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Biochemical Significance of Cystatin-C and High-Sensitive CRP in Patients with Acute Coronary Syndrome; any Clinical Correlation with Diagnosis and Ejection Fraction

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ABSTRACT

Introduction: Acute coronary syndrome (ACS) is a life-threatening disorder that remains a source of high morbidity and mortality despite advances in treatment. Risk assessment of ACS done by using risk factors and risk markers, CRP is not merely an inflammatory marker but may also participate in the pathogenesis of atherosclerosis and myocardial injury. Additionally, Cystatin-C (Cy/C) is a biomarker recognized for monitoring renal impairment, recently, showed an associated significance with the incidence of coronary vascular atherosclerosis and systolic ventricular dysfunction.

Aim of the study: This study designed to inspect the clinical impact of Cy/C and Hs/CRP circulating levels in patients with ACS and to evaluate their association with diagnosis, ejection fraction (EF) and the angiographic number of stenosed coronaries.

Materials and methods: This is a *single-center* cross-sectional study involved 136 ACS patients and 94 controls. Comparisons of measures of body mass index and serum measurements of Cy/C, Hs/CRP, uric acid (UA), creatinine, and estimated glomerular-filtration-rate (eGFR) between the two groups. Further echocardiographic examinations and angiographic imaging were performed by interventionalists for ACS patients. According to their levels; Cy/C was classified into quartiles and Hs/CRP into tertiles.

Results: Mean patients' age was 57.3±13 years. The mean plasma UA values were significantly higher in patients. The eGFR was significantly lower in ACS people. Mean sera levels of both Hs/CRP and Cy/C were significantly higher in cardiac patients. There was a higher incidence of associated risk factors in terms of tobacco use, hypertension and diabetes mellitus. Around two-thirds of the patients had lower Cy/C quartiles, despite no variation in the distribution of all study variables according to Hs/CRP tertiles. Univariable regression analysis shows no significant correlation among uric acid, eGFR, LVEF, and Hs/CRP with Cy/C levels in ACS patients. ROC analysis for both Cy/C and Hs/CRP biomarkers compared to UA showed higher sensitivity and specificity significantly.

Conclusion: Both Cy/C and Hs/CRP were significantly higher in ACS patients than controls. No significant correlation was observed among uric acid, eGFR, and Hs/CRP with Cy/C levels in ACS patients.

Keywords: cystatin-C; clinical correlation; acute coronary syndrome

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INTRODUCTION

Cystatin C (Cy/C) is a protein member of competing cysteine proteases inhibitors known to be a novel marker of renal function that appears to have even robust association with mortality and/or cardiovascular events than creatinine or estimated glomerular filtration rate (eGFR) in aged and admitted acute coronary syndrome patients (ACS). Whether higher incidences of cardiovascular diseases (CVD) accompanied elevated Cy/C is due to augmented ischemic load, raised risk of atheromatosis, or because of another mechanism is indefinite [1, 2]. Almost, all cells producing Cy/C at a continual rate in form of micromolecules, (hence simply filtered by glomeruli), remaining in high concentrations in blood, saliva, semen, cerebrospinal as well as synovial fluids. Cy/C is an immunmodulators, and enhance production NO. Notably, Cy/C is produced and secreted by cardiac cells, where its synthesis is upstretched during myocardial necrosis [1].

Including under the umbrella of ACS are three clinical entities, unstable-angina, and -(ST & non-ST) segment-elevation myocardial infarction (MI), which are principal reasons for death and incapacity universally [1]. The incremental role of Cy/C outside classical kidney and cardiac biomarkers leftovers discreetly revealed. Both Cy/C and creatinine are hematological markers of kidney functions, however, dislike creatinine, Cy/C is not prejudiced by age, gender, race and body mass and may be more appropriate for identifying mild-moderate variations in eGFR [3]. A nearer look at the current data indicates that elevated values Cy/C values in ACS patients are associated with high risk for cardiovascular events and lethality independent of renal-function [4].

Inflammation shares a contributory role in the progression of arteriosclerosis and ACS and is intricated in the inflammatory bases of ischemic cardiac necrosis. Nevertheless, the correlations of CRP levels, with cardiomyocyte dysfunction and/or necrosis continue to be entirely well-demarcated [4].

This study intended to inspect the relationships between levels of circulating Hs/CRP and Cy/C in patients with ACS.

MATERIALS AND METHODS

Patients were recruited in this single-center study, from Babylon heart center for interventional cardiology; all had prearranged admission for diagnostic or therapeutic catheterization.

Study subjects

All the 136 patients were diagnosed as ACS by expert cardiologists and referred for further interventional catheterization. Well-matched healthy controls (n=94) were chosen, being free of any cardiac disabilities. In addition, the ACS patients were free from any renal impairment.

Description of ACS

The whole ACS patients met the diagnostic canons recognized by the 2014 American Heart Association /American College of Cardiology guidelines for managing non-ST-segment elevation ACSs and the 2013 ACC Foundation/AHA guideline for the management of ST-segment elevation MI [1].

Biochemical classifications of study analytes

Serum measurements of Cy/C in the study patients' classified into quartiles [(<0.9), (0.9-1.49), (1.5-1.99) and (>2.0) mg/l] [1]. The study population was also stratified according to their levels of serum uric acid early at their admission, into tertiles: first (<5.0), second (5-6) and the third (> 6.0) mg/dl. Moreover, Hs/CRP levels were classified into tertiles (< 1.0), (1-3) and (> 3) mg/L.

Measurements of other biochemical assays

All blood assays were completed fasting blood on the day of admission which was drawn and deposited at -70 ºC. Elabscience® Human Cy/C ELISA kit used to measure Cy/C, while Hs/CRP was evaluated by using CALBIOTECH® high sensitivity CRP ELISA kit. Both creatinine and uric acid were evaluated using conventional procedures. Estimated glomerular-filtration-rate (eGFR) was assessed by application of a new estimation method established by adjusting the "Modification-of-Diet-in-Renal-Disease (MDRD) Study" equation, depending on data from "Chinese CKD" patients [5]:

$eGFR\ (ml/min\ per\ 1.73\ m^2) = 175 \times Scr^{-1.234} \times age^{-0.179}$; *[Serum creatinine -mg/dl….and if female* × *0.742]*

The frozen blood samples not exposed to more than a couple cycles of "freeze/thaw" while the entire biochemical analytes were completed as quantified by the manufacturing directions.

Echocardiographic study

All echocardiograms were performed at rest. Standard echocardiographic examination was done by means of a VIVID 7 apparatus (GE Healthcare®) with a 2.5/3.5 MHz probe. A single qualified physician performed echocardiography: M-mode; unaware of the study protocol. The left ventricular ejection fraction (LFEF %) was measured by means of "modified-Simpson's-technique". A cut-off-point < 40 LVEF % designated for expressing LV systolic dysfunction. Henceforth, ACS patients were divided into two subgroups: EF < 40% *vis* ≥ 40% [6].

Other study parameters

Age, sex, BMI, tobacco smoke behaviors, and history of DM, and hypertension were documented by the authors from patients' records. Blood samples were gained for measuring creatinine and Cy/C.

Biostatistics assay

All statistical investigation was completed with SPSS IBM-Version 25-software. The ACS patients were clustered into quartiles based on their serum Cy/C levels, and tertiles based on serum uric acid and Hs/CRP. Numerical differences across quartiles and/or tertiles were evaluated by ANOVA for continuous variables and chi-square for dichotomous covariables. Judgments of continuous data (given as means) were achieved by means of *t*-tests for independent-samples. *ROC*-curve studies were completed to create both specificity/sensitivity testing. Univariate logistic regression analysis applied anywhere instructed.

Ethical Consideration

Up-To-Date permission at the beginning was gained from each applicant (or family assistant) for ACS subjects & control groups separately, and the whole study was approved officially by the scientific committee for research ethics at the University of Babylon. Organized consent for blood samples to be collected and the local hospital ethical committee agreed on the entire study.

RESULTS

The mean patients' age was 57.3±13 years, which is not different from that of the control group. Likewise, BMI was statistically similar between the two study groups. The males' number was 92 (67.7%), which were less significant than that of the control 83 (88.29%). The mean levels of serum creatinine among ACS and control subjects were parallels. The mean plasma uric acid values were significantly higher in the patients' group (*p*-0.001). Correspondingly, the estimated GFR was significantly lower in ACS people compared to healthy persons (109.6) to (127) ml/min per 1.73 m^2, sequentially (*p*-0.001). Mean sera levels of both Cy/C and Cy/C were significantly higher in cardiac patients mutually (*p*-0.001). The higher incidence of associated risk factors in terms of significant current Tobacco use (*p*-0.05), and highly significant concomitant hypertension and diabetes mellitus in ACS patients (0.001) were evident (table-1).

Table-1: Baseline characteristics of studied subjects

Characteristics	Acute coronary syndrome patients N=136	Health controls N=94	*P*-value
Age	57.3±13	31.9±12	0.05
Sex/Male No (%)	92 (67.7)	83 (88.3)	0.05
Body mass index (kg/m^2)	28.3±4.6	26.8±4.6	NS

Current smoking No (%)	57 (41.9%)	42 (44.6%)	0.05
Creatinine (mg/l)	0.87±0.4	0.86±0.1	NS
Uric acid (mg/ml)	5.9±1.8	4.4±1.3	0.001
Estimated GFR (ml/min/1.73 m^2)	106.7±31.6	124.3±22	0.001
Cystatin-C (mg/ml)	1.38±1	0.5±0.2	0.001
HSCRP (mg/ml)	7.8±7.2	0.06±0.1	0.001

The issue of classification of the subjects' blood levels of Cy/C into four quartiles is well exposed in figure-1, which showed that around two-thirds of the ACS patients had lower Cy/C quartiles than those with higher quartiles, despite the fact, that table-2 revealed no significant variation in distribution of all study variables according to Hs/CRP tertiles. However, when the study variables of ACS patients distributed according to the serum Cystatin-C quartiles some significant differences among variables regarding male sex, Tobacco use habit, and incidence of hypertension.

Table-2: Characteristics of ACS patients distributed according to the serum Hs/CRP tertiles and Cystatin-C quartiles

Characteristics		Hs/CRP, (mean± SD or No %)			*P*-value	Cystatin-C Quartiles, (Mean±SD or No %)				*P*-value
		< 1.0 (N=20)	1.0 - 3.0 (N=10)	> 3.0 (N=106)		I (N=49)	II (N=40)	III (N=21)	VI (N=26)	
Age (years)		63±3.2	64.2±6.7	55.3±10.5	NS	54.9±15.3	57.4±11.1	62.8±10	58.3±13	NS
Male No (%)		12 (13.0)	7 (7.6)	73 (79.3)	NS	30 (32.6)	20 (21.7)	17 (18.5)	25 (27.2)	0.013
BMI kg/m^2		28.1±5.4	28.7±3.7	27.4±4.7	NS	27.2±4.5	28±3.9	26.2±4.6	26.8±3.9	NS
Current Smoker No (%)		3 (8.3)	3 (8.3)	30 (83.4)	NS	10 (35.7)	10 (35.7)	4 (14.2)	4 (14.2)	0.005
LVEF%		51.3±9.5	53±6.7	50.7±12.7	NS	49.9±13.5	54.7±7.3	51.5±6.3	51.9±8.3	NS
LVEF≤40 Mean (%)		6 (30)	2 (20)	24 (22.6)	NS	13 (26.5)	6 (15)	7 (33.3)	7 (27)	NS
LVEF>40 Mean (%)		14 (70)	8 (80)	82 (77.4)		36 (73.5)	34 (85)	14 (66.7)	19 (73)	
Uric acid		6.0±1.7	5±1.6	5.8±1.8	NS	5.9±1.8	5.9±2.1	5.1±1.4	6.1±1.8	NS
First tertile No (%)		4 (20)	7 (70)	40 (37.7)	NS	16 (32.6)	18 (45)	10 (47.6)	11 (42.3)	NS
Second tertile No (%)		8 (40)	1 (10)	34 (32.1)		15 (30.6)	6 (15)	8 (38.1)	2 (7.7)	
Third tertile No (%)		8 (40)	2 (20)	32 (30.1)		18 (36.8)	16 (40)	3 (14.3)	13 (50)	
Hs/CRP						9± (5.4)	5.7± (4.6)	5.2± (3.3)	11.4± (14.3)	NS
Cystatin-C		1.5±0.69	1.2±0.79	1.6±1.1	NS					
Creatinine		1.1±0.7	1.1±0.8	0.8±0.3	NS	0.79±0.4	0.79±0.2	0.97±0.9	0.99±0.2	NS
eGFR		97.9±34.1	96.4±50.3	105.2±26.9	NS	101.4±39.2	97.5±35.9	109±25.6	115±31.9	NS
Number of stenosed coronaries	*1-vessel*	7/20	5/10	18/106	NS	11/49	12/20	9/21	5/26	NS
	2-vessels	0/20	0/10	55/106		28/49	12/20	1/9	11/26	
	3-vessels	11/20	5/10	21/106		6/49	8/20	9/21	7/26	
	4-vessels	2/20	10/10	12/106		4/49	820	2/21	3/26	
< 60 No (%)		2 (10%)	3 (25%)	7 (6.6%)	NS	8 (16.3)	5 (35)	1 (4.8)	3 (11.5)	NS
> 60 No (%)		18 (90%)	7 (75%)	99 (93.4%)		41 (83.7)	26 (65)	20 (95.2)	23 (88.5)	
DM No (%)		3 (8.1)	4 (10.8)	30 (81.1)	NS	8 (5.8)	26 (19.1)	10 (7.4)	13 (9.6)	NS
Hypertension No (%)		7 (15.2)	5 (10.9)	34 (73.9)	NS	11 (8.1)	21 (15.4)	19 (14)	23 (17)	0.02

NS: not significant, DM: Diabetes Mellitus, BMI: Body Mass Index, LVEF: Left Ventricular Ejection Fraction

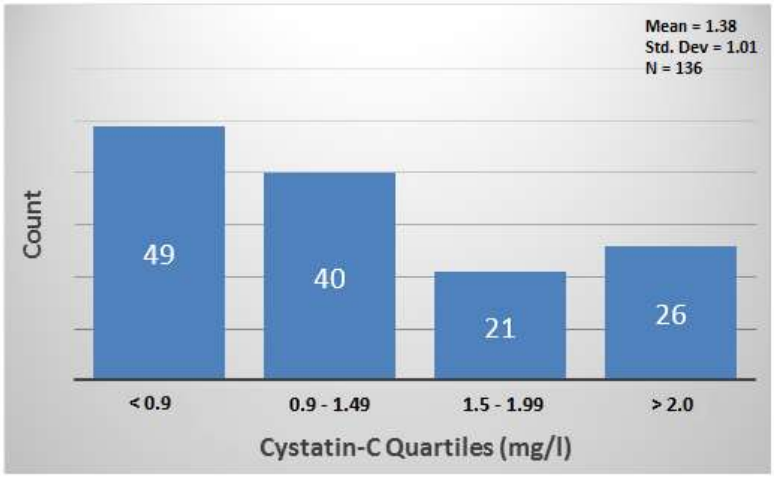
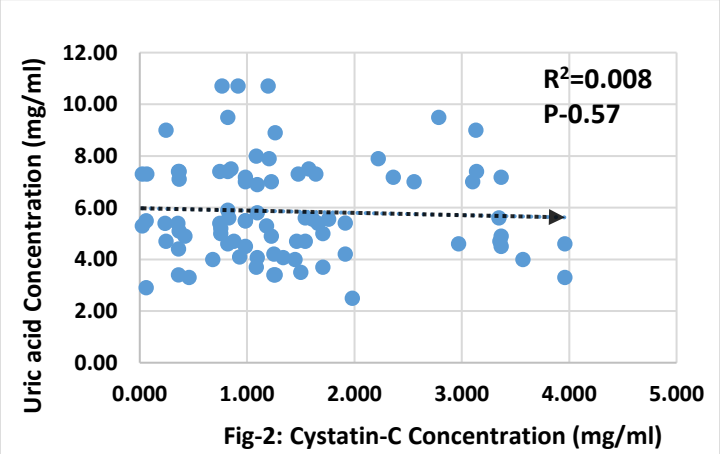
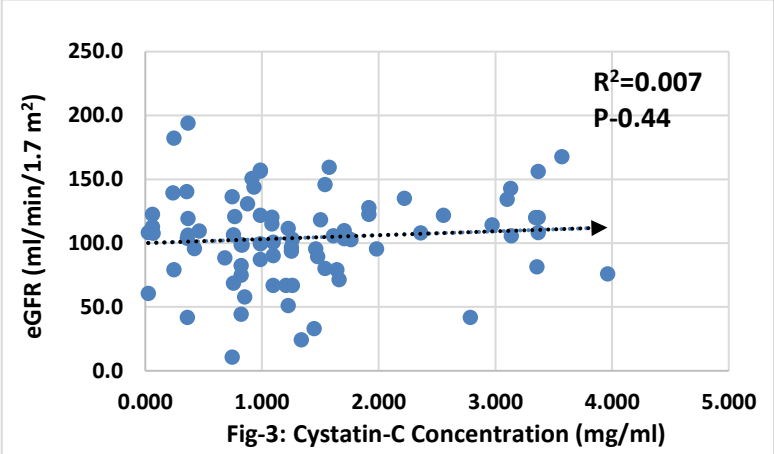
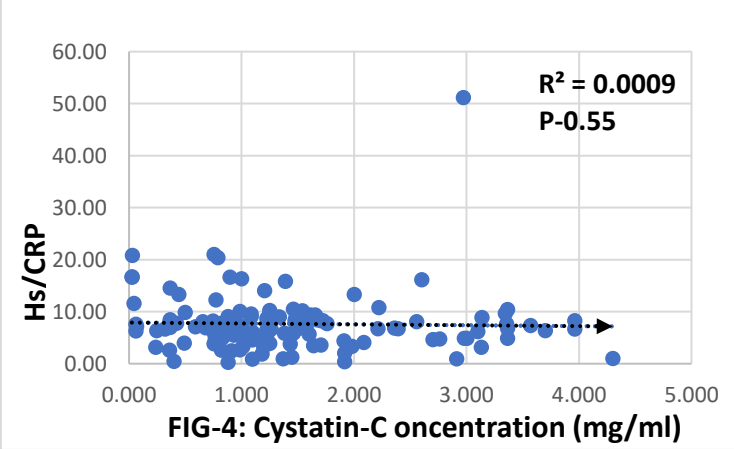


Figure-1: Cystatin quartiles in ACS patients

Univariable regression analysis shows no significant correlation among uric acid, eGFR, and Hs/CRP with Cy/C levels in ACS patients (figures 2, 3, & 4)







Of note, gender shows a significant impact on the level of Cy/C in the blood, where it was higher in males. In the same meaning, the incidence of both diabetes mellitus and hypertension were more among males, unlike all other study variables which were distributed almost evenly between both sexes (table-4).

Table-3: Gender distribution of the study parameters in patients with acute coronary syndrome

Characteristics	Age	LVEF	BMI	Creatinine	eGFR	Hs/CRP	Cystatin-C	Uric acid	Diabetes (No)	Hypertension (No)
Male (mean)	58.5	49.8	27.4	0.9	105.2	8.0	1.6	5.8	38	40
Female (mean)	51.9	52.0	26.8	0.8	109.9	7.2	0.9	5.9	19	34
Significance	NS	NS	NS	NS	NS	NS	0.05	NS	0.05	0.05

ROC curve analysis (figure-5) for both Cy/C and Hs/CRP biomarkers compared to uric acid showed higher sensitivity and specificity significantly: [82% 91% and 92%, 97% to 62%, 74%] with AUC [0.863 and 0.999 to 0.729] and 95% CI [0.812-0.913 and 0.997-1.000 to 0.663-0.794] consecutively (*p*-0.000).

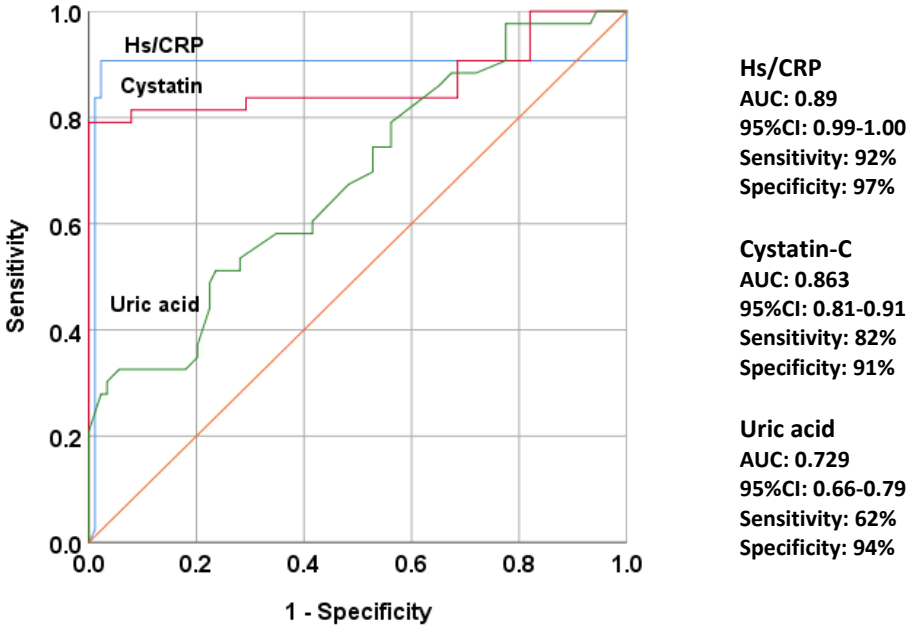


Figure-4: Areas under the curves (AUC) and 95% confidence interval (CI) of study biomarkers in patients for detection of more than one coronary vessel affection and left ventricular ejection fraction as analyzed by ROC

DISCUSSION

As a genuine syndrome, ACS represents a source of high morbidity even with substantial therapy-advances take into attention assessment through risk factors or risk biomarkers [7]. top of these factors, few recent risk-issues initiated to be correlated with ACS [8] reported that Cy/C is less impacted by factors including age, gender, and muscular-mass than blood creatinine and consequently, might be a worthy indicator of cardiovascular events especially in the aging that mismatch our results. High Cy/C measures shown to be independently associated with demographic aspects including age, female-gender, BMI, besides tobacco-smoke, even in individuals without renal dysfunction as exposed by [9].

In this study, Cy/C levels in all participants were mimic the situations of other surveys [10]. Like our-outcomes, {Huang, 2019 #93} reported significant higher Cy/C in ACS patients. That reflects atherotic changes in ACS subjects as Cy/C is expressed in all of the nucleated cells controlling cysteine-proteases activity and hence, regulates in inflammatory responses that intern, contributes an essential role in atherogenesis. Accordingly, Cy/C induces a "hyper-inflammatory state" that subsidizes susceptible coronary-sclerotic-plaque [11] as well as a higher prevalence of coronary-vascular events, although the precise explanations of such relationships are unspecified [12]. Other studies suggest that increased levels of Cy/C may indicate an increased risk of heart disease, heart failure, stroke, and mortality [13].

The positive correlation of Cy/C with SUA and creatinine observed in this work; are in agreement with other studies [14]. The later found significant relations of Cy/C with creatinine and SUA mutually in Japanian undergraduates. Our study exposed a negative correlation of Cy/C with eGFR in agreement with [13], who reported higher Cy/C levels which could reflect early GFR problems. Recent shreds of evidence supported a graded-association among high Cy/C measures and elevated risk of CVD in individuals with high eGFR [15, 16].

The higher incidence of associated risk factors in terms of concomitant hypertension and diabetes in ACS patients was consistent with the finding of [17] who showed a

higher prevalence of risk factors in individuals with higher Cy/C concentrations [18] used Cy/C with 1mg/L cut-off of and obtained a sensitivity of 77.8% in detecting ACS patients, but with low specificity (62%) and the accuracy of 0.831. Our study used a cutoff value of 0.955 (at a higher cut-off we obtained a lower specificity). The sensitivity was 86%, specificity was 84% which reflects the predictive value of Cy/C as a biomarker for ACS, which agreed with [19] findings, who reported a modest predictive rate of Cy/C as a single biomarker for ACS.

Our study showed the association of higher Cy/C level (at the 4 quartiles) in ACS patients with the lower systolic function of the left ventricle reflected by lower EF and this is in agreement with [20] who found that higher Cy/C levels were associated with lower EF. This also has agreed with [21] who concluded that Cy/C could be a marker of heart failure.[22] also showed the association between higher Cy/C level and the lower EF. From these findings, we can conclude that Cy/C can detect the systolic dysfunction of the LV.

Our results showed that the Cy/C level not related to the number of diseased arteries. Which was in contrast to other studies that concluded that the Cy/C level tended to increase as the number of diseased arteries increased [19]; [23] As well as [22] who found that patients with a higher Cy/C levels revealed a higher number of diseased vessels compared to those with lower Cy/C levels. These discrepancies might be explained to-some-extent by the small sample size.

There are main arguments that can be advanced to support that the inflammatory process plays a dynamic role in all stages of atherogenesis. The higher CRP levels in ACS subjects are undoubtedly pointed toward ongoing inflammation associated with coronary-events along with a higher possibility of a vulnerable plaque [24]. In our work, Hs/CRP outcomes are in agreement with the overwhelming pieces of evidence corroborating such associations. CRP subsidizes coronary-sclerosis evolution by employing proinflammatory assets, modulation of innate-immunity, complement activation, enhancing platelet activation, thrombogenesis, as well as vessels-remodeling/angiogenesis. Still, whether CRP exhibits a regulator or amplifier effect on the innate-immunity has to be fully explicated [25]. Notwithstanding the aforesaid pathways, there is no evidence that high Cy/C values clinically are associated with cardiovascular disease by inflammation [26] specially if Hs/CRP levels already raised at baseline, an uneven increase of this marker, increased CRP due to other concomitant etiology.

No correlation between inflammatory marker (Hs/CRP) and Cy/C in our work seems to be similar to several other surveys ([18] [27]. While other studies investigate the levels of serum Cy/ C in ACS revealed positive correlation [28] [29]

Earlier revisions have found no association between Cy/C and CRP after adjusting for renal function whereas, further studies described a correlation between these two biomarkers in patients not affected by IHD. Of late, [30] showed that the inflammatory status of a patient does not influence the role of Cy/C as a marker of GFR and [31] assumed that inflammation does not seem to be involved in the association between Cy/C and coronary-sclerosis. [32]. Yet, the pathogenesis of this mutual association between the cardiac and renal systems (i.e. cardiorenal syndrome) is still uncertain. [33].

The absent correlation of Cy/C with BMI perhaps been prejudiced by the diverse representation of both sexes amongst the ACS patients encompassed in our work. This association is dependent on renal function in some revisions, and it has been proposed that Cy/C, as a biomarker of kidney function, would be better to reveal the relation of the inflammation identified in ACS patients without overt kidney dysfunction [27]. However, the correlation was weak between Cy/C and Hs/CRP in a study published in 2010 [10].

In the current study, the overall accuracy, specificity, and sensitivity results of the Hs/CRP test for the detection of ACS were nearly comparable to those generated by our previous work [34]as well as a current study held in Pakistan [35].

On reasonable grounds, there is no compelling reason to claim that such high sensitivity/specificity and a reputable accuracy rate render the investigator conclude that Hs/CRP test basically appropriate as a screening tool for ACS. Moreover, Hs/CRP test is attainable in numerous identical laboratories. Additionally, there is overwhelming evidence corroborating the concept that Hs/CRP levels correlated with the severity of CAD. In the meantime, baseline Hs/CRP values are not prone to the time-of-day discrepancy, even after years of storage! [36, 37]. Based on this evidence, it can be considered as a valuable and applicable measure for screening and diagnosing AMI.

A study by Zethelius et al, 2008 evaluated whether a grouping of bioindicators, like Cy/C, NT-Proatrial natriuretic peptide, troponin-I, and Hs/CRP together, enhanced ACS stratification versus the traditional risk factors [38]. Those investigators revealed that Cy/C addition to the system expressively augmented prognostic value [28].

In contrast with the previous report from Karbala holey province [39], we exposed no correlation between Hs/CRP levels and angiographic findings of ACS, which to some extent like two other studies [40, 41]. Our results could be explained either by the lower number of patients with unstable angina enrolled in our study; or due to IVUS based diagnosis used by other authors. The limited increase in Hs/CRP levels in unstable angina patients and in non-Q-wave-MI could be due to low-grade myocardial necrosis which occurs during the ischemic attack. [40].

A closer look to our finding, one can realize that Hs/CRP was no correlated to LVEF that is inconsistence with what had been published by [42], while serum concentrations showed an inverse correlation with LVEF in another study [43]

Thus, it should be established whether reduced Hs/CRP measures are associated with reduced risk of ACS and the assistance of labeling the risk of IHD with Hs/CRP has to be resolute. Nevertheless, additional analyses are essential to explicate its precise contribution CVD and to investigate whether those with mild/moderate kidney dysfunction and

an ACS must be managed in-a-way different to those with preserved kidney function [44].

CONCLUSION

Both Cy/C and Hs/CRP mutually as well as SUA, eGFR are significantly higher in ACS subjects compared to healthy groups. Cystatin-C could be a useful marker for diagnosing CAD, however, it is not an influential predictor of LVSD. It appears imperative to put gender and tobacco use into consideration in estimation Cy/C level as our study exposed it is affected by these factors. In the current study, it could be claimed that Hs/CRP represents a substantial biomarker in causation besides the strong association with IHD. Hs-CRP biomarker can be measured as a useful biomarker for diagnosing and screen ACS patients.

LIMITATIONS

Some limitations had better be considered in inferring our results. *First*, our study design was cross-sectional conducted at a single center. *Second*, some inflammatory biomarkers like interleukin-6 and tumor necrosis factor-α were not applied in this study. Even though such markers perhaps do not disturb our conclusions, they could make an impression on the estimation of Hs/CRP and Cy/C in this work. *Third*, our study is limited in that it was directed in a small-sample-size. However, it possibly will not able to exclude whether the observed difference would reach a significant level if larger sample size is inspected. Accordingly, the correlation between Cy/ C, Hs/CRP levels and severity of CAD requests to be re-investigated in a population-based prospective analysis to establish the exact contribution of Cy/C and Hs/CRP in preventive cardiology.

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