was determined by utilizing FOX2 reagent as described previously Harma et al. (2005). For preparation of FOX2 reagent ammonium ferrous sulphate was dissolved in H_2SO_4, and to this solution butylated hydroxytoluene (BHT) in methanol was added. After addition of xylenol orange, working solution was obtained. Assay mixture of plasma and FOX2 reagent was incubated at 25 °C, followed by centrifugation at 12,000 g for 10 min. Supernatant was collected to read the absorbance at 560 nm.

2.4. Determination of TAS in plasma

TAS in plasma was estimated by following the method of Erel (Ozcan, 2004). The coloured ABTS radical (2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) is reduced to the colourless form by the antioxidants content of the sample. This bleaching of coloured ABTS is recorded as absorbance at 740 nm. Assay calibration was done using standard antioxidant solution (traditional Trolox equivalent).

2.5. Oxidative stress index

OSI was described as the ratio of total peroxide to TAS. OSI (arbitrary unit) = [total peroxide /TAS] x 100 Aslan et al. (2011).

2.6. Assay of cytokines

Serum level of hsCRP (Calbiotech, USA), VEGF (Chongqing Biospes Co., Ltd, China), and cytokines TNF α, IL 1β (R & D Systems, USA), IL 6 and IL 10 (Diaclone, France) concentrations were measured using sandwich enzyme-linked immunosorbent assay kits, according to the manufacturer's instructions. The level of minimal detectable dose was 0.005 mg/L for hsCRP, 10 pg/mL for VEGF, 1.6 pg/mL for TNF??, < 1 pg/mL for IL 1β, 2 pg/mL for IL 6, and 0.98 pg/mL for IL 10. Pro-inflammatory/anti-inflammatory cytokine ratio was calculated by dividing the values of TNFα by that of IL 10.

2.7. Statistical analysis

Graph Pad Prism software (Graph Pad Software, San Diego, USA) was used for statistical analysis. Data values of the groups were checked for normal distribution using Shapiro Wilk test. To find the differences between the values of means among the groups, one way ANOVA followed by post hoc Tukey's test was used for parametric data and Kruskal Wallis test followed by Dunn's multiple comparison test was used for non- parametric data. Correlation between parameters was assessed by Pearson and Spearman correlation test. Receiver operating characteristic curves were used for determining the predictive value of parameters for distinguishing the two groups GS ≤ 100 and GS > 100. For all analysis, results were considered significant if $p \leq 0.05$.

3. Results

Baseline characteristics of the study population are shown in Table 1. Age and body mass index (BMI) was not found to be different significantly in the three groups. The prevalence of hyperlipidemia was highest among risk factors in the two groups of patients, followed by hypertension (HTN), smoking, diabetes mellitus (DM) and family history of coronary artery disease (CAD) in group GS ≤ 100; but followed by HTN, smoking, CAD family history and DM in group GS > 100.

We found augmented plasma level of total peroxide in different groups of the patients, about 0.48 fold higher in group GS ≤ 100 and 0.87 fold higher in group GS > 100 as compared to control group ($p < 0.01$). The elevation of total peroxides in group MI ≤ 45 years was 0.79 times than group control ≤ 45 years and 0.65 times in group MI > 45 years versus (vs.) control > 45 years group. Also, total peroxide content in groups GS ≤ 100, MI ≤ 45 years was significantly lowered in relation to groups GS > 100, MI > 45 years, respectively (Figs. 1 and 2,). Furthermore, higher age control group (control > 45 years) was also having 0.50 times greater concentration of total peroxide as compared to lower age control group (control ≤ 45 years). On the other hand, TAS was significantly diminished in patients (0.30 fold decrease in group GS ≤ 100 and 0.42 fold decrease in GS > 100 group) than healthy controls. The decline in TAS was 0.28 and 0.40 times in groups MI ≤ 45 years and MI > 45 years with respect to groups control ≤ 45 years and control > 45 years, respectively. The mean TAS values in groups GS > 100, MI > 45 years were found to be lessened when compared with groups GS ≤ 100, MI ≤ 45 years, respectively ($p < 0.01$). Ratio of total peroxide/TAS, OSI was found to be greatly elevated in MI patients (1.22 and 2.59 fold in groups

Table 1
Clinical characteristics of healthy volunteers and MI patients.

Variables	Control (n = 57)	GS ≤ 100 (n = 66)	GS > 100 (n = 59)	p
Age	50.28 ± 9.71[a*]	50.98 ± 11.51[a*]	52.50 ± 12.76[a*]	ns
Sex M/F	40/17	49/17	47/12	ns
Body Mass Index (Kg/m^2)	23.28 ± 2.48[a*]	24.07 ± 3.12[a*]	24.28 ± 3.29[a*]	
Family history of coronary artery disease	4	8	10	
Hypertension		14	15	
Diabetes mellitus		12	9	
Hyperlipidemia		20	25	
Smoking		14	13	
Admission blood glucose (mg/dl)	94.03 ± 20.15[a*]	127.56 ± 31.91[b*]	133.98 ± 29.17[b*]	0.001
Systolic blood pressure (mm Hg)	126.64 ± 18.09[a*]	149.45 ± 24.58[b*]	155.18 ± 25.92 [b*]	0.001
Diastolic blood pressure (mm Hg)	83.56 ± 13.90*	87.16 ± 14.01*	89.16 ± 15.73*	ns

*Values are expressed as mean ± SD. The different letters (a, b) indicate statistically significant difference among groups.

GS ≤ 100 and GS > 100, respectively) as compared to control individuals. Upon comparison with age matched control groups, OSI in groups MI ≤ 45 years and MI > 45 years was raised to 1.67 and 2.00 fold, respectively). Remarkable escalation in OSI was obtained in groups GS > 100, MI > 45 years with respect to GS ≤ 100, MI ≤ 45 groups, respectively ($p < 0.01$)

hsCRP level was highly elevated in patients of MI than control group ($p < 0.01$). Moreover, heightened levels of hsCRP in groups GS > 100 (3.40 times vs. healthy controls), MI > 45 years (3.19 times vs. control ≤ 45 years) as compared to groups GS ≤ 100 (2.25 times vs. healthy controls), MI ≤ 45 (1.96 times vs. control > 45 years) respectively, were also statistically significant. The mean serum VEGF level in patients was increased (0.81 fold in patients with GS ≤ 100 and 0.90 fold in group GS > 100) in relation to healthy individuals to a noteworthy extent ($p \le 0.01$). The enhanced VEGF content was 0.89 & 0.83 times in groups MI ≤ 45 years and MI > 45 years with respect to age matched control groups. However, the elevated content of VEGF in groups GS > 100, MI ≤ 45 years, compared to groups GS ≤ 100, MI > 45 years respectively was not found to be significantly different (Figs. 1 and 2).

The cytokines TNFα, IL 1β, and IL 6 levels were significantly higher in the MI subjects relative to control and the inflated content of these cytokines in groups GS > 100, was exhibiting statistical significance as compared to GS ≤ 100 (Fig. 3). The elevation in TNF α, IL 1β, and IL 6 concentration in group GS ≤ 100 than controls was 0.47, 0.52 and 0.48 fold, respectively. Also, with reference to healthy individuals, increase in the level of TNFα, IL 1β, and IL 6 was 0.98, 0.88 and 0.76 fold, respectively in group GS > 100. As compared to respective age matched control groups, the enhancement in TNFα level was 0.53 times, 0.62 times in IL 1β level, 0.58 times in IL 6 level in group MI ≤ 45 years and 0.81 (TNFα), 0.73 (IL 1 β), 0.64 (IL 6) times in group MI > 45 years. However, level of these cytokines were enlarged in group MI > 45 years as compared to the group MI ≤ 45 years, but this enlargement does not reached significance level of $p < 0.05$ except that

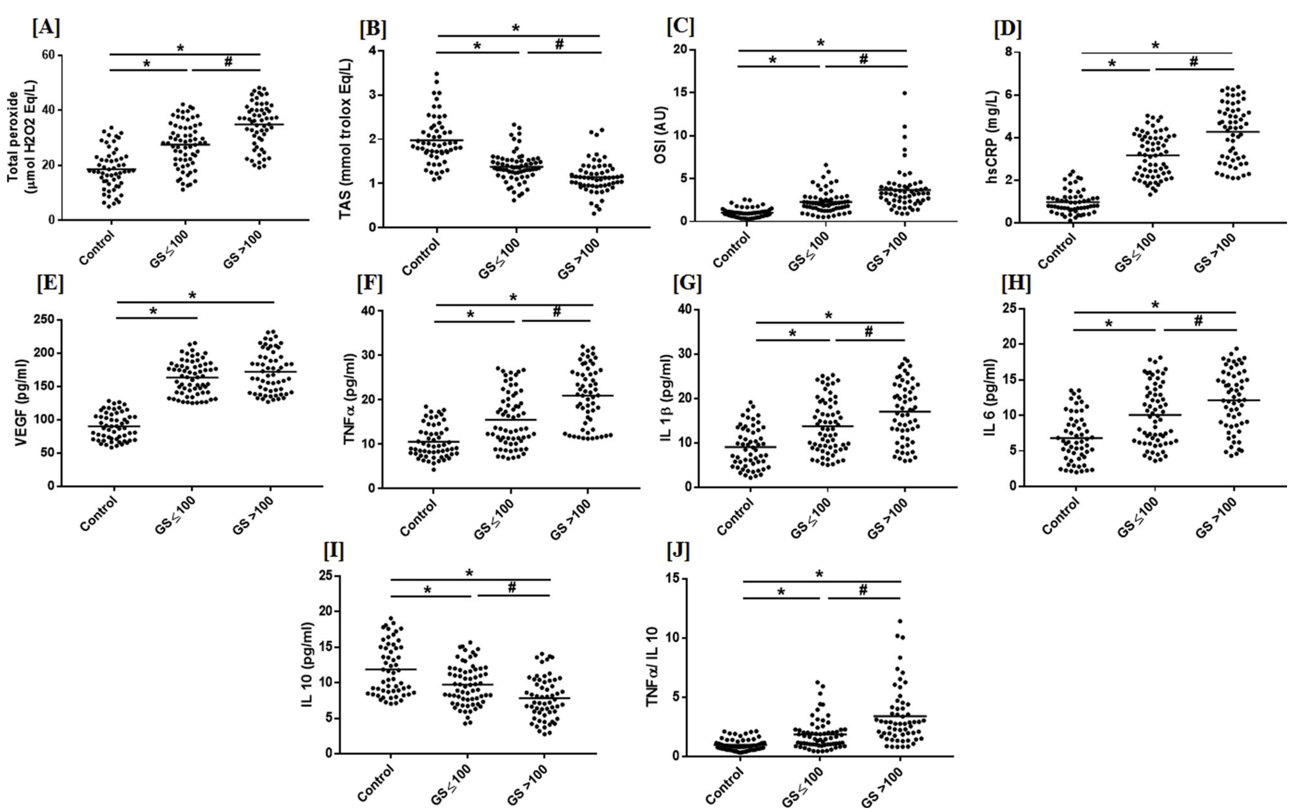


Fig. 1. Level of (A) total peroxide, (B) TAS, (C) OSI, (D) hsCRP, (E) VEGF, (F) TNFα, (G) IL 1 β, (H) IL 6, (I) IL 10, (J) TNFα/IL 10 ratio in control, GS ≤ 100, and GS > 100 groups. *$p \le 0.05$ vs. control, # $p \le 0.05$ vs. GS > 100 group.

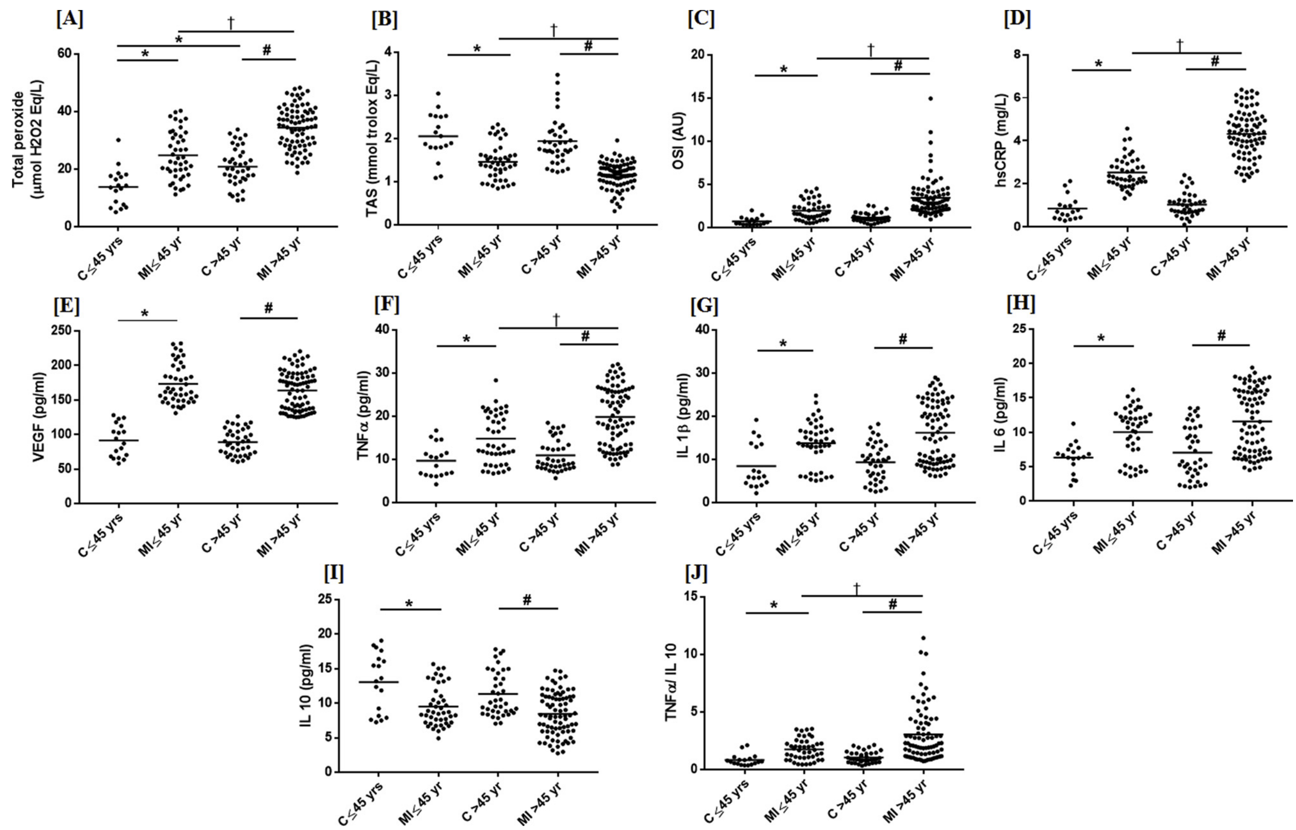


Fig. 2. Level of (A) total peroxide, (B) TAS, (C) OSI, (D) hsCRP, (E) VEGF, (F) TNFα, (G) IL 1 β, (H) IL 6, (I) IL 10, (J) TNFα/IL 10 ratio in control ≤ 45 years, MI ≤ 45 years, control > 45 years, MI > 45 years. * p ≤ 0.05 vs. control ≤ 45 years, # p ≤ 0.05 vs. control > 45 years, † p ≤ 0.05 vs. MI > 45 years.

for TNFα. The anti-inflammatory cytokine IL 10 showed significant decrement in the patients (0.18 fold in GS ≤ 100 group and 0.34 fold in group GS > 100) with respect to healthy volunteers. The groups GS > 100 had much reduced amount of IL 10, compared to groups GS ≤ 100 ($p < 0.01$). Furthermore, relatively higher IL 10 level in group MI ≤ 45 years (0.27 times decrease vs. control ≤ 45 years) than group MI > 45 years (0.25 times decrease vs. control > 45 years) does not differed significantly. We measured ratio of TNFα/IL 10 and found it to be heightened in the patients (0.89 fold elevation in GS ≤ 100 vs. control; 2.46 fold increase in GS > 100 vs. control; 1.10 fold enlargement in MI ≤ 45 years vs. control ≤ 45 years, 1.90 fold rise in MI > 45 years vs. control > 45 years). Also, the groups GS ≤ 100, MI ≤ 45 years showed significantly lower TNFα/IL 10 ratio with respect to groups GS > 100, MI > 45 years (Figs. 1 and 2).

Correlation analysis showed GS to be in significant positive correlation with total peroxide (r = 0.601, p ≤ 0.001), OSI (r = 0.572, p ≤ 0.001), hsCRP (r = 0.651, p ≤ 0.001), TNFα (r = 0.600, p ≤ 0.001), IL 1β (r = 0.439, p ≤ 0.001), IL 6 (r = 0.431, p ≤ 0.001), and TNFα/IL 10 ratio (r = 0.573, p ≤ 0.001) and inverse association with TAS (r = -0.502, p ≤ 0.001), IL 10 (r = -0.418, p ≤ 0.001) (Table 2). However, VEGF (r = -0.089, p ≤ 0.32) was not found to be significantly correlated with GS. All these parameters along with age, BMI, gender, excluding VEGF were incorporated in multiple linear regression analysis. Out of all these variables only total peroxide and hsCRP were found to be significant predictors of high GS. Through ROC curve analysis, optimum cut off value of total peroxide was 33.78 (AUC = 0.733, 61% sensitivity & 74% specificity), OSI was 2.57 (AUC = 0.729, 66% sensitivity & 71% specificity), hsCRP was 3.61 (AUC = 0.744, 64% sensitivity & 60% specificity), TNFα/IL 10 was 2.10 (AUC = 0.734, 64% sensitivity & 72% specificity) for determining high GS (Fig. 4).

4. Discussion

ROS generated during infarction potentially impede myocardial contraction through interaction with membrane proteins and lipids, thereby initiating peroxidative cellular damage. This also results in generation of oxidized derivatives of proteins, DNA, and lipids, which serve as signal for induction of inflammatory response by activation of complement components and leukocytes which secrete an array of molecules like cytokines, chemokines, matrix metalloproteinase in affected region leading to induction of structural alteration in heart's chamber (Elmoselhi et al., 2003). In present study, we determined the relationship between oxidative stress parameters, inflammatory cytokines and severity of the disease and we found comparatively elevated total peroxide in plasma of patients having higher GS and age, this again points in the direction of oxidation of lipid molecules by free radical species which may also contribute in alteration of cardiomyocyte permeability, similar to our previous study (Shahzad et al., 2018). We also found total peroxide level to be significantly enhanced in higher age controls than lower age control individuals demonstrating increased lipid peroxidative damage with age. In consonance with our results, Junqueira et al reported a positive correlation between age and thiobarbituric acid reactive substances (TBARS) (Junqueira et al., 2004). Additionally, oxidative stress is considered a risk factor for ageing and increase in oxidative stress with aging also contribute to the development of chronic inflammation and diseases.

TAS reflects clearly the redox status of the plasma and we observed significantly lower value of TAS in all patients than healthy volunteers which is indicative of impairment of the defense system that neutralize free radicals because of such grave production of free radicals that antioxidant molecular system falls short in capturing. Moreover, OSI was significantly increased in patients with higher age and GS as compared to lower age and GS groups, respectively. Similar to our study, Aksoy et al. evaluated Total Oxidant Status (TOS), TAS and OSI

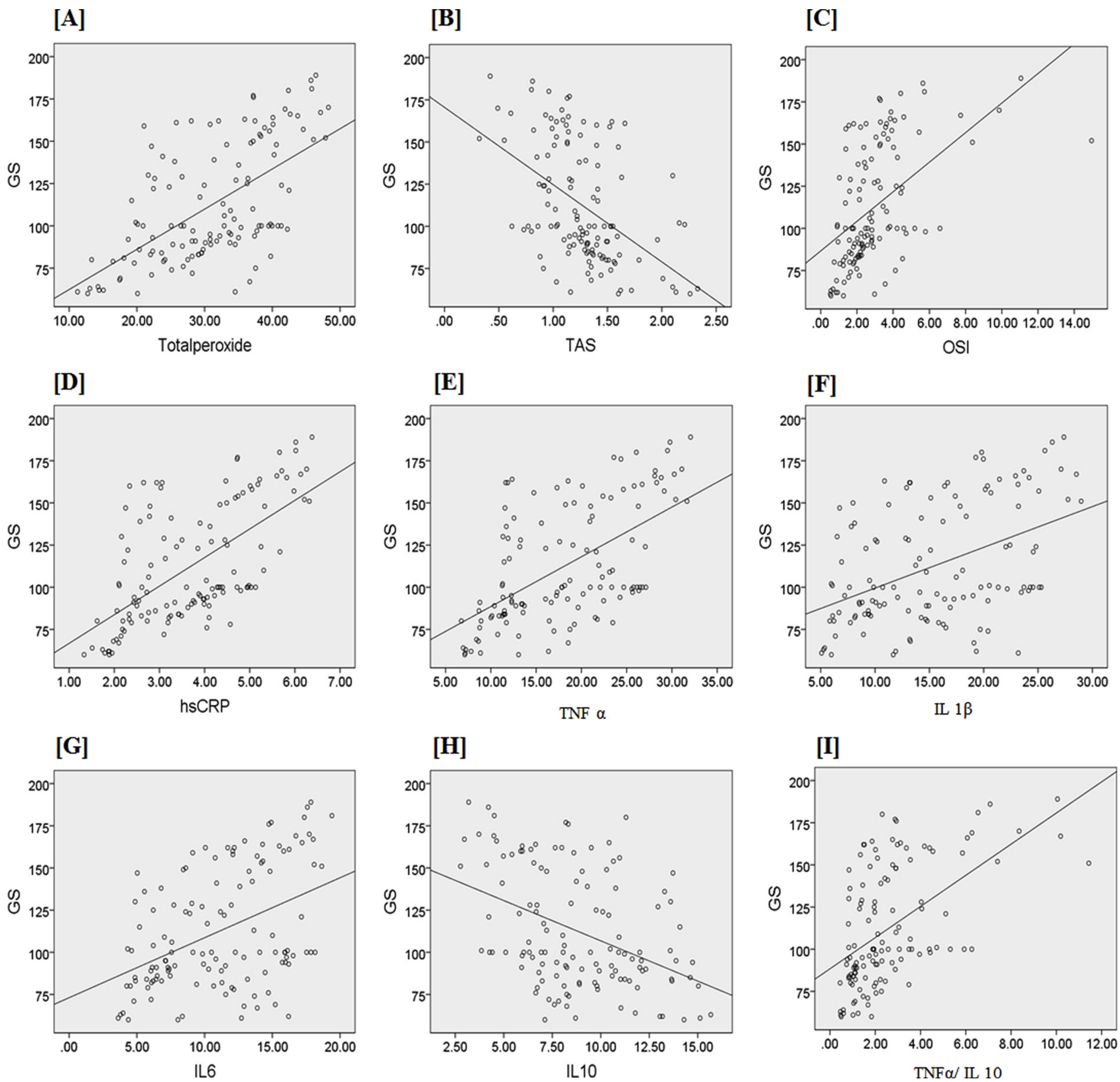


Fig. 3. Correlation of GS with total peroxide (A), TAS (B), OSI (C), hsCRP (D), TNFα (E), IL 1β (F), IL 6 (G), IL 10 (H), TNFα/IL 10 ratio (I).

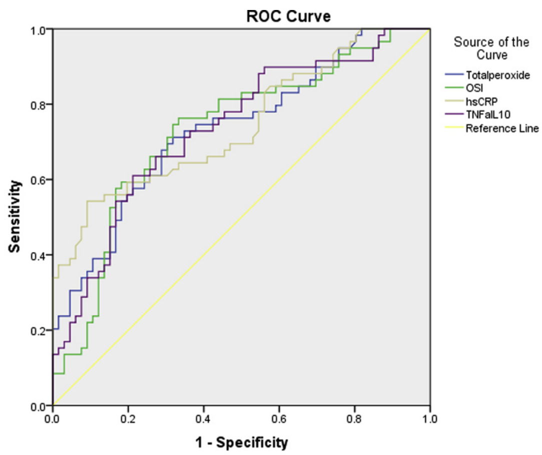
Table 2
Bivariate and multivariate relationship between GS and clinical, oxidative, inflammatory parameters in MI patients.

	Correlation coefficient	*p*	Standardized β regression coefficient[#]	*p*
Age	0.357	0.001		
Body Mass Index (BMI)	0.186	0.03		
Gender	0.048	0.59		
Total peroxide	0.601	0.001	0.486	0.001
TAS	−0.502	0.001		
OSI	0.572	0.001		
hsCRP	0.651	0.001	0.685	0.001
VEGF	−0.089	0.32		
TNFα	0.6	0.001		
IL 1β	0.439	0.001		
IL 6	0.431	0.001		
IL 10	−0.418	0.001		
TNFα/IL 10	0.573	0.001		

TNFα/IL 100.5730.001.

in patients having lower and higher SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery) score, and found patients with higher syntax score to be more engrossed with oxidative stress than lower syntax scorers and the score was positively correlated with TOS and OSI (Aksoy et al., 2012). We also got GS, index of disease severity to be in direct linkage with total peroxide, OSI and in indirect linkage with TAS.

Other study reported TAS and TOS or total peroxide content to be significantly lowered in CAD patients than healthy controls and Gensini score of the patients, a determinant of CAD severity correlated negatively with TAS but positively with age and TOS of heart tissue (Demirbag et al., 2010). We also found decreased TAS in MI patients but elevated total peroxide in patients than controls. This contrast in our results might be because our study included patients with occluded arteries which culminated in infarction, revealing further that atherosclerotic patients initially had decreased amount of both plasma antioxidants and oxidants but with progression of atherosclerotic plaques into ischemic injury, deficit in antioxidants continues with rise in oxidants. Since, we obtained total peroxide, OSI to be elevated in GS $>$ 100, MI $>$ 45 years groups, respectively than groups GS $\leq$ 100, MI $\leq$ 45, this illustrates oxidant related stress to be associated not only with disease severity but also with patients age.



Area Under the Curve					
Test Result Variable (s)	Area	Std. Error[a]	Asymptomatic Sig.[b]	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
Total peroxide	0.733	0.045	.000	0.645	0.820
OSI	0.729	0.046	.000	0.638	0.819
hsCRP	0.744	0.044	.000	0.658	0.831
TNFα/IL 10 ratio	0.734	0.045	.000	0.647	0.822

a. Under the nonparametric assumption
b. Null hypothesis true area= 0.5

Fig. 4. The diagnostic value of OSI and TNFα/IL 10 ratio to differentiate between MI patients having GS > 100 and GS ≤ 100.

VEGF functions as potent mitogen for endothelial cells. It stimulates collateral formation and tissue repair after wounding in cardiac muscles insulted by ischemia (Hojo et al., 2000). We found VEGF level significantly elevated at admission in MI patients relative to control group but VEGF elevation in group GS > 100 with respect to group GS ≤ 100 was not significant indicating that VEGF level at admission does not indicate higher GS. Also, higher age MI patients had reduced VEGF level than their lower age counterparts though not significant but presenting the view that elderly MI patients may had less chances of VEGF mediated angiogenesis and endothelial cell proliferation in the disease course. Our finding is supported by work of Yin et al which reported raised VEGF level in ischemic and hypoxic patients. They suggested the increased VEGF level in MI patients to be having protective nature of promoting angiogenesis (Yin et al., 2000).

Inflammatory markers like hsCRP is valuable prognostic marker in case of cardiovascular events. In one prospective study, patients going through peripheral vascular surgery having elevated hsCRP levels were found to have greater frequency of adverse cardiac events than those having lower hsCRP level (Zanati et al., 2009). We obtained much greater increase in serum hsCRP content in MI patients relative to controls and significant difference was noted in groups with GS > 100 and higher age as compared to their lower counterparts, revealing that the degree of inflammation is much larger in MI patients with higher GS and age. In the same line of thought, CRP and IL 6 levels after MI were reported to be higher in patients having age more than 70 years as compared to patients less than 70 years, along with exaggerated left ventricular remodeling (Mahara et al., 2006).

This study also revealed significant elevation in pro-inflammatory cytokines namely TNFα, IL 1β, IL 6 apart from inflammatory marker hsCRP reflecting the upsurge in their secretion, in MI patients. TNFα and IL 1 β, not only escalate the endothelial expression of adhesion molecules but also causes simultaneous secretion of soluble chemo attractants, production of matrix metalloprotease, enhancement of vascular permeability and all this further augments inflammation (Dinarello, 2011). Also, TNFα, IL 1β stimulate the production of IL 6, which further potentiate procoagulant effects by increasing the reactivity of platelets (Oleksowicz et al., 1995). We observed higher TNFα, IL1β and IL 6 in group GS > 100 relative to group GS ≤ 100, parallel to earlier finding (Alturfan et al., 2014). This shows that extensive ischemic damage to myocardial tissue can leads to local production and release of proinflammatory cytokines.

In contrast to IL 1β and IL 6, IL 10 functions by negatively modulating the immune response, it inhibits T cell mediated cytokine synthesis, promotes differentiation of antiatherogenic regulatory T (Treg) cells (Yamashita et al., 2003). We detected decreased plasma level of IL 10 in MI patients than healthy volunteers. Significantly dropped serum level of IL 10 was also witnessed in Unstable Angina Pectoris (UAP) patients who subsequently developed cardiovascular events than those without events (Anguera et al., 2002). Availability of lower amount of IL 10 to suppress chemoattraction of lymphocytes, and inhibition of proinflammatory molecules secretion might had been the reason for heightened amount of proinflammatory cytokines found in MI patients. Moreover, we found altitude in TNFα/IL 10 ratio in MI patients than controls, demonstrating the disturbance of intricate balance of pro and anti-inflammatory pathways with shifting of equilibrium towards pro-inflammatory state. Thus, TNFα, IL 1β, IL 6 levels were upregulated and that of IL 10 was down-regulated in MI patients possessing high severity in terms of GS and in those having higher age (Fig. 5). But the concentration of IL 1β, IL 6 and IL 10 were not significantly distinguished in the two age group representing that elderly and young MI patients may have similar state of inflammation after infarction, unlike oxidative stress.

Correlation analysis revealed GS to be prominently associated with oxidative stress and inflammation. Upon incorporation of all these parameters along with age, gender and BMI, only total peroxide and hsCRP were found to be significant predictors of high GS. ROC curve analysis revealed higher AUC for hsCRP, followed by TNFα/IL 10 ratio, total peroxide and OSI for screening of high GS in MI patients. Thus, total peroxide, OSI, hsCRP and TNFα/IL 10 ratio potentially differentiate MI patients with high GS than those having lower GS with sufficient accuracy. So, on the basis of Hosmer and Lemeshow's criteria (0.7 ≤ AUC < 0.8), total peroxide, OSI, hsCRP and TNFα/IL 10 ratio are acceptable parameters for identifying MI patients with high GS. Hence, these parameters could be used clinically for prediction of low and high GS in MI patients. Accordingly, total peroxide, OSI, hsCRP and inflammatory cytokines reflect disease severity in terms of GS in MI patients (Fig. 5).

Among strategies to reduce ROS induced detrimental effect in Cardiovascular Diseases (CVDs), antioxidant therapy has gained much recognition. In a previous study, intake of resveratrol containing grape

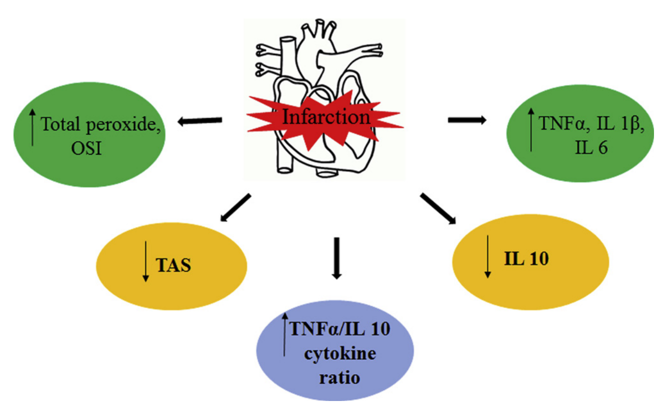


Fig. 5. Schematic representation of oxidative and inflammatory damage occurring during ischemic injury in MI. Increased level of total peroxide requires countering action of larger amount of antioxidants, thereby resulting in depleted TAS and elevated OSI. In addition, the content of inflammatory cytokines (TNFα, IL 1β and IL 6) increase with decrease in anti-inflammatory cytokine IL 10, leading to increased ratio of TNFα/IL 10 ratio.

extract was found to lower down the oxidation of low density lipoprotein (LDL) and increased anti-inflammatory molecules in patients of CAD (Tomé-Carneiro et al., 2012). Moreover, the generation of pro-inflammatory cytokines like TNF-α and IL-6 by Peripheral Blood Mononuclear Cells (PBMCs), were also diminuted in CAD patients, who consumed resveratrol (Tomé-Carneiro et al., 2013). The consumption of olive oil with larger phenolic composition by healthy individuals lead to decrease in ratio of total cholesterol to High Density Lipoprotein (HDL) and oxidative stress biomarkers (Weinbrenner et al., 2004). Furthermore, olive oil intervention inhibited endothelial dysfunction and decreased inflammatory parameters and in this way assisted in the treatment of atherosclerotic patients (Widmer et al., 2013). However, a meta-analysis showed no significant protection of curcumin on oxidation of LDL and level of total cholesterol in a heterogenous population (Sahebkar, 2014). The protection conferred by phytochemicals in CVDs are mainly attributed to their antioxidant, anti-inflammatory, and antithrombotic properties. In contrast to the preclinical studies, clinical trials for treatment by antioxidants in diseased condition are still under controversy. The limitation of the clinical studies depends on size and heterogeneity of the sample plus dose, bioavailability, pharmacological properties and absorption kinetics of antioxidant compounds. Thus, there is a requirement for larger and well controlled human studies for determining clinical usefulness of antioxidant treatment in CVDs.

The limitation of our study is that patients admitted to a single center and relatively small in number were enrolled and also correlation of GS with many other pro and anti-inflammatory cytokines was not evaluated.

5. Conclusion

Our study demonstrated that the levels of oxidative stress markers and inflammation significantly intensify in MI patients with rise in GS score and increasing age. So, OSI and TNFα/IL 10 cytokine ratio are easily measurable, cheap laboratory parameters and can be used in assessment of risk and severity in terms of GS in MI patients.

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Declarations of interest

None.

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