Original Article

Role of High Mobility Group Box-1 (HMGB1) in Obesity and Metabolic Syndrome

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ABSTRACT

Objective: To determine the role of hmgb1 in obesity and metabolic syndrome.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the Mohammad Islam Medical College Gujranwala and Sialkot Medical College Sialkot during Jan 2019 to March 2020.
Materials and Methods: 40 blood samples of adults Metabolic syndrome (MS) subjects and 20 samples of obese subjects between the ages 25-50 were obtained from M. Islam Teaching Hospital, Gujranwala and Sialkot Medical College Sialkot. 20 healthy subjects served as the control group. Fasting serum samples were analyzed for lipid profile, fasting blood glucose (FBG), and insulin and HMGB1 levels. Insulin and HMGB1 were estimated by commercially available ELISA kits. Insulin resistance was calculated by HOMA-IR index.
Results: Blood pressure showed significant differences among the three groups of subjects and was shown to be highest in the MS group. Significantly increased levels of FBS (124.13±8.77 mg/dl) were observed in the MS group as compared to obese and normal subjects (85.95±2.68 mg/dl and 84.50±1.06 mg/dl, respectively). Lipid profile revealed that triglycerides, LDL and cholesterol levels were significantly higher (213.78 ± 11.62mg/dl, 133.30 ± 6.45mg/dl and 218.98 ± 5.66mg/dl respectively) and HDL levels were relatively low in MS patients (44.18 ± 1.03) in comparison with obese triglycerides, LDL, cholesterol and HDL levels (133.85±6.31mg/dl, 106.15±4.31mg/dl, 166.00±5.56 mg/dl and 45.70±1.53mg/dl respectively) and normal subjects triglycerides, LDL, cholesterol and HDL levels(122.05±4.25mg/dl, 108.05 ± 3.56mg/dl, 152.15±6.00mg/dl and 46.65±1.07mg/dl respectively). Mean HMGB1 levels were maximal in patients with MS (19.68±2.58 mg/dl) and were significantly different from mean levels in subjects with obesity alone (11.06±1.12 mg/dl) and healthy subjects (13.28±0.65 mg/dl). Significantly elevated levels of insulin and insulin resistance were evident in patients suffering from MS (13.59±1.49 mg/dl and 4.06±0.54 mg/dl, respectively) as compared to healthy subjects (10.22±1.29 mg/dl and 2.13±0.26 mg/dl, respectively) and the obese group (9.95 ± 1.67 mg/dl and 2.06±0.32 mg/dl, respectively).
Conclusion: The current study demonstrates significantly higher levels of serum HMGB1 levels in MS patients in comparison with those of obese and control groups. The study suggests a role of HMGB1 as a pro-inflammatory cytokine in patients with MS. Significantly increased insulin resistance in MS patients further indicates that the HMGB1 related inflammatory pathway may be involved in pathogenesis of diabetes type 2.

Key Words: HMGB1, Metabolic syndrome (MS), obesity, insulin resistance, pro-inflammatory cytokine

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INTRODUCTION

The incidence of obesity has increased phenomenally during the past 3 decades and has emerged as a pandemic worldwide, as indicated by the World Health Organization (WHO). Obesity is generally associated with a number of co morbidities such as cardiovascular disease (CVD), diabetes mellitus type 2 (DM2), high blood pressure and some forms of cancer, amongst others resulting in a high mortality rate and posing a tremendous health and economic burden. Essentially, diseases that are related to or that are the outcome of obesity have become the main source of high mortality due to obesity [(1)]. DM2 has been shown to have a strong association with obesity and the main causal factor has been assumed to be development of insulin resistance. Insulin resistance also leads to a variety of other disorders including high blood pressure, impaired lipid metabolism, cardiovascular illness and polycystic ovarian syndrome [(2)].

Obesity may be defined as accumulation of an excess of adipose tissue mass resulting from an imbalance

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between caloric intake and caloric expenditure[3]. Obesity is also linked to a state characterized by chronic low grade inflammation and meta-inflammation evidenced by increased pro-inflammatory and decreased anti-inflammatory markers [(4)]. In the meta-inflammatory state the usual signs of inflammation are not present that are redness, increased body temperature, pain and loss of function, but it creates a pro-inflammatory state mainly in the liver and adipose tissue as well as in muscles and pancreas. Basically the dysregulation between metabolism and immunity is considered as the starting point for obesity and the resulting disorders [(5)]. Proinflammatory factors that are increased in obesity include IL-6, TNFα, IL-1, leptin and others. Recently a new protein, the high-mobility group box 1 (HMGB1) was identified as a proinflammatory mediator and shown to have the ability to activate several immune cells and production of various cytokines [(6)].

MATERIALS AND METHODS

This case control study was carried out on 80 subjects of both sexes 25-50 years of age. The study was approved by the Ethical Committee Sialkot Medical College Sialkot. The patients were recruited from M. Islam Teaching Hospital, Gujranwala & Sialkot Medical College Sialkot strictly on a voluntary basis.

The patients were selected on the basis of BMI > 35 with or without metabolic syndrome and hence were severely obese. Height and weight measurements were recorded and BMI was calculated by using the standard formula i.e weight in kg/ height in meter². A group of age-matched subjects was included in the study as control. The subjects were, therefore, categorized to the following 3 groups:

Group I: Severely obese with metabolic syndrome (BMI > 35) n=40.

Group II: Severely obese (BMI>35) n=20

Group III. Controls (BMI 20-25) n=20

Informed written consent was taken from all the subjects recruited in the study. Blood pressure was measured by using the standard mercury sphygmomanometer by auscultatory method.

Fasting blood sample of each participant in the study was obtained by venepuncture after a 12-hour overnight fast, into a gel clotting vial. Serum was separated by centrifugation at 3000 rpm for 15 to 20 minutes after 1 hour of sample collection. The remaining samples were ale quoted into tubes and stored at -80^{o}C until analyzed.

NCEP ATPIII criteria defines metabolic syndrome as presence of three or more of the following risk factors:

1. Fasting blood glucose ≥ 5.6 mmol/l (100 mg/dl)
2. Blood pressure ≥ 130/85 mmHg
3. Triglycerides ≥ 1.7 mmol/l (150mg/dl)
4. HDL-cholesterol Men: <1.03 mmol/l (40mg/dl), Women: <1.29 mmol/l (50mg/dl)
5. Central Obesity (Alberti et al.,2005)

Serum samples were analyzed for fasting blood glucose level by using the glucose oxidase method and lipid profile by automated enzymatic methods. Analysis of HMGB-1 level in human serum is carried out by ELISA kit (Bioassay Technology Laboratory, Korain Biotech Co. Birmingham, England) serum Insulin levels were also determined by ELISA using a commercially available kit (AccuBind ELISA Microwells, Monobind Inc. Lake Forest, CA 92630, USA). All assays were performed by following the manufacturers' standard kit protocol. Inter-assay coefficient of variation was less than 10% in all cases. HOMA1-IR index was calculated by using the formula: HOMA1-IR = fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/ L)/22.5.

Inclusion Criteria: A group of age-matched subjects was included in the study as control.

Exclusion Criteria: Patients with hepatic and infectious or endocrine diseases other than diabetes or impaired glucose tolerance, syndromic obesity, pregnancy, and lactation, were excluded from the study.

RESULTS

The physical characteristics of all subjects are summarized in Table 1.

The study subjects and controls were within the same age range (25-50yr). The BMI of subjects with MS and obesity was greater than 35, and, therefore, belonged to the category of severe obesity. The BMI of normal healthy controls was less than 25. Both, systolic and diastolic blood pressure were markedly and significantly higher in patients with MS (150±2.67 and 98.13±1.38 mmHg, respectively) compared to subjects with severe obesity (121.50±1.81 and 81.00±1.43 mmHg respectively) and the control group (119.50±1.53 and 77.00±1.05 mmHg, respectively). No significant difference was found in the mean blood pressure values of subjects presenting obesity and those of normal subjects.

Table No.1: Physical Characteristics

Variables	Metabolic syndrome (group-1) n=40 (mean ± sem)	Obese (group-2) n=20 (mean ± sem)	Controls (group-3) n=20 (mean ± sem)	P-value*
Age (years)	42.75 ± 0.926	37.50 ± 1.445	37.40 ± 1.035	<0.0001
Height (ft)	5.20 ± 0.42	5.17 ± 0.04	5.45 ± 0.06	0.001
Weight(kg)	95.20 ± 1.88	90.20 ± 1.708	63.65 ± 1.85	<0.0001
Bmi (kg/m^2)	37.98 ± 0.70	36.33 ± 0.41	22.90 ± 0.25	<0.0001
Systolic bp (mmhg)	150 ± 2.67	121.50 ± 1.81	119.50 ± 1.53	<0.0001
Diastolic bp (mmhg)	98.13 ± 1.38	81.00 ± 1.43	77.00 ± 1.05	<0.0001

*Analysis of variance (ANOVA); Significant difference among the three groups; P<001

Patients with MS were hyperglycemic – mean FBG levels were 124.13±8.77mg/dl and were significantly different from the other two groups (P<0.001). FBG levels in obese and normal subjects were not significantly different and were within the normal range (85.95±2.68 and 84.50±1.06 mg/dl, respectively).

Mean values of components of lipid profile and total cholesterol levels are shown in Table 2 and Figures 2,3,4,5.

Serum triglycerides were significantly raised in patients with MS compared to the obese and control group (213.78±11, 133.85±6.31 and 122.05±4.25 mg/dl, respectively). Although mean triglyceride levels were higher in obese than in control group, the difference was statistically not significant. Low-density lipoprotein (LDL) levels were significantly (p=0.002) higher in metabolic syndrome group (133.30 ± 6.45mg/dl) as compared to obese group (106.15 ± 4.31mg/dl) and control group (108.05 ± 3.56mg/dl). However, in our subjects we did not find any significant difference in levels of HDL although they tended to be lower in patients with MS as compared to the other two groups, severely obese and control (44.18±1.03 vs 45.70±1.53 and 46.65±1.07 mg/dl, respectively). Mean serum total cholesterol levels were markedly and significantly higher (P<0.001) in the MS group compared to the subjects with obesity alone and the control subjects. The mean levels of cholesterol in control, obese and metabolic syndrome groups were 152.15±6.00, 166.00±5.56 and 218.98±5.66 mg/dl, respectively.

Mean serum HMGB1 levels were shown to be maximal in patients with MS (19.68±2.58 ng/ml) and differed significantly (P< 0.05) from those of subjects with obesity (11.06±1.12 ng/ml) and normal body weight (13.28±0.65 ng/ml). Interestingly, HMGB1 levels were shown to be normal in patients presenting obesity alone.

Table No.2: Biochemical Spectrum

	Metabolic syndrome (group-1) n=40 (mean ± sem)	Obese (group-2) n=20 (mean ± sem)	Controls (group-3) n=20 (mean ± sem)	P-value*
Fasting blood sugar (fbs)	124.13 ± 8.77	85.95 ± 2.68	84.50 ± 1.06	<0.0001
Triglycerides	213.78 ± 11.62	133.85 ± 6.31	122.05 ± 4.25	<0.0001
Ldl	133.30 ± 6.45	106.15 ± 4.31	108.05 ± 3.56	0.002
Hdl	44.18 ± 1.03	45.70 ± 1.53	46.65 ± 1.07	0.326
Cholesterol	218.98 ± 5.66	166.00 ± 5.56	152.15 ± 6.00	<0.0001
Insulin	13.59 ± 1.49	9.95 ± 1.67	10.22 ± 1.29	0.166
Hmgb1	19.68 ± 2.58	11.06 ± 1.12	13.28 ± 0.65	0.021
Insulin resistance	4.06 ± 0.54	2.06 ± 0.32	2.13 ± 0.26	0.005

*Analysis of variance (ANOVA); Significant difference among the three groups; P<0.05

Table No.3: Multiple Comparison of Dependent Variables by Scheffe Test

Dependent Variable	Comparison A		Comparison B		Comparison C	
	Metabolic syndrome	Obese	Metabolic syndrome	Control	Obese	Control
SBP (mg/dl)	150 ± 16.94	121.50 ± 8.12	150 ± 16.94	119.50 ± 6.86	121.50 ± 8.12	119.50 ± 6.86
	P = 0.000*		P = 0.000*		P = 0.89	
DBP (mg/dl)	98.13 ± 8.74	81.00 ± 6.40	98.13 ± 8.74	77.00 ± 4.70	81.00 ± 6.40	77.00 ± 4.70
	P = 0.000*		P = 0.000*		P = 0.24	
FBS (mg/dl)	122.30 ± 55.82	85.95 ± 12.02	122.30 ± 55.82	84.50 ± 4.77	85.95± 12.02	84.50 ± 4.77
	P = 0.000*		P = 0.000*		P = 0.99	
Triglycerides (mg/dl)	213.78 ± 73.53	133.85 ± 28.23	133.85 ± 28.23	122.05 ± 19.00	133.85 ± 28.23	122.05 ± 19.00
	P = 0.000*		P = 0.000*		P = 0.79	
LDL(mg/dl)	133.30 ± 40.79	106.15 ± 19.29	133.30 ± 40.79	108.05 ± 15.94	106.15 ± 19.29	108.05 ± 15.94
	P = 0.01*		P = 0.02*		P = 0.98	
HDL(mg/dl)	44.18 ± 6.56	45.70 ± 6.87	44.18 ± 6.56	46.65 ± 4.79	45.70 ± 6.87	46.65 ± 4.79
	P = 0.67		P = 0.36		P = 0.89	
Cholesterol (mg/dl)	218.98 ± 35.79	166.00 ± 24.87	218.98 ± 35.79	152.15 ± 26.86	166.00 ± 24.87	152.15 ± 26.86
	P = 0.000*		P = 0.000*		P = 0.38	
Insulin (μ IU/ml)	13.35 ± 9.69	10.00 ± 7.91	13.35 ± 9.69	12.81 ± 10.43	10.00 ± 7.91	12.81 ± 10.43
	P = 0.27		P = 0.33		P = 0.99	
HMGB1 (ng/ml)	19.20 ± 18.08	12.27 ± 8.26	19.20 ± 18.08	20.08 ± 11.16	12.27 ± 8.26	20.08 ± 11.16
	P = 0.004*		P = 0.02*		P = 0.10	
IR	4.06 ± 0.54	2.06 ± 0.32	4.06 ± 0.54	2.13 ± 0.26	2.06 ± 0.32	2.13 ± 0.26
	P = 0.02*		P = 0.03*		P = 1.00	

Table No.4: Correlation among Independent Predictors of Metabolic Syndrome and Obesity

	SBP	DBP	FBS	TG	LDL	HDL	TC	Insulin	HMGB1	Insulin Resistance
SBP	1	.842**	.256*	.478**	.394**	-.113	.589**	.184	.227*	.273*
DBP	-	1	.236*	.493**	.402**	-.055	.608**	.184	.276*	.267*
FBS	-	-	1	.230*	.302*	-.021	.264*	.033	.189	.439*
Triglycerides	-	-	-	1	.258*	-.367**	.488**	.139	.157	.247*
LDL	-	-	-	-	1	.060	.589**	.060	-.093	.124
HDL	-	-	-		-	1	-.013	-.070	-.038	-.073
Cholesterol	-	-	-	-	-	-	1	.311**	.103	.386**
Insulin	-	-	-	-	-	-		1	.214	.850**
HMGB1	-	-	-	-	-	-	-	-	1	.346**

SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, FBS=Fasting blood glucose, TG= Triglycerides LDL= Low density lipoprotien, HDL= High density lipoprotien, HMGB1= High Mobility group box 1 IR= Insulin resistance. **. Correlation is significant at the 0.01 level (2-tailed). *. Relationship was significant at the zero point zero five level (2-tailed).

Although mean serum insulin levels were higher in patients with MS (13.59±1.49 µIU/ml) but the difference from obese and normal group (9.95±1.67 and 10.22±1.29 µIU/ml) was not statistically different, Insulin resistance as determined by HOMA-IR was found significantly greater with incidence of MS (4.1±0.5) whereas it was within the normal range in obese and control groups (2.1±0.26 and 2.1±0.3).

DISCUSSION

Insulin resistance and excessive adiposity are ascribed as the main etiological factors in the pathogenesis of MS often leading to CVD [7,8,9,15]. These conditions are characterized by an increase in inflammatory cytokines [10,1,12,16]. Only a few studies have been carried out to demonstrate the role of HMGB1 in MS [13,14,17]. More recently raised levels of HMBG1 have been shown to act as a significant biomarker for development of MS [16,17]. MS is frequently preceded or accompanied by excessive adiposity. This study was primarily carried out to assess HMBG1 in obese subjects with and without MS. We have therefore investigated using HMBG1 levels as a supplementary criterion to assess the severity of MS and that of obesity without any apparent co-morbidities. Few studies have been carried out previously to determine the role of HMGB1 in adult MS. This study shows that serum HMGB1 levels are significantly higher in the MS group as compared to obese and control groups. These findings are consistent with a previous study in which role of HMGB1 was evaluated in children predisposed to MS. In this study serum HMGB1 levels were found to be significantly raised and closely related to other parameters of MS as shown by [18]. Some evidence also suggests that obesity is associated with higher serum levels of HMGB1 [18]. MS is characterized by low grade inflammation, oxidative stress and pro-inflammatory state. HMGB1 acts as a pro-inflammatory cytokine released in response to stress and inflammation with enhancement of further inflammatory cytokines and disease progression [18].

Interestingly, in the present study no significant increase in levels of HMGB1 were observed in subjects with 'pure obesity' indicating role of other factor in elevation of HMGB1 in patients with MS. HMGB1 levels were significantly increased in our MS patients compared to the obese and control groups

Insulin resistance is believed to be the second most important factor in the pathogenesis of MS [17]. It has been suggested that HMGB1 plays an important role in insulin resistance through NF-κβ pathway activation and its levels are found to be positively correlated with HOMA-IR [16]. The results of current study showed that insulin resistance as determined by HOMA-IR, is robustly associated and positively correlated with levels of HMGB1. On the other hand, IR as observed in patients presenting obesity alone was not significantly different from that of normal controls. A similar picture was obtained with serum insulin levels that were markedly higher in patients with MS compared to the other two groups of subjects.

CONCLUSION

Metabolic syndrome has emerged as a global forthcoming public health disorder over past few Decades. Low grade inflammation being the prominent characteristic of metabolic syndrome leading to release of a cascade of cytokines enhancing the disease progression. Based on the findings of present study it is concluded that all the cardiovascular risk factors are vigorously higher in metabolic syndrome. HMGB1 is also found to be significantly raised and involved in individual component of MS. Depending upon the stimulus; HMGB1 is released extracellularly and binds to its specific receptors leading to activation of NF-κβ signaling pathway which is central regulatory pathway of inflammation.HMGB1 has strong positive correlation with HOMA-IR and it is found to be raised in subjects having high fasting blood glucose. Significantly increased insulin resistance in MS patients revealed that HMGB1 related inflammatory pathway may be involved in pathogenesis of diabetes type 2.

Emergence of HMGB1 as a strong proinflammatory cytokine has opened a new window for future therapeutic interventions. HMGB1 blocking therapy should be considered pharmacologically to limit the inflammatory process. Blocking HMGB1 will result in improvement of all the components of metabolic syndrome. Also in future, study should be conducted on a larger population to further explore the role of HMGB1 in metabolic syndrome.

Author's Contribution:

Concept & Design of Study:	Rabea Nasir
Drafting:	Usman Nasir, Ghazala Khalid
Data Analysis:	Rashid Siddique, Nimra Ikram, Akmal Khurshid Bhatti
Revisiting Critically:	Rabea Nasir, Usman Nasir
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REFERENCES

1. Klune JR, Dhupar R., Cardinal J., Billiar T. R., Tsung A. HMGB1: endogenous danger signaling. Molecular Med 2008;14(7-8):476–484.
2. Mantell LL, Parrish WR, Ulloa L. HMGB-1 as a therapeutic target for infectious and inflammatory disorders. Shock 2006;25(1):4–11.
3. Kang R, Chen R, Zhang Q, et al. HMGB1 in health and disease. Molecular Aspects of Med 2014;40: 1–116.
4. Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. J Leukocyte Biol 2013;93(6):865–873.
5. Tang Y, Zhao X, Antoine D, et al. Regulation of posttranslational modifications of HMGB1 during immune responses. Antioxidants Redox Signaling 2016;24(12):620–634.
6. Ito I, Fukazawa J, Yoshida M. Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. J Biological Chem 2007; 282 (22):16336–16344.
7. Andersson U, Wang H, Palmblad K, et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Experimental Med 2000;192(4):565–570.
8. Štros M. HMGB proteins: interactions with DNA and chromatin. Biochimica et Biophysica Acta 2010;1799(1-2):101–113.
9. Giese K, Cox J, Grosschedl R. The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. Cell 1992;69(1):185–195.
10. Javaherian K, Liu LF, Wang JC. Nonhistone proteins HMG1 and HMG2 change the DNA helical structure. Science 1978;199(4335):1345–1346.
11. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. Ann Review Immunol 2011;29:139–162.
12. Nogueira-Machado JA, Volpe CMDO, Veloso CA, Chaves MM. HMGB1, TLR and RAGE: a functional tripod that leads to diabetic inflammation. Expert Opinion on Therapeutic Targets 2011;15(8):1023–1035.
13. Orlova VV, Choi EY, Xie C, et al. A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. The EMBO J 2007;26(4):1129–1139.
14. Schiraldi M, Raucci A, Muñoz LM, et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. J Exp Med 2012;209(3):551–563.
15. Yang H, Wang H, Czura CJ, Tracey KJ. The cytokine activity of HMGB1. J Leukocyte Biol 2005;78(1):1–8.
16. Yang D, Chen Q, Yang H, Tracey KJ, Bustin M, Oppenheim JJ. High mobility group box-1 protein induces the migration and activation of human dendritic cells and acts as an alarmin. J Leukocyte Biol 2007;81(1):59–66.
17. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Ann Review Immunol 2010;28:367–388.
18. Yu M, Wang H, Ding A, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 2006;26(2):174–179.