Original Article

The Expression of Soluble C-Type Lectin-Like Receptor-2 (sCLEC-2) in Metabolic Syndrome

Syeda Tehreem Kanwal[1], Zil-e-Rubab[1], Syed Zaryab Ahmed[1], Moazzam Ali[2], Aliya Irshad Sani[1] and Ghulam Hussain Soomro[3]

ABSTRACT

Objective: To find out the levels of "soluble C-type lectin-like receptors-2 (sCLEC-2)" in subjects of metabolic syndrome.

Study Design: Cross-Sectional Study

Place and Duration of Study: This study was conducted at the Ziauddin University and Hospital, Karachi from 30th April 2021 to 1st January 2022.

Materials and Methods: The study comprised 45 patients fulfilling the criteria of metabolic syndrome (MetS) based on AHA**,** which includes the following risk factors: diabetes mellitus, hypertension, central obesity or dyslipidemia. A group of 10 healthy individuals was also recruited as a healthy group. The study was approved by the ethics review committee (ERC) reference code: 3440321TKBC. Demographic and anthropometric data were gathered following informed consent. A physical examination was performed. Venous blood (5 ml) was drawn by using an aseptic technique. Serum was obtained and sCLEC2 levels were assessed by ELISA kit (Bioassay Technology Laboratory BT LAB Cat. No. E7510Hu). The mean, standard deviation (SD), median, and interquartile range (IQR) were used to report numerical data, along with the r and p values. P-value < 0.05 was considered as statistically significant.

Results: The mean of sCLEC-2 levels among 45 MetS individuals was 371.8 pg/ml ± 320 pg/ml whereas the median was calculated to be 237 pg/ml IQR of 358.6 pg/ml. In healthy subjects, the mean sCLEC-2 level was 90.6 pg/ml (±37.09 pg/ml) and the median was 98.6 pg/ml (IQR 45 pg/ml). The serum sCLEC-2 levels were significantly high in metabolic syndrome than the healthy individual (*p* value 0.0001).

Conclusion: The increased soluble CLEC-2 expression in metabolic syndrome patients as compared to healthy individuals might be linked to platelet activation and development of atherosclerosis in metabolic syndrome.

Key Words: Metabolic Syndrome (MetS), "soluble C-type lectin-like receptor 2 (sCLEC-2)", Diabetes Mellitus, Hypertension, Dyslipidemia, BMI

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INTRODUCTION

Metabolic syndrome (MetS) is a set of risk factors that include hyperglycemia, obesity, dyslipidemia, and hypertension (Gui et al., 2017)[1]. Metabolic syndrome (MetS) has a strong correlation with coronary artery disease (CAD) which is caused by atherosclerotic plaque buildup (Mahalle et al., 2014)[2]. A proportion of 20 to 25% of the adult population is affected by MetS, globally(do Vale Moreira et al., 2020)[3]. South Asians are more likely to develop MetS due to their high body fat percentage, abdominal obesity, and insulin resistance than the general population (Enas et al., 2007)[4]. According to one study, the prevalence of MetS in the Pakistani population has increased from 35% to 64% over last decades. (Hai et al., 2019)[5]

Platelets play a significant role in the advancement of atherothrombosis by inducing hyper-reactivity or activation, which is a major component in the development of CAD in MetS patients. This result is due to the intricate interaction between obesity and MetS features: insulin resistance, inflammation, and oxidative stress are all factors that contribute to endothelial dysfunction (Santilli et al., 2012)[6]. Platelet activation is characterised by shape changes, microparticle shedding, and overexpression of receptors which are now being studied with different atherothrombotic diseases.(Rubenstein and Yin, 2011)[7] C-type lectin-like receptor 2 (CLEC-2) is one of the platelet membrane activation proteins, it is a type of pattern recognition receptor (PRRs) (Gitz et al., 2014)[8]. Pattern recognition receptors have been reported in the platelet membrane activation and one of them is CLEC which functions via a tyrosine kinase-dependent pathway. It causes effective activation of platelets via

1. Department of Biochemistry / Research[2], Ziauddin University, Karachi.
3. Department of Cardiology, Ziauddin Hospital, Karachi.

Correspondence: Zil-e-Rubab, Professor, Research Department of Ziauddin University, Karachi.
Contact No: 03323009192
Email: moazzam.ali@zu.edu.pk

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the tyrosine kinase-dependent pathway, which strongly relates to platelet activation.(Martyanov et al., 2020)[9]. Studies showed the significant relationship of sCLEC-2 to thrombus stabilization and its mechanism of shedding during platelet activation. This makes it an ideal marker for detecting atherosclerosis prior to the event. (Lombard et al., 2018)[10] Several studies have linked platelet activation membrane receptors to various thrombotic diseases. However, there is a dearth of studies evaluating soluble CLEC-2 in metS, which is considered a transitional stage between being healthy and developing a morbid event such as a myocardial infarction or stroke.(Poddar and Banerjee, 2020)[11] (Fryar et al., 2012)[12] Therefore, we aimed to find out the levels of sCLEC-2 levels in a group of metabolic syndrome patients**.**

MATERIALS AND METHODS

Total of 45 individuals were recruited in the current study via consecutive sampling techniques from Ziauddin medical hospital, Karachi, Pakistan. The ethics review committee (ERC) at Ziauddin University (reference code: 3440321TKBC), approved the study protocols, based on the Declaration of Helsinki. All of the subjects had at least three risk factors out of four to be diagnosed with metabolic syndrome. For the purpose of determining the mean CLEC-2 levels in healthy subject, 10 healthy individuals were also recruited after taken informed consent.

The inclusion of subjects were based on diagnostic criteria for metabolic syndrome, which include having at least three of the following conditions: waist circumference of greater than 120 cm (or more than 88 cm for women) (B) Fasting blood sugar less than 100 mg/dL or on antidiabetic medications (C) HDL-C less than 50 mg/dL in females or 40 mg/dL in men (D) Triglycerides less than 150 mg/dL or on antidyslipidemic therapies (E) SBP less than 130 mmHg or DBP less than 85 mmHg or on antihypertensive medication (Benjamin et al., 2019)[13]. Whereas, patients having septicemia, autoimmune disease, malignancy, pregnancy and any cardiovascular diseases other than coronary artery disease were excluded from the study on the basis of medical records.

Sample Preparation and ELISA: Using aseptic approach, 5ml of blood was drawn from a venepuncture. 2.5ml blood was discharged into an EDTA tube for CBC, which was thoroughly mixed by inverting the tubes 8 to 10 times to avoid coagulation. For ELISA, another 2.5ml of blood was collected in a gel tube and centrifuged at 3000 rpm for 15 minutes at room temperature. Supernatants were carefully collected and divided into 250 mL aliquots in Eppendorf tubes. All of the samples were kept at -20°C. Each tube was used once only test to avoid freezing-thawing cycles. The serum level of sCLEC-2 was determined according to the manufacturer's protocol using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory BT LAB Cat.No. E7510Hu).

Statistical analysis: Statistical analysis was carried on SPSS version 20. The normality of data was assessed by Shapiro-wilk test. Continuous variables were presented as the median and interquartile range. Categorical data expressed in frequency and percentages. The comparison between the two groups was assessed by Mann Whitney test and spearman correlation was performed. (P-value less the 0.05 consider significant)

RESULTS

The study subjects were predominantly males 24 (53.3%) than females 21(46.7%) with median age of 54 (IQR 24.7) years. Whereas, majority of study subjects had smoking and positive family history of coronary artery disease. All the study subjects had hypertension followed by diabetes and hyperlipidemia, indicating that these comorbidities are prominent in metabolic syndrome as depicted in Table 1.

Patients of MetS have higher values of Body mass index (BMI) (27.9 IQR 5), Systolic B.P (140 IQR 20 mmHg), Waist circumference (40 IQR 7.5 inches), Fasting blood sugar (120 IQR 66 mg/dl), Random blood sugar (231 IQR 104 mg/dl), HbA1c (8.3 IQR 5), Urea (38.5 IQR 36.5 mg/dl), Creatinine (1.2 IQR .79 mg/dl) and lipid profile [Total Cholesterol 160 IQR 60, Triacylglycerol (TAG) 145 IQR 84.5, HDL 40 IQR 11.2, LDL 120 IQR 98] then the normal ranges. All these factors showed no significant correlation with the sCLEC-2 concentration except for HbA1c which showed p value 0.05 with correlation coefficient r 0.297.

Table No.1: The sCLEC-2 levels in relation to demographic and clinical characteristics of participants

Variable	Metabolic syndrome Frequency n (percentage %)	CLEC-2 levels	P=value
Gender: Male Female	24 (53.3%) 21(46.7%)	M= 257.7(I.R 502) F= 213.6 (I.R 181)	0.275
Family History: Yes No	28 (62.2%) 17(37.8%)	Yes= 231.3 (I.R 114.6) No= 329.1 (I.R 384.1)	0.271
Smoking: Yes No	28(62.2%) 17(37.8%)	Yes= 257 (I.R 408.6) No= 206 (I.R 68)	0.440
Hyperlipidemia: Yes No	36 (80%) 9(20%)	236.7(I.R 352) 233.4 (I.R 151.7)	0.650
Diabetes Mellitus: Yes No	43(95.6%) 2 (4.4%)	Yes= 242 (I.R 311.3) No= 143.2 (I.R 90)	0.098

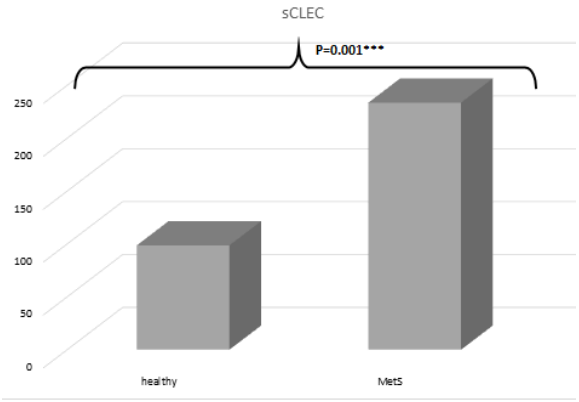

Figure No.1: Serum levels of sCLEC-2 in Metabolic syndrome and healthy subjects

The expression of sCLEC-2 was assessed in the serum of 45 patients with metabolic syndrome and 10 healthy people. The median was 233 pg/ml (IQR 275.5 pg/ml) and the mean was 371.8 pg/ml (±320 pg/ml) in patient of MetS. Whereas the sCLEC-2 levels in healthy individuals was observed as mean 90.6 pg/ml (±37.09 pg/ml) and the median was 98.6 pg/ml (IQR 45 pg/ml). As shown in figure 1, the difference between the MetS and healthy groups is statistically significant with p value 0.0001 at 95% CI. (Fig 1)

DISCUSSION

The syndrome refers to a group of metabolic abnormalities such as dyslipidemia, hypertension, and insulin resistance (IR), which are all linked to central fat deposition and contribute as a risk of coronary artery disease (CAD). The primary mechanism involved in CAD development is atherosclerosis. Membrane activating receptors are considered to be potential tool for assessing under lying atherosclerosis and CLEC-2 is one of them (Watson et al., 2010)[14]. Studies verified that CLEC-2 and its ligand have significant role in initiation and progression of atherosclerotic plaque but its clinical importance in sub-clinical coronary artery disease is still not fully established specially in patients of metabolic syndrome(Fox et al., 2020)[15], which has highest chances of developing atherosclerotic plaque. In current study, it was observed that the s-CLEC-2 levels were seen to be higher in MetS when compare to healthy group, with highly statistically significant (p=0.001) difference between them. Similar to our study, Kazama et al. measured sCLEC-2 levels in 25 diabetes patients and compared them to 10 healthy people. They found that DM patients had greater levels than healthy people, however the differences were not significant in their investigation. The rationale to explain our observation is the assumption that MetS is a collection of several disorders, as opposed to DM, which is a single disease entity. It might be due to the recruitment of diabetic patient based on their HbA1c (>8.0%) rather than diabetic patient with complications that mainly involve vascular disorders. (Kazama et al., 2015)[16]

Platelets expressed CLEC-2, when they are exposed to inflammatory conditions (Meng et al., 2021)[17]. The Inoue et al., explained the expression of CLEC-2 ligand (S100A13), on vascular smooth muscle at the site of atherosclerotic lesions, especially, during the conditions of oxidative stress.(Inoue et al., 2015)[18] This supports the finding of current study, as MetS is a well-known oxidative condition. Following the process of platelet activation, CLEC-2 shed in to the blood in soluble form in proportional to the atherothrombotic activity and this can be detected by ELISA. Inoue et al. stated that sCLEC-2 have higher prognostic, therapeutic and diagnostic benefits in clinical setting compare to other platelet membrane activation receptor like GPVI in patients of stable angina, acute coronary syndrome and healthy individual.(Inoue et al., 2019)[19]. Literature suggested, CLEC-2 have role beyond homeostasis as during the development of deep vein thrombosis (Suzuki-Inoue et al., 2018)[20].

Clec-2 levels, on the other hand, were shown to be significantly higher in patients with coronary artery disease (CAD) and ischemic heart disease (IHD) as compared to healthy individuals, which were similar to MetS levels in the current investigation. (Zhang et al., 2019, Wu et al., 2019)[21,22]. This stresses the predictive usefulness of increased clec-2 levels, since levels were comparable to MetS but also in other advanced illnesses such as CAD and IHD, implying that these levels might be useful early biomarker for identifying high-risk CAD proband. (Fei et al., 2020)[23]

In conclusion, considering the existing data sCLEC-2 levels might be an indicator for corresponding platelet activation which most probably be related to the underlying atherothrombotic process, therefore, more studies should be design or conducted for analyzing the sCLEC-2 levels in Pakistani population and its relation with different parameters of atherosclerosis.

CONCLUSION

The increased soluble CLEC-2 expression in metabolic syndrome patients as compared to healthy individuals might be linked to platelet activation and development of atherosclerosis in metabolic syndrome.

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Author's Contribution:

Concept & Design of Study:	Syeda Tehreem Kanwal, Zil-e-Rubab
Drafting:	Zil-e-Rubab, Syed Zaryab Ahmed
Data Analysis:	Moazzam Ali, Aliya Irshad Sani, Ghulam Hussain Soomro
Revisiting Critically:	Syeda Tehreem Kanwal, Zil-e-Rubab

Final Approval of version: Syeda Tehreem Kanwal

Conflict of Interest: The study has no conflict of interest to declare by any author.

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