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Evaluation of Serum Levels of Endothelial Adhesion Molecules: Leukocyte Adhesion Molecule-1 (LAM-1), CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) in Fibrotic Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author TEM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MES and AFA managed the analyses of the study. Authors HAO and TEH managed the literature searches. Author MAZ collected the samples. All authors read and approved the final manuscript.

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ABSTRACT

Leukocyte adhesion molecule-1 (LAM-1), CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) are adhesion molecules and constitute important steps in the liver inflammation due to chronic hepatitis C. We measured soluble intercellular adhesion molecule (LAM-1), (LFA-1) and (Mac-1) as well as cholesterol and triglyceride concentrations in the serum of 120 patients with fibrosis. A study was carried out to analyze levels of LAM-1, LFA-1 and Mac-1in fibrotic patients, and find whether increasing with cholesterol and triglycerides. 120 serum samples from fibrotic patients were

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classified according to levels of Cholesterol and Triglycerides concentration into four groups. Positive LAM-1 samples were found in 90% of patients in first group, 83% in the second group, 73% in the third group and 46% in the fourth group. These levels were significantly higher than their levels in control group ($p<0.0001$) indicating extremely significant. Level of LAM-1in group (1) was extremely significant compared to group (4) (356 ± 70.5 vs. 209± 5 $p < 0.0001$ ES). High LFA-1level was found in 76% in first group, 73% in the second group, 70% in the third group, and 40% in the fourth group. The levels of MAC-1 in first group were significantly greater than their levels in control group ($p<0.0001$), and +ve MAC-1 samples were found in 66% in first group, 53% in the second group, 46% in third group, and 36% in fourth group. AST and ALT were significantly higher in first group, compared to healthy group (95.68 ± 33.32 vs. 31.77 ± 8.11, $p < 0.001$) for AST and (78.6 ± 29.86 vs. 28.55 ± 7.15 $p < 0.001$) for ALT indicating very significant relationship, while no significant was detected between the fourth group and healthy individuals (33.56±8.16 vs. 28.55 ± 7.15 $p = 0.05$ NS). Our study showed a significant increase in levels of LAM-1 and LFA-1 rather than MAC-1 in fibrosis compared to healthy individuals. The results showed the ability to circulate LAM-1 and LFA-1 to predict fibrosis disease and evaluated the relationship between circulating adhesion molecules and fibrotic patients.

Keywords: Endothelial adhesion molecules; LAM-1; LFA-1; Mac-1.

1. INTRODUCTION

Adhesion molecules play a significant role in the recruitment of neutrophils to the site of inflammation. Neutrophils' localization is dynamic and involves multiple steps. In each step a different family of adhesion molecules takes part [1,2].

Cell adhesion molecules (CAMs) are proteins located on the cell surface involved in binding with other cells or with the extracellular matrix (ECM) in the process called cell adhesion. Cell adhesion molecules are necessary to help cells stick to each other and their surroundings [3].

CAMs are classified into different families, Selectin family of CAMs [as L-Selectin (LAM-1), P-Selectin & E-Selectin], Mucin-like family of CAMs [as Glycam-1, PSGL-1 & MadCAM-1], Immunoglobulin superfamily of CAMs [as ICAM1, VCAM1 & MadCAM-1] and Integrin family of CAMs [as LFA-1 & Mac-1]. Integrins are expressed on leukocytes and they help in leukocyte adherence to vascular endothelium [4].

L-selectin, also known as LAM-1, LAM-1 belongs to the selectin family of proteins. LAM-1 is a cell adhesion molecule found on lymphocytes [5]. Lymphocyte function-associated antigen 1 (LFA-1) is found on all T-cells and also on B-cells, neutrophils, macrophages, and NK cells. LFA-1 is involved in recruitment to the site of infection. It binds to ICAM-1 on antigen-presenting cells and functions as an adhesion molecule [6]. Macrophage-1 antigen (Mac-1) belongs to the integrin family of proteins, it`s a complement receptor ("CR3") consisting of CD11b (integrin αM) and CD18 (integrin β2) [7].

In the inflammatory state, LFA-1and MAC-1 are play essential roles in promoting migration of immunological cells to target site [8,9].

Raised serum levels of CAMs (LAM-1, LFA-1, and MAC-1) have been reported in patients with infections, inflammatory, neoplastic diseases and fibrosis disease [10,11]. Also, CAMs are considered as markers of atherosclerosis [12]. Measuring serum levels of them may be important for prognosis of these diseases.

Fibrosis is the most common chronic disease and is emerging as an early atherosclerosis` marker [13,14].

1.1 Aim

The present study was based on the hypothesis of that circulating levels of MAC-1, LAM-1 and LFA-1may be useful markers for increased expression of cellular CAMs in patients with fibrosis. To test the hypothesis, we examined the relationship between levels of circulating MAC-1, LAM-1, LFA-1 and the extent of fibrosis as identified by highly cholesterol and triglycerides (TG). We also tested whether the levels of circulating AMs predict the risk of incident fibrosis disease.

2. MATERIALS AND METHODS

150 serum samples were collected (120 serum samples from fibrotic patients and 30 controls)

from gastroenterology center, Mansoura university. Liver fibrosis is diagnosed by liver biobsy. Liver biopsy is still considered the gold standard for the assessment of necroinflammation and fibrosis. Fibrotic patients are classified according levels of cholesterol and triglyceride. Samples are centrifuged at 1500 rpm for 15 minutes and stored at -20 C untill used. Then ALT, AST and Alb were detected by biochemical analysis

2.1 Quantification of Soluble Adhesion Molecules

Serum levels of circulating LAM-1, LFA-1 and MAC-1, were determined by a commercially available sandwich ELISA technique (RayBiotech.Inc, for MAC-1 and LAM-1 and R&D systems.Inc.Minneapolis for LFA-1).

The procedure were performed according to the manufactures' instructions. Briefly, a conjugated monoclonal antibody against LAM-1, LFA-1 and MAC-1 was added to micro-titre plates coated with a murine monoclonal IgG antibody recognizing a different epitope of the corresponding molecule. After incubation with samples or standards in appropriate dilution, the color reaction was developed with tetramethylenebenzidine (TMB), and the plates were read on an automated multiscanner at 450 nm.

2.2 Analysis of Serum Markers

Routine biochemical determinations as levels of liver enzymes, (including ALT & AST) and albumin were performed in sera samples by standard automated methods using an automatic analyzer (Hitachi 902 autoanalyzer S.N. 1048008).

2.3 Statistical Methods

Data are expressed as means + SD. Unpaired t test were used for between – group analysis at baseline. A value of $p<0.0001$ regards as extremely significant, $p< 0.001$ very significant and $p<0.05$ regards as significant.

3. RESULTS

The fibrotic patients, with normal cholesterol and triglycerides (group4), with high both cholesterol & triglyceride (group1), and healthy individuals had a mean age (+SD) of 43.33 ± 5.87, 52.65 ± 8.12, and 40.6 ± 7.7 years respectively. Serum AST and ALT were significantly higher in fibrotic patients with high TG and cholesterol (group4) , compared to healthy individuals. 95.68 ± 33.32 vs 31.77 ± 8.11 , $p < 0.001$, for AST and 78.6 ± 29.86 vs 28.55 ± 7.15 $p < 0.00$, for ALT indicating very significant relationship. No significant was reported between ALT levels of fibrosis (with normal TG and cholestrol) and healthy individuals (33.56 $\pm$ 8.16 vs 28.55 $\pm$ 7.15 $p = 0.05$ ***NS***) (Table 1). While, ALT and AST levels of fibrotic patients with high either TG or cholesterol (group 2 or 3) had moderate significant compared to healthy individuals (for ALT, $p <0.05$ ***S*** & $p <0.05$ ***S***) & (for AST, $p <0.05$ ***S*** & $p <0.05$ ***S***) for high cholesterol (group 2) and high TG (group 3) respectively.

Serum albumin concentrations of fibrotic patients with abnormal both TG and cholesterol was lower compared to that of healthy individuals (3.299 ± 0.38 vs 4.15 ± 0.27 $p >0.05$ ***NS***), indicating no statistical significance.

3.1 Evaluation of LAM-1, LFA-1 and MAC-1 Levels

LAM-1, LFA-1and MAC-1 were determined in 120 fibrotic patients which were classified to four groups: the first group includes 30 samples with high both TG and cholesterol, the second group includes 30 samples with normal TG and abnormal cholesterol, the third group includes 30 samples with abnormal TG and normal Cholesterol, and the fourth group includes 30 samples with normal both TG and cholesterol using ELISA. Sera from 30 healthy individuals were used as healthy controls.

LAM-1: As shown in Table (2), the level of LAM-1, in group (1) ranged from 277-422 and mean+SD 356 + 70.5, from 243 – 395 with mean+SD 310 + 82 for group (2), ranged from 175 – 331with mean+SD, 226 + 95, for group (3) and 134 – 275 with mean+SD, 209 + 59, for group (4). These concentrations were significantly higher than their concentration in control group that had LAM-1 levels from 85 – 220 and 133.5 + 56.89 as mean+SD, indicating extremely significant ($p<0.0001$).

Cut off value was defined as 2 SD above the mean of the healthy individuals. Cut off value for the LAM-1 in the fibrotic patients was 225. So, positive samples of serum LAM-1 are those above 225. The +ve LAM-1 samples were reported in 27 out of 30 (90%) patients in the first group, 25 out of 30 (83%) in

Table 1. Demographics and baseline characteristics for patients and controls

Parameter	Control n= 30	Fibrotic patients			
		Normal TG & cholesterol (n = 30) *P value*	Normal TG & abnormal cholesterol (n = 30) *P value*	Abnormal TG & normal cholesterol (n = 30) *P value*	Abnormal TG & cholesterol (n = 30) *P value*
Age (yr)	40.6± 7.7	43.33 ± 5.87	44.6 ± 6.76	43.2 ± 6.11	52.65 ± 8.12
Sex M/F	18/12	17/13	9/21	8/22	11/19
Alb. (mg%)	4.15 ± 0.27	3.78 ± 0.25	3.76 ± 0.26	3.71 ± 0.27	3.299 ± 0.38 *P > 0.05* ***NS***
ALT (IU/l)	28.55 ± 7.15	33.56 ± 8.16 P=0.1421 ***NS***	39.7 ± 10.93 *p* <0.05 ***S***	44.7 ± 12.4 *p* <0.05 ***S***	78.6 ± 29.86 *P*<0.0001 ***ES***
AST (IU/l)	31.77 ± 8.11	39.78 ± 13.50 P=0.1225 ***NS***	50.88 ± 18.2 *p* <0.05***S***	63.3 ± 18.23 *p* <0.05 ***S***	95.68 ± 33.32 *P*<0.0001 ***ES***

NS: Not significant; ES: Extremely significant

Table 2. Prevalence of LAM-1, LFA-1and MAC-1 in serum of Fibrotic patients and healthy control

Group	No	LAM-1		LFA-1		MAC-1	
		Positive > 225		Positive > 279.65		Positive > 432.3	
		Range mean + SD *P* value	+Ve / -Ve	Range mean + SD *P* value	+Ve / -Ve	Range mean + SD *P* value	+Ve / -Ve
Healthy control	30	85 – 220 133.5 ± 56.89	0/30	178 – 265 208 ± 54.5	0/30	94 – 426 321 ± 110	0/30
Fibrosis with high TG and chol	30	277-422 356 ± 70.5 *P* <0.0001 ***ES***	27/3	355 -545 445 ± 95 *P* <0.0001 ***ES***	23/7	178 - 778 488 ± 289 *P* <0.0001 ***ES***	20/10
Fibrosis with normal chol and high TG	30	243 – 395 310 ± 82 *P* <0.0001 ***ES***	25/5	332 – 489 420 ± 65 *P* <0.0001 ***ES***	22/8	157 – 687 441 ± 245 *P* <0.0001 ***ES***	16/14
Fibrosis with normal TG and high chol	30	175 – 331 226 ± 95 *P* <0.0001 ***ES***	22/8	285 – 434 354 ± 82. *P* <0.0001 ***ES***	21/9	143 – 634 421 ± 210 *P* <0.0001 ***ES***	14/16
Fibrosis withnormal both TG and chol	30	134 – 275 209 ± 59 *P* <0.0001 ***ES***	14/16	243 – 387 312 ± 68 *P* <0.0001 ***ES***	12/18	125 – 567 354 ± 198 *P* <0.0001 ***ES***	11/19

the second group, 22 out of 30 (73%) in the third group, and 14 out of 30 (46%) in the fourth group. The specificity of LAM-1 was 100%. Level of LAM-1 in fibrotic patients was extremely significant in group 1 (with high both TG and cholesterol) compared to those in group 4 (with normal both TG and cholesterol) (356 + 70.5 vs 209 + 59 p < 0.0001). The overall accuracy of the test equal to 93%, 85%, 80%, and 70% for the four groups respectively

LFA-1: The level of LFA-1, in group (1) ranged from 355-545 and mean+SD 445 + 95, from 332 – 489 with mean+SD, 420 + 65 for group (2), ranged from 285 – 434 with mean+SD, 354 + 82, for group (3) and 234 – 387 with mean+SD, 312 + 68, for group (4).These concentrations were significantly higher than their concentrations in control group that had LFA-1 levels from 178 – 265 and 208 + 54.5 as mean+SD, indicating extremely significant (p<0.0001) (Table 2).

Cut off value for the LFA-1in the fibrotic patients was 279.65. So, +ve samples of serum LFA-1 are those above 279.65. The positive LFA-1samples were found in 23 out of 30 (76%) patients in the first group, 22 out of 30 (73%) in the second group, 21 out of 30 (70%) in the third group, and 12 out of 30 (40%) in the fourth group. The specificity of LFA-1 was 100. Level of LFA-1in fibrotic patients with high TG and cholesterol was extremely significant compared to fibrotic patients was extremely significant in group 1 (with high both TG and cholesterol) compared to those in group 4 (with normal both TG and cholesterol) (445 + 95 vs 312+ 68 p < 0.0001 ES). The overall accuracy of the test equal to 89%, 82%, 75%, and 66% for the four groups respectively.

MAC-1: The level of MAC-1, in group (1) ranged from 178-778 and mean+SD 488 + 289, from 157 – 687 with mean+SD , 441 + 245 for group (2), ranged from 143 – 634 with mean+SD, 421 + 210, for group (3) and 125 – 567 with mean+SD, 354 + 198, for group (4). These concentrations were significantly higher than theie concentrations in control group that had MAC-1 levels from 94 – 426 and 321 + 110 as mean+SD, indicating extremely significant (p<0.0001) (Table 2).

The cut off value for the MAC-1 in the fibrotic patients was 432.3, So, positive samples of serum MAC-1 are those above 432.3. The +ve MAC-1 samples were reported in 20 out of 30 (66%) patients in the first group, 16 out of 30 (53%) in the second group, 14 out of 30 (46%) in the third group, and 11 out of 30 (36%) in the fourth group. The specificity of MAC-1 was 100%. Level of MAC-1 in fibrotic patients with high TG and cholesterol was extremely significant compared to fibrotic patients was significant in group 1 (with high both TG and cholesterol) compared to those in group 4 (with normal both TG and cholesterol) (488 ± 289 vs 354 ± 198 *p* =0.0020 ***VS***). the overall accuracy of the test equal to 79%, 70%, 67%, and 45% for the four groups respectively.

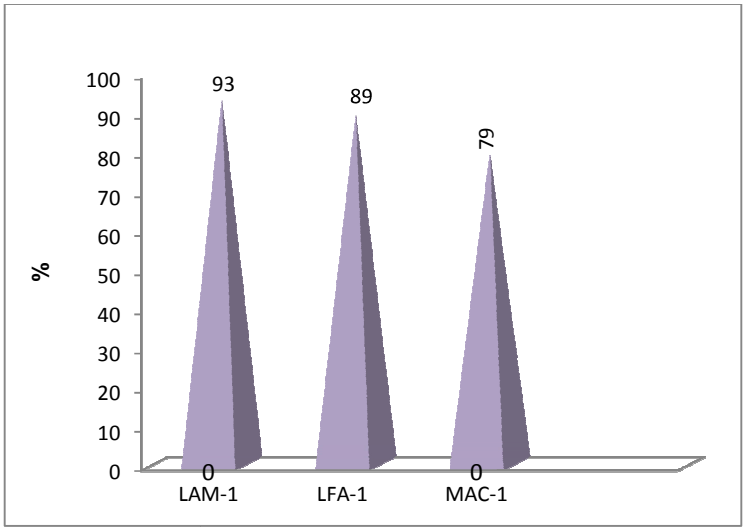

Fig. 1. Accuracy of ELISA for detecting LAM-1, LFA-1and MAC-1 in fibrosis with abnormal TG and cholesterol compared to controls

4. DISCUSSION

Cellular adhesion is essential in maintaining multicellular structure. Cellular adhesion can link cells in different ways and can be involved in signal transduction. Cell adhesion is also essential for the pathogenesis of infectious organisms [15,16]. Adhesion molecules , such as LAM-1, LFA-1and MAC-1**,** play essential roles in the migration and attachment of leukocytes [17,18].

Adhesion molecules, LAM-, LFA-1 and MAC-1 have been increased in inflammatory processes [19] and were detected in individuals with atherosclerosis and individuals with high hyperlipidaemia [20,21].

Fibrosis of the liver is excessive accumulation of scar tissue that results from ongoing inflammation and liver cell death that occurs in most types of chronic liver diseases. Nodules, abnormal spherical areas of cells, form as dying liver cells are replaced by regenerating cells. This regeneration of cells causes the liver to become hard. Fibrosis refers to the accumulation of tough, fibrous scar tissue in the liver [22,23]. The fibrosis has risen rapidly with the obesity, where it has been reported in up to 15 % of non-obese individuals and up to 75% of obese individuals [24,25].

So, in this research, we concerned on the analysis of the serum levels of different endothelial CAMs in fibrotic patients that suffered from high both TG and cholesterol or only one of them compared to healthy control.

In our study, the serum levels analysis of these three adhesion molecules in fibrotic patients with abnormal both cholesterol and TG showed significantly higher levels compared to controls group ($p<0.0001$). Serum concentrations of LAM-1 and LFA-1were higher in fibrotic patients with high cholesterol and TG values (group1), with sensitivity 90% and 76% respectively and accuracy 93% and 89% respectively, while these concentrations were decreased in case of fibrotic patients with Normal cholesterol and TG values (group 4), with sensitivity 46% and 40% respectively and accuracy 70% and 66% respectively. Raised levels of LAM-1reflect endothelial activation, while increased levels of LFA-1could reflect endothelial or immune activation (T-lymphocyte activation) [10,19].

The sensitivity and accuracy of MAC-1 concentrations in fibrotic patients with high Cholesterol and TG were 66% and 79% respectivelly and were 36% and 45% in fibrotic patients with normal cholesterol and TG.

High serum levels of LAM-1 and LFA-1compared to MAC-1 levels has already been reported in patients with asthma [26] or ischemic heart disease [27,28].

Recent investigations have reanimated the view that there exists a possible link between atherosclerosis and inflammation. The recruitment of leukocytes into areas of inflammation is mediated by interacting sets of cell adhesion molecules [29]. In atherosclerosis, focal expression of key adhesion molecules particularly triggered by plasma atherogenic lipoproteins has been detected and these molecules may mediate the recruitment of mononuclear cells to the plaque. Among these adhesion molecules, LAM-1 & LFA-1. have been suggested to play an important role in atherogenesis [30,31].

The present research exhibited a significant increase in concentrations of LFA-1 and LAM-1 in fibrotic patients compared with healthy individuals and these results are in harmony with the results of Musso et al. [10].

Girón-González et al showed that, in cirrhotic patients, endothelial activation has an essential role in modifications of the circulatory status. Also, it has a role in the de novo expression of LFA-1and MAC-1 to mediates the transmigration of inflammatory cells, inducing tissue damage of liver [32]. Many studies reported that, in chronic liver disease, LAM-1and LFA-1expressions are up-regulated, where they have roles in the pathogenesis of chronic hepatitis and fibrotic patients [33,34], increased levels of LAM-, LFA-1 and MAC-1 have been reported in individuals with atherosclerosis and hyperlipidaemia [21].

Williams et al reported that high serum levels of soluble LAM-1 are associated with chronic hepatitis and liver fibrosis [31,35]. Diet-related lipoprotein particles induce the expression of adhesion molecules [31].

In fibrotic patients with high both TG, cholesterol, serum ALT and AST were significantly higher compared to controls, indicating very significant relationship ($p < 0.001$) for AST and ($p < 0.001$) for ALT. While, in fibrotic patients (with normal TG and cholestrol), ALT & AST

levels showed no difference compared to controls (P=0.1421 & P=0.1225) indicating no significance. These result were in accordance with the results of Wyszomirska et al. [36].

According to World Health Organization (WHO), high levels of triglyceride (≥ 150 mg/dl) is one of the diagnostic criteria of the metabolic syndrome [37,38].

The increased expression of MAC-1 on the endothelial surface is the earlist indicator of endothelial activation in the hyper cholesterolaemic rabbit [39].

In several studies, the high expressions of AMs, such as LAM-1, LFA-1 and MAC-1, were reported [10,32]. Where these adhesion molecules has roles in the pathogenesis of fibrotic liver. In our research, very less concentrations of adhesion molecules were found in the normal liver in contrast to fibrotic patients, where high concentrations of them found.

Our study reported a significant increase in levels of LAM-1 and LFA-1 rather than MAC-1 in fibrosis compared to healthy individuals. The results showed the ability of circulating LAM-1 and LFA-1 to predict fibrosis disease and evaluated the relationship between circulating adhesion molecules and fibrotic patients.

5. CONCLUSION

Our study showed a significant increase in levels of LAM-1 and LFA-1 rather than MAC-1 in fibrosis compared to healthy individuals. The results showed the ability to circulate LAM-1 and LFA-1 to predict fibrosis disease and evaluated the relationship between circulating adhesion molecules and fibrotic patients.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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