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Original Article

Comparison among the Atherogenic Index of Plasma, Ratios of Lipoproteins, Apolipoproteins and Cholesterol Retention Factor (CRF) inPatients with Metabolic Syndrome

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ABSTRACT

Objective: To evaluate various lipid, lipoprotein and apolipoprotein ratios among participants with and without metabolic syndrome.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Naval Hospital, Islamabad Pakistan, from Jan 2019 to Dec 2020.

Methodology: We selected 164 patients with metabolic syndrome. Metabolic syndrome was diagnosed as per "NCEP-defined metabolic syndrome criteria". Insulin resistance was calculated using Homeostasis Model Assessment for Insulin Resistance (HOMA-IR). Various apolipoproteins were calculated using the turbidimetric method on Humastar-200 and lipoproteins by direct detergent methods.

Results: The differences for Apo-B/Apo-A1 were found to be higher among metabolic syndrome subjects [{Metabolic syndrome (n=83):0.55±0.22} vs {Non-Metabolic syndrome (n=81):0.50±0.29}, *p*=0.271]. Area Under Curve (AUC) was highest for the atherogenic index of plasma [0.744], followed by both total cholesterol/HDLc [0.641], Cholesterol Retention Factor (CRF) having [0.641], Apo-B/Apo-A1[0.593] and HDLc/LDLc [0.418].

Conclusion: Differences between subjects with and without NCEP-defined metabolic syndrome were significant for the atherogenic index of plasma (AIP), HDLc/LDLc, and Cholesterol Retention Factor (CRF).

Keywords: Atherogenic index of plasma (AIP), Apo-B/Apo-A1, Cholesterol retention factor (CRF), HDLc/LDLc, HOMA-IR.

How to Cite This Article: *Khan SH, Afzal AH, Gilani M, Akhtar FN, Shahid R. Comparison among the Atherogenic Index of Plasma, Ratios of Lipoproteins, Apolipoproteins and Cholesterol Retention Factor (CRF) in Patients with Metabolic Syndrome. Pak Armed Forces Med J 2023; 73(3): 637-641. DOI: https://doi.org/10.51253/pafmj.v73i3.6280*

INTRODUCTION

Metabolic syndrome, considered to be associated with insulin resistance, is one of the commonest metabolic disorders plaguing our modern world.[1,2] Lipids, apolipoproteins and lipoproteins are the key derangements observed in metabolic syndrome. The major alterations resulting in pathogenic fat deposition remain the transfer of fatty acids and proteins from one lipoprotein to another, causing resistance to insulin action.[3,4] Apolipoproteins and lipoprotein lipid rations can emerge as strong predictors of insulin resistance-induced changes within the blood.[5] These changes as ratios can be more predictive to define metabolic syndrome and thus can help diagnose metabolic syndrome.[6] A Chinese study has found the Apo-B/Apo-A1 ratio to be helpful in diagnosing metabolic syndrome. Similarly, Jung *et al.* observed the same ratio as useful in diagnosing "Metabolic Syndrome" in the Korean population.[7] Provided supporting data for Apo-B/Apo-A1 for diagnostic significance in insulin resistance syndrome; other lipid and lipoprotein ratios have been observed to have superior performance. Kim *et al.* have demonstrated Non-HDL-cholesterol/HDL cholesterol to have better utility in identifying subjects with metabolic syndrome than the Apo-B/Apo-A1 ratio.[8] Furthermore, the research becomes more contradicting as few authors, like Alemzadeh *et al.* have not observed using the Apolipoprotein ratio to be better than lipoprotein and lipid ratios.[9]

We, therefore, planned a study to evaluate the various lipid, lipoprotein and apolipoprotein ratios among subjects with and without metabolic syndrome. Furthermore, we evaluated the ratio between these candidate markers by evaluating their utility by measuring Area Under Curve (AUC).

METHODOLOGY

The cross-sectional study was conducted at Naval Hospital, Islamabad Pakistan, from January 2019 to December 2020. The Ethical Review Committee approved the study project.

Inclusion Criteria: The target population included subjects who visited the hospital without any disease as accompanying patients or came to have an executive routine/annual check-up.

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Received: 11 Feb 2021; revision received: 16 Mar 2022; accepted: 04 Apr 2022

Exclusion Criteria: Patients who had a history of intake of any medicine for any metabolic ailments or supplements of any sort or have some chronic disease like an autoimmune ailment or endocrine disorder or had gynaecological or surgical procedures carried out in the last 3-6 months were excluded. Pregnant ladies and adolescents below 16 tears were also excluded from the study.

The selected volunteers were asked to report for further testing on any day in medical fasting status in the next few days. They were briefed about the study requirements, data collection procedures, clinical examination and future use of data for research, and aspects related to data confidentiality, followed by a signed written consent. Once these patients reported in medical fasting status, they were interviewed per the written questionnaire, followed by various anthropometric measurements, including hip and waist circumference, BMI and blood pressure. The medical officers also examined for signs of any chronic ailments. Finally, selected subjects were sampled for 10ml of blood in gel tubes and EDTA container, which was further used for measurements of lipid profile, Apo-A1, Apo-B, Lipoprotein (a) [Lp(a)] and serum insulin (Table-I).

Table-I: Methods and Instruments used for Measurement of Various Biochemical Parameters

Parameters	Method	Instrument
Total cholesterol	CHOD-PAP method	Selectra-ProM
Serum triglycerides	GPO-PAP method	
HDLc	Direct detergent based method	
LDLc	Direct detergent based method	
Apo-A1	Turbidimetry method	Humastar-200
Apo-B	Turbidimetry method	
Lp(a)	Turbidimetry method	
Serum insulin	Chemi-luminescent method	Immulite R-1000

During analysis, we needed to recheck some samples for technical reasons, and some patients were called back. However, we lost six patients to follow-up. Cholesterol Retention Factor [(LDLc-HDLc)/LDLc] was used as defined by Freeman *et al.*[10] Finally, we calculated insulin resistance by using Mathew's *et al.* method for "Homeostasis Model Assessment for Insulin Resistance" (HOMA-IR).[11] Metabolic syndrome was defined as per "National Cholesterol Education Program (NCEP), Adult Treatment Panel (ATP) III criteria".[12]

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Independent sample t-test was also utilized to measure the differences between subjects with and without NCEP-defined metabolic syndrome for various ratios, including Apo-B/Apo-A1, HDLc/LDLc, total cholesterol/HDLc, triglyceride/HDLc and Cholesterol Retention Factor. A General Linear Model (GLM) was utilized using Apo-B/Apo-A1 as the dependent variable with the presence/absence of metabolic syndrome as a fixed variable and gender as a covariate. In order to see which variable closely depicts the presence of metabolic syndrome, we calculated Area Under Curve (AUC) for various cholesterol indices by employing Receiver Operating Curve (ROC) analysis. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

The main outcome measures in our study were: a-atherogenic index of plasma, 2-total cholesterol/HDLc, 3-Cholesterol Retention Factor (CRF), 4-Apo-B/Apo-A1 and 5-HDLc/LDLc. The differences for LDLc, Apo-A1 and Lp(a) were significant among gender groups (Table-II).

Table-II: Gender Distribution with respect to BMI, Glycemic, Lipids, Lipoproteins, Apolipoproteins and Insulin Resistance (n=164)

Parameters	Gender	n	Mean±SD	*p*-value
Age (years)	M	97	45.18±10.11	0.002
	F	67	40.23±10.04	
Body Mass Index (BMI)	M	97	26.01±3.63	0.001
	F	67	31.11±6.38	
Total cholesterol (mmol/L)	M	97	4.63±1.06	0.255
	F	67	4.46±0.77	
Serum triglycerides (mmol/L)	M	97	2.29±1.36	0.228
	F	67	1.84±0.93	
Low density lipoprotein cholesterol (mmol/L)	M	97	2.82±0.85	0.014
	F	67	2.96±0.74	
High density lipoprotein cholesterol (mmol/L)	M	97	0.81±0.24	0.409
	F	67	0.84±0.23	
Non-HDL cholesterol (mmol/L)	M	97	3.82±0.98	0.131
	F	67	3.62±0.74	
Apo-A (mg/dl)	M	97	126±21.97	0.014
	F	67	135±25.24	
Apo-B (mg/dl)	M	97	66.44±21.75	0.095
	F	67	60.84±19.96	
Lp(a) in (mg/dl)	M	97	13.23±9.15	0.023
	F	67	16.60±9.40	
Fasting insulin (IU/L)	M	97	13.72±10.39	0.165
	F	67	16.06±10.86	
HOMA-IR	M	97	3.77±3.12	0.246
	F	67	4.50±4.42	

The evaluated ratio and calculated cholesterol, we find the differences between HDLc/LDLc, atherogenic

index of plasma (Triglycerides/HDLc) and Cholesterol Retention Factor (CRF) as significant (Table-III).

Table-III: Difference in ratios among Apo-B/Apo-A1, HDLc/LDLc, Cholesterol Ratio, Atherogenic Index of plasma, Cholestrol Retention Factor (CRF) (n=164)

Parameters	n	Mean±SD	*p*-value
Apo-B/Apo-A1			
Present	83	0.55±0.22	0.271
Absence	81	0.50±0.29	
HDLc/LDLc			
Present	83	0.29±0.09	0.027
Absence	81	0.33±0.14	
Total cholesterol/HDLc			
Present	83	6.23±2.05	0.118
Absence	81	5.67±2.48	
Atherogenic Index (Triglyceride/HDLc Ratio)			
Present	83	3.24±1.67	0.001
Absence	81	2.36±1.92	
Cholestrol Retention Factor [(LDLc-HDLc)/LDLc]			
Present	83	0.78±0.11	0.012
Absence	81	0.72±0.15	

Considering the effects of gender as significant, we developed a GLM model where gender was used as a covariate; the differences for Apo-B/Apo-A1 were found to be higher among metabolic syndrome subjects than subjects with metabolic syndrome subjects and the male population (Figure-1).

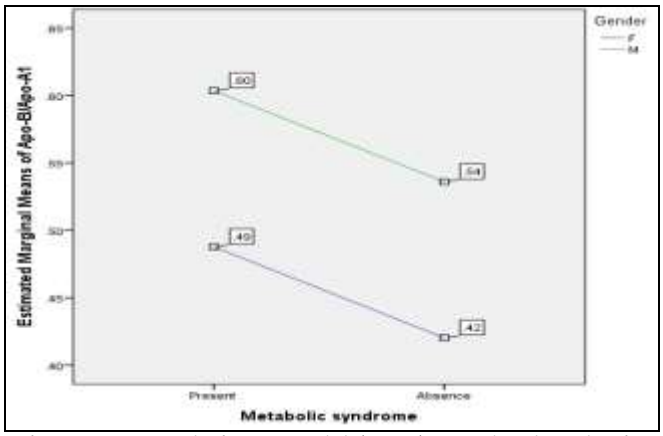

Figure-1: General Linear Model (GLM) was developed using the Apo-B/Apo-A1 ratio as dependent variable, and presence or absence of metabolic syndrome as categorical factor with gender as covariate (Model Significance, *p*=0.095)

AUC, as evaluated by ROC analysis, indicated the Atherogenic index of plasma with the highest Area Under Curve (AUC) [0.744], followed by both total cholesterol/HDLc and Cholesterol Retention Factor (CRF) having AUC=0.641, Apo-B/Apo-A1(AUC:0.593) and HDLc/LDLc (AUC:0.418) (Figure-2).

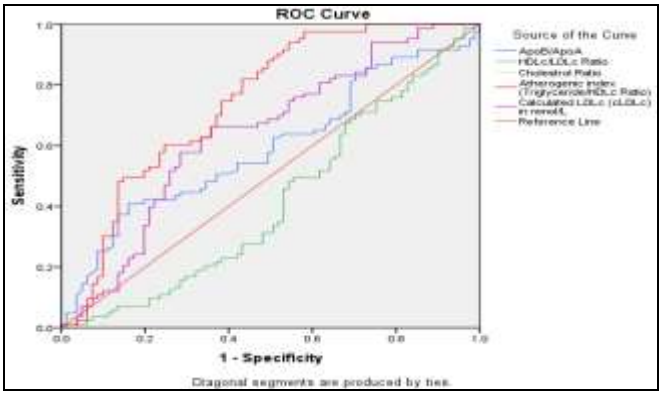

Figure-2: Receiver Operator Curve (ROC), evaluation of various lipid and lipoprotein indices to depict metabolic syndrome, highlighting Atherogenic index of plasma with highest Area Under Curve (AUC) [0.744], followed by both total cholesterol/HDLc and Cholestrol Retention Factor (CRF) having AUC=0.641, Apo-B/Apo-A1 (AUC:0.593) and HDLc/LDLc (AUC:0.418)

DISCUSSION

Various lipid parameters and indices utilized in our study highlighted that indices generally perform better than simple use lipid parameters in terms of risk prediction. Our study identified the Atherogenic index of plasma (AIP), HDLc/LDLc and Cholesterol Retention Factor (CRF) to predict better risk among subjects with or without NCEP-defined metabolic syndrome. Further, we were able to identify AIP to have the highest AUC for diagnosing metabolic syndrome in our study. AIP has also been observed to predict metabolic syndrome by Zhang *et al.* and Fernández-Macías *et al.*[13,14]

Contrary to previous results, we could not demonstrate a significant association for Apo-B to Apo-A1 ratio for diagnosing metabolic syndrome. The possible explanations could include the following: Firstly, gender seems to have an impact on some of the protective lipoproteins and apolipoproteins, but it appears that Apo-A1 appears to have a greater effect on gender than HDLc as depicted by the GLM analysis, which helps us segregate the gender phenomena more clearly. Anagnostis *et al.* have also indicated low levels of Apo-A1 in the premenopausal age group.[15] A study targeting only females in both pre and post-menopausal age groups can further explain this controversy. Moreover, some data points to racial differences in apolipoproteins, which could be a differentiating area between the lipid patterns among our population and the Caucasian population.[16,17] Furthermore, there is

data which indicates the mutations within specific Apo-A or Apo-B, which determine the lipid levels and the predilection towards metabolic syndrome rather than the simple concentration.[18] Taken together, all this contributes towards the heterogeneous relation between lipid and lipoprotein levels and cardiovascular disease risks. However, our data highlight that apolipoprotein and lipoprotein data need a genderbased reference range.

Current reliance on lipoproteins and not on apolipoproteins was suggested in several studies to be a better methodology to predict underlying cardiovascular risks; however, our study has identified AIP to be a better ratio to diagnose metabolic syndrome, which has been associated with CVD morbidity and mortality. Our study also highlighted a very specific need to evaluate not just the apolipoprotein data among our population but also to understand the possible differences in various lipid ratios underlying the sub-continental obesity paradox.

ACKNOWLEDGEMENTS

The authors acknowledge the work of Lab technicians Mr Ibrahim and Mr Amin for all their support.

LIMITATIONS OF STUDY

Firstly, metabolic syndrome's development and multifactorial nature may not be truly represented by one single biomarker, and the current IDF or NCEP-defined criteria may be ideal for diagnosing metabolic syndrome. However, consideration may be made to include AIP, which is more representative of metabolic syndrome and may replace the existing requirement of separate triglyceride and HDLc. Secondly, this is a small-scale cross-sectional study to outline the association between variables and thus can suffer from a lack of long-term follow-up data and type-II statistical error. The latter could be one reason for Apo-B/Apo-A1-related non-significant data. Finally, we need more regional data to highlight the nature of the characteristics of apolipoproteins and the interactions between various lipid components.

CONCLUSION

Differences between subjects with and without NCEP-defined metabolic syndrome were significant for the atherogenic index of plasma (AIP), HDLc/LDLc, and Cholesterol Retention Factor (CRF). Area Under Curve (AUC) to depict metabolic syndrome was highest for AIP, followed by total cholesterol/HDLc, CRF, Apo-B/Apo-A1 and HDLc/LDLc.

Conflict of Interest: None

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SHK & AHA: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

MG & FNA: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

RS: Critical review, drafting the manuscript, interpretation of data, concept approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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