ISSN: 03875547
Volume 45, Issue 02, April, 2022

Study of I CAM and Homosyctine in patiants with Cardio vascular disease

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Keywords:
Homosyctine

ABSTRACT

The current study is aimed to investigate the effect of Intercellular cell adhesion molecule and Homo cysteine amino acid in patients with cardio vascular disease by using Serological methods [EELISA]. The study included the collection of (100) blood samples from study group (patiants with cardio vascular disease) in center care unit (ccu) in Baquba hospital. the study collected sample from healthy people (30) samples. The study investigated lawer significant (I CAM) value on patiants group compared with healthy group value (0.001). The results of HCY also decreased significant on the study group.

1. INTRODUCTION

1.1 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are multidimensional disorders that primarily impact the key aspects of the human circulatory system, like the heart, blood arteries, and blood itself. CVDs could be congenital or acquired during the course of a person's life. The biggest and most common cardiovascular developed issues are atherosclerosis, rheumatic heart disease, and cardiovascular inflammation [1].

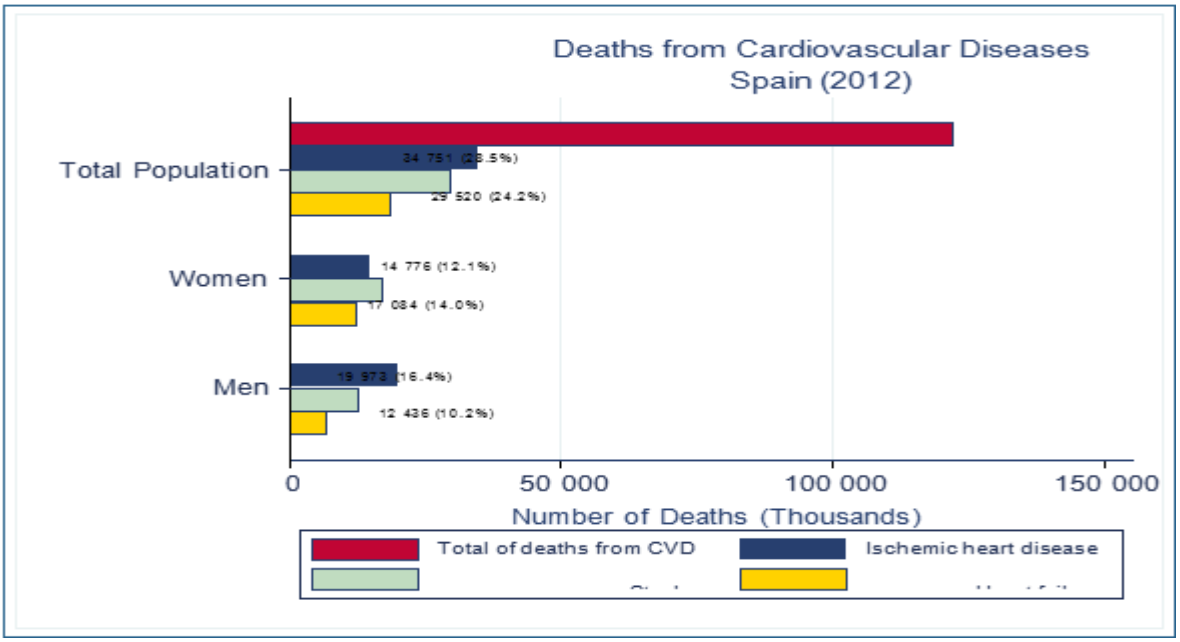

Figure 1 shows the number of people who died in Spain in 2012 as a result of the most frequent cardiovascular disorders [2].

1.2 Intercellular Adhesion Molecule 1 (ICAM-1)

Intercellular adhesion molecule-1 (ICAM-1), (CD54), a 90 kDa cell surface glycoprotein, [3] it is another endothelial adhesion molecule that is upregulated at sites of atherosclerosis [4]. Studies have shown that levels of soluble ICAM-1 correlate with the extent of atherosclerosis in humans, [5] and that ICAM-1 knockdown is associated with a reduction in the size of vascular lesions in apoE-deficient mice [6]. The selective binding of $\alpha_L\beta_2$ integrin, which is expressed by lymphocytes, monocytes, and neutrophils [7], [8] to ICAM-1 activates xanthine oxidase (XO) and generates $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) within the endothelial cell [9], [10].

The concept of the critical role of adhesion molecules in endothelial dysfunction has been reported [11]. It has been shown recently that cellular adhesion molecule levels, including ICAM-1 suggested to be increased in patients with CVD [12]

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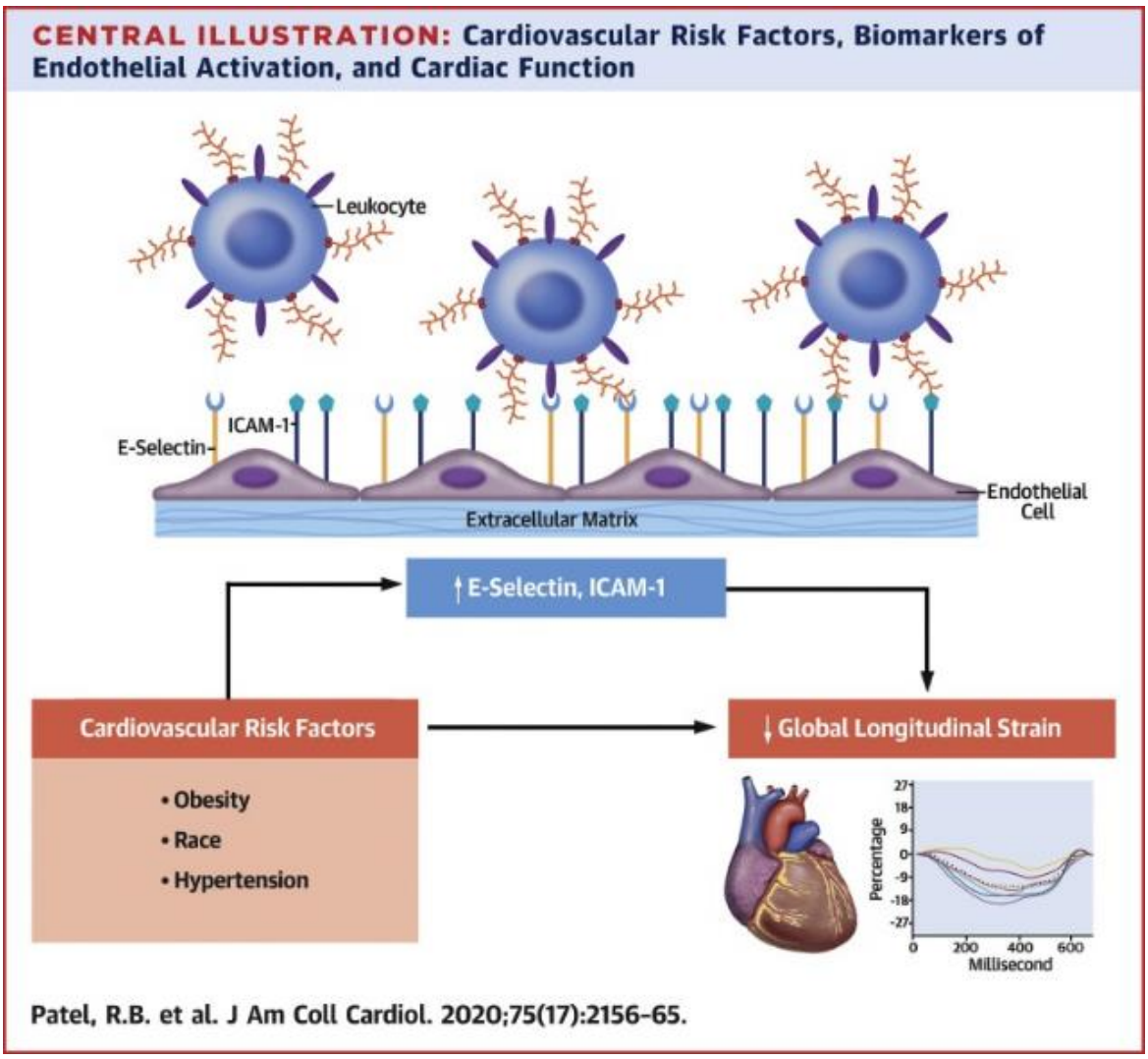

Figure (2) cardiovascular risk factors, Biomarkers to Endothelial activation (I-cam), [4]

2. Homocysteine

The amino acid methionine's blood metabolism can induce atherosclerosis; nevertheless, its relevance as a cardiovascular risk factor is yet unknown. Low folic acid and B vitamin levels are associated with greater homocysteine levels. The data regarding the usefulness of several therapies, like daily folic acid supplements, in lowering homocysteine levels, and hence the risk and progression of CVDs, is conflicting [13].

2.1 Blood sample collection

All participants' blood is drawn in the morning and before breakfast. About (10-15) milliliters of venous blood was drawn from patients and control individuals and left at room temperature for (15) minutes in a gel tube (without anticoagulant), after which serum was separated by centrifugation at (3000 xg) for (15) minutes, divided into (4) aliquots, and stored frozen at (-20 °C) until utilized for biochemical determination.

2.2 PROCEDURE of I CAM by using Eliza

1- Washing: Fill each well with 200 ul of Washing Solution. Aspirate the wells to remove any remaining liquid, then wash the plate three times with 300 ul of Washing Solution per well. After the last wash, flip the plate and wipe with a paper towel to remove any leftover solution.

2- Reaction: In every well, add 100 ul of standard, blank, and sample in duplicate. The Plate Sealer

should be used to seal the plate. Incubate for at least 2 hours at room temperature.

3- Washing: As in step 1, aspirate the wells to remove any liquid and wash the plate four times.

NOTE: To produce low background readings, the plate must be vigorously washed following the incubation phases.

4- Detection: Per well, add 100 ul of the diluted detection antibody. Then use the Plate Sealer to seal the plate. Incubate for 2 hours at room temperature.

5- Washing: As in step 1, aspirate and wash the plate four times

6- Conjugates: Per well, add 100 ul of diluted Streptavidin-HRP. The Plate Sealer should be used to seal the plate. Incubate at room temperature for 30 minutes (or at 37°C for 30 minutes).

7- Washing: As in step 1, aspirate and wash the plate four times.

8- Color Development: To each well, add 100 ul of TMB or pink-ONE TMB solution. In order for the color to develop properly, incubate at room temperature. Fill each well with 100 ul of the stop solution.

9- Reading: Measure observance at 450 nm with a microplate reader.

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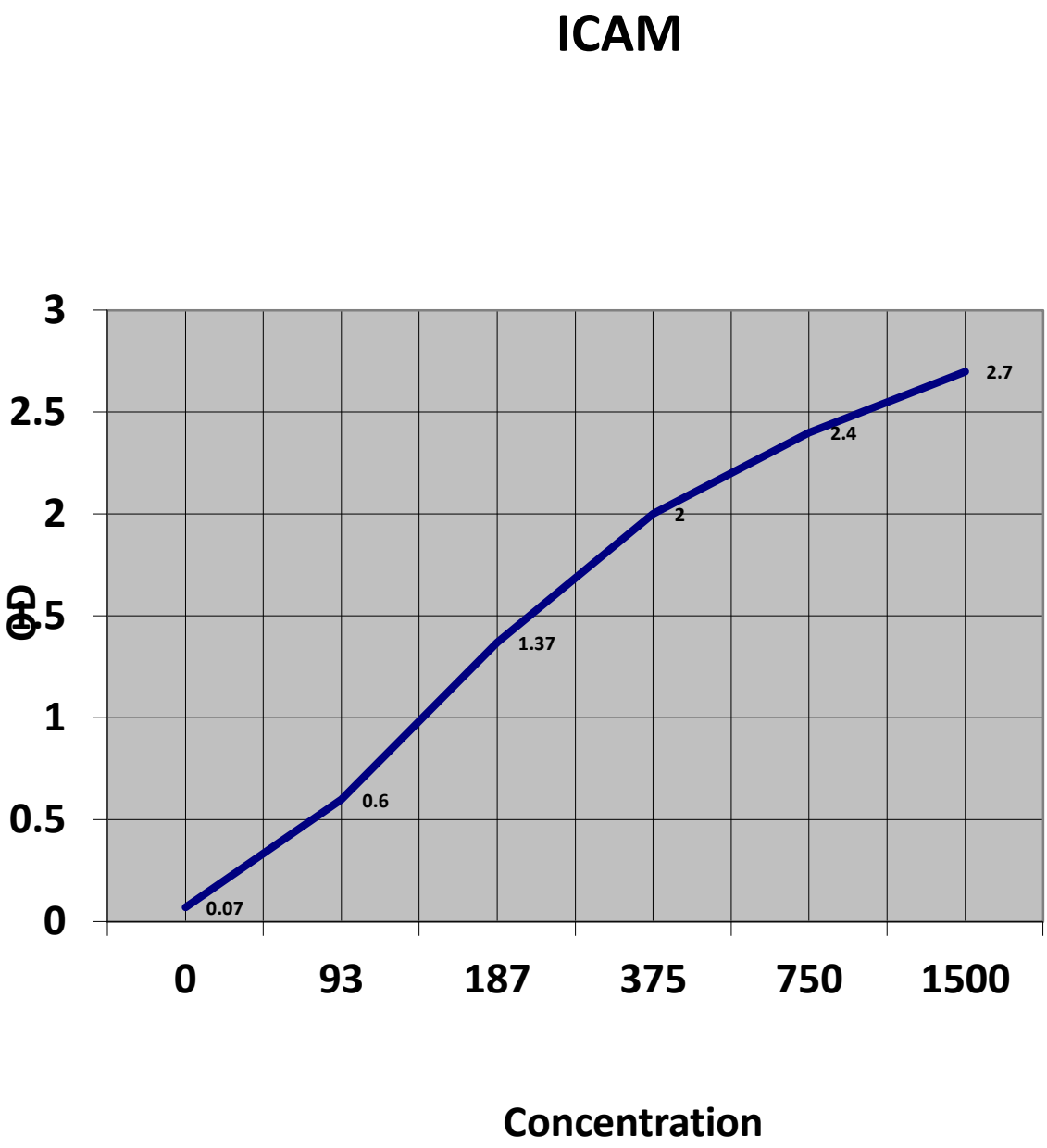

Figure (3) standard CURVE OF I CAM

2.3 Procedure of HCY by using Eliza

1.Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells!

2.Add Sample and Biotin- labeled Antibody: Add 50µL of Standard, Blank, or Sample per well. The blank well is added with Sample/Standard dilution buffer. Immediately add 50µL Biotin-labeled Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding

inside wall touching and foaming as much as you can.)
3.Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
4.HRP-Streptavidin Conjugate (SABC): Add 100µL SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.
5.Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.
6.TMB Substrate: Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 15-20 minutes. (The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
7.Stop: Add 50µL Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8.OD Measurement: Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution.

2.4 The results of serum ICAM, in study groups (patients compared with controls)

The results of the statistical analysis showed a significant decrease in the ICAM level in the patients (205.26 ± 11.47) compared to the control (219.62 ± 43.02respectively) group figure (4). and this result dis agree with [14] and in other study [15] observed a weakly associated between ICAM-1 and several cardiovascular risk markers in the fact intercellular adhesion molecule-1 (ICAM-1) Vascular cell adhesion molecules (ICAM-1) which are glycoproteins integral to the cell membrane, are responsible for the adhesion of different cells onto the endothelial surface Cell adhesion molecules facilitate the attachment of circulating leucocytes to the endothelium, to sites of inflammation, and their subsequent movement and accumulation in arterial walls, all of which are processes pivotal in the development and progression of atherosclerosis [15].

The results in this study was agree with [16], [17] were observed increasing in levels of CKMB

Table (1) ICAM, in study groups

Parameters	Group	Mean ± Std.	P-value
ICAM	Control	219.62 ± 43.02	0.001
	Patients	205.26 ± 11.47	

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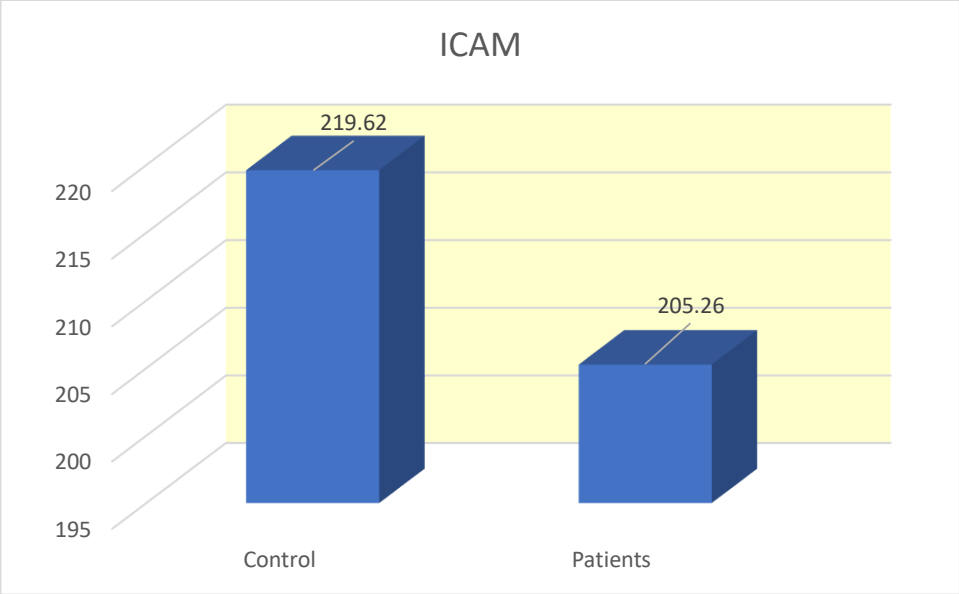

Figure (4) serum ICAM level in study groups

2.5 The results of serum HCY in study groups (patients compared with controls)

The HCY showed decrease significant in patients (10.33 ± 1.46), when compared with control (62.45 ± 4.11) p-value <0.05, while several studies observed that the CVD an in depended of HCY as [18] were observed Hyper homocysteinemia may lead to an enhancement of the adverse effects of risk factors like hypertension, smoking, lipid and lipoprotein metabolism, as well as promotion of the development of inflammation. The prevalence of hype rhomocysteinemia may vary significantly between populations, and most likely depend on age, diet, and genetic background as well. Increasing age, male sex, smoking, coffee consumption, high blood pressure, unfavourable lipid profile, high creatinine and faulty diet are some of the factors associated with increased homocysteine levels figure (5) table (2).

Table (2) serum HCY in study groups

Parameters	Group	Mean ± Std.	P-value
HCY	Control	62.45 ± 4.11	0.000
	Patients	10.33 ± 1.46	

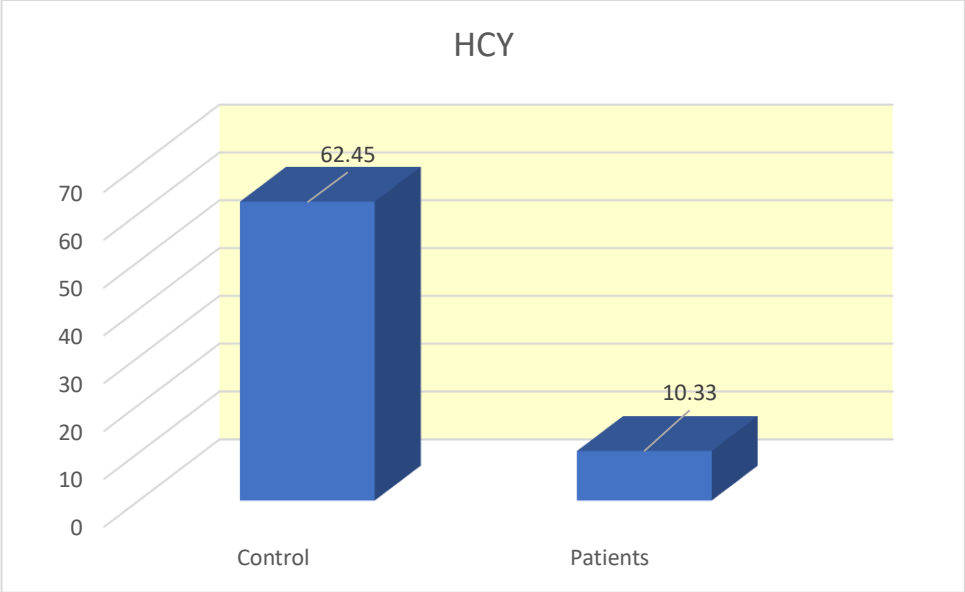

Figure (5) serum HCY level in study groups

3. Conclusion

The results shows decreases of Intercellular cell Adhesion molecule-1 (I CAM-1) in patients with atherosclerosis compared with control in Iraq while the Homosistyne HCY showed decrease significant in patients when compared with control.

4. References

[1] Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury part I: basic mechanisms and in vivo monitoring of ROS. Circulation. 2003;108(16):1912–1916.

[2] Cardiovascular disease [Internet]. Eciclopaedia Britannica. [cited 2014 Dic 18.

[3] Matheny HE, Deem TL, Cook-Mills JM. Lymphocyte migration through monolayers of endothelial cell lines involves VCAM-1 signaling via endothelial cell NADPH oxidase. J Immunol. 2000;164:6550–6559.

[4] Cook-Mills JM, Johnson JD, Deem TL, Ochi A, Wang L, Zheng Y. Calcium mobilization and Rac1 activation are required for VCAM-1 (vascular cell adhesion molecule-1) stimulation of NADPH oxidase activity. Biochem J. 2004;378:539–547.

[5] Khodabandehlou Khosrow, Masehi-Lano Jacqueline J, Poon Christopher, Wang Jonathan, Chung Eun Ji. Targeting cell adhesion molecules with nanoparticles using in vivo and flow-based in vitro models of atherosclerosis. Experimental Biology and Medicine. 2017;242(8):799–812. doi: 10.1177/1535370217693116.

[6] Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol. 2007;27:2292–2301.

[7] Rohde LE, Lee RT, Rivero J, Jamacochian M, Arroyo LH, Briggs W, Rifai N, Libby P, Creager MA, Ridker PM. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 1998;18:1765–1770.

[8] Bourdillon MC, Poston RN, Covacho C, Chignier E, Bricca G, McGregor JL. ICAM-1 deficiency reduces atherosclerotic lesions in double-knockout mice (ApoE(-/-)/ICAM-1(-/-)) fed a fat or a chow diet. Arterioscler Thromb Vasc Biol. 2000;20:2630–2635.

[9] Lawson C, Wolf S. ICAM-1 signaling in endothelial cells. Pharmacol Rep. 2009;61:22–32.

[10] Rahman A, Fazal F. Hug tightly and say goodbye: role of endothelial ICAM-1 in leukocyte transmigration. Antioxid Redox Signal. 2009;11:823–839.

[11] Wakabayashi Y, Fujita H, Morita I, Kawaquchi H, Murota S. Conversion of xanthine dehydrogenase to xanthine oxidase in bovine carotid artery endothelial cells induced by activated neutrophils: involvement of adhesion molecules. Biochim Biophys Acta. 1995;1265:103–109.

[12] Wang Q, Doerschuk CM. Neutrophil-induced changes in the biomechanical properties of endothelial cells: roles of ICAM-1 and reactive oxygen species. J Immunol. 2000;164:6487–6494.

[13] Koenig W. High-sensitivity C-reactive protein and atherosclerotic disease: from improved risk prediction to risk-guided therapy. Int J Cardiol. 2013;168(6):5126-34.

[14] Je´ ssica Cavalcante dos Santos, Marina Sampaio Cruz, Raul Hernandes Bortolin, Katiene Macedo de Oliveira, Je´ ssica Nayara Go´ es de Arau´ jo,I Victor Hugo Rezende Duarte, Ananı´lia Medeiros Gomes da Silva,I Isabelle Cristina Clemente dos Santos,I Juliana Marinho de Oliveira Dantas,II Maria Sanali Moura de Oliveira Paiva,II Adriana Augusto Rezende,I Mario Hiroyuki Hirata,III Rosario Dominguez Crespo Hirata,III Andre´ Ducati Luchessi and Vivian Nogueira SilbigerI “Relationship between circulating VCAM-1, ICAM-1, E-selectin and MMP9 and the extent of coronary lesions” CLINICS;73(2018)

[15] Setor K. Kunustor ,Stephan J.L Bakker and Robin P.F. Dullaart " Soluble vascular Cell Adhesion Molecules May be Protective of future cardiovascular Disease Risk :Findings from the prevend prospective cohort study " Original article .V.24 pp 804-818 (2017).

[16] Saima Sharif, Muhammad Saqib , Shagufta Naz, Misbah Manzoor,Afshan Kaleem and Mehwish Iqtedar "Relationship of IGF-1 and CKMB in Ischemic heart disease subjects "Endocrinology & Metabolism International Journal volume 6 (2018).

[17] Lizzy M.Brewster, Yentl C.Haan, Aeillko H. Zwinderman , Bert Jan Van den Born, Gert A. van Montfrans " CK (Creatine Kinase) Is Associated With Cardiovascular Hemodynamics " ahajournals .org,(2020)

[18] Paul Ganguly and Sreyoshi Fatima Alam " Role of homocysteine in the development of cardiovascular disease"Nutrition journal " 14: 6(2015).