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STUDY E3 SUMO-PROTEIN LIGASE (NSMCE2), LIPID PROFILE AND MALONDIALDEHYDE IN PATIENTS WITH ATHEROSCLEROSIS

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ABSTRACT

The present work includes a clinical study of the E3 Sumo-Protein Ligase enzyme[NSMCE2] in 60 clinically diagnostic atherosclerosis patients compared with 30 healthy as controls, also this study committed to the measurement several parameters levels such as lipid profile, MDA and relationship to E3 Sumo-Protein Ligase, the results observed that the serum triglyceride, total cholesterol, LDL, VLDL and LDL/HDL ratio showed significant increase in patients group in comparison to control group, while HDL showed significant decrease in patients group in comparison to control group. The level of malondialdehyde(MDA) were significant increase ($p<0.001$) in patients group in comparison to control group. The level of NSMCE2 were highly Significant increase ($p<0.0001$) in patients group in comparison to healthy control group. The aim of the present study is to investigate the E3 Sumo-Protein Ligase and some biochemical parameters in patients with atherosclerosis compared with control.

KEYWORDS: Atherosclerosis, E3 Sumo-Protein Ligase, NSMCE2, Lipid profile, MDA.

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INTRODUCTION

Atherosclerosis is a chronic inflammatory disease arise from builds up of fatty substances, cholesterol, calcium, and other substances found in the blood were plaque inside artery walls over time, plaque hardens and narrows arteries\(^1\,^2\). Producing pathological conditions such as acute myocardial infarction, unstable angina or sudden cardiac death\(^1\). Early diagnosis of atherosclerosis may be highly benefit from many clinical parameters, like serum cholesterol, LDL-cholesterol, plasma triglycerides, HDL-cholesterol, MDA and related enzymes. Atherosclerosis starts when high blood pressure, smoking, or high cholesterol damages the endothelium. At that point, cholesterol plaque formation begins\(^1\). Invasion, crosses damaged endothelium. The cholesterol enters into the area of the damaged vessel and deposit there\(^3\). Inflammatory cells, like macrophages, will also enter the damaged area, causing inflammation and engulfing the lipids. Once inside the vessel wall, LDL-cholesterol particles get stuck and their content becomes more prone to oxidation. The damage caused by the oxidized LDL-cholesterol molecules triggers a cascade of immune responses which over time can produce an atheroma. Eventually, the artery becomes inflamed. As the lipids begin to accumulate on the endothelial surface, a thick plaque will form. The white blood cells stream in to digest the LDL-cholesterol\(^4\,^5\). Over years, the accumulating mess of cholesterol, dyslipidemia, cells debris, white blood cells, calcium, and other substances, initiate a plaque in the walls of arteries. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area which causes a narrowing of the artery, reducing blood flow, increasing blood pressure\(^6\,^7\), finally lead to stroke, CAD, thrombosis\(^9\,^10\). According to the theory of oxidative stress, atherosclerosis is the result of the oxidative modification of low density lipoproteins (LDL) in the arterial wall by reactive oxygen species (ROS)\(^11\), not only from the endothelial cells, but also from the smooth muscle cells and the adventitial cells\(^11\). Endothelial dysfunction predisposes to long-term atherosclerotic lesions and has been proposed as an important diagnostic and prognostic factor for coronary syndromes\(^12\). The uptake by macrophages is easier compared to non-oxidized lipoproteins\(^12\,^13\). The determination of Malondialdehyde (MDA) is used for monitoring lipid peroxidation in biological samples\(^14\). Therefore, MDA is considered an important oxidative stress biomarker as is the total antioxidant status and plays a key role in modifying LDL-cholesterol, which mediates the pathophysiological changes by nonenzymatic and auto-oxidative glycosylation\(^15\,^18\). Oxidized LDL-cholesterol has been shown to accumulate in atherosclerotic lesions, and a growing body of evidence indicates that oxidized LDL-cholesterol is involved in the pathogenesis of coronary artery disease, acute coronary syndrome, and vulnerable plaque\(^15\,^19\). Small Ubiquitin-like Modifier (SUMO) proteins are a family of small proteins belongs to the ubiquitin (Ub) and ubiquitin-like (Ubl) protein family\(^20\,^21\). SUMO proteins are covalently attaches to certain lysine residues of specific target proteins in cells and alters a number of different functions depending on the substrates\(^20\). The SUMOylation is a dynamic and reversible process regulated by both conjugation and de-conjugation enzymes via a three-step process and three enzyme reactions, E1 (activation), E2 (conjugation), and E3 (ligation)\(^22\,^23\). The SUMOylation is a part of important regulatory mechanisms that modify proteins in the nucleus and regulate multiple cellular processes such as nucleo-cytoplasmic signal transduction, apoptosis, stress responses, protein stability, subcellular localization of proteins, protein-protein interactions, protein-DNA interactions, and transcriptional activity of transcription factors and progression through the cell cycle\(^22\,^23\).

MATERIALS AND METHODS

These study was conducted on a cohort of 60 patients with atherosclerosis and 30 healthy persons to be used as control ranging
between (40-75) years. These patients were hospitalized at Research Institute for educational laboratories in the city of Medicine of the Ministry of Health. Seven milliliter of blood were collected and allowed to clot for 10-15 min. at room temperature, centrifuged for (10) min. at (3000xg). The serum were separated and stored at [-20]°C until used. Serum cholesterol level, Serum Triglycerides (TG) level was measured by fixed enzymatic end point method supplied by Biolabo\textsuperscript{24,25}. The chylomicrons and lipoproteins of VLDL and LDL-cholesterol contained in the sample are precipitated by the addition of phosphotungastic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contains HDL-cholesterol from which the cholesterol can be determined using the cholesterol enzymatic reagent, the same method for total cholesterol estimation \textsuperscript{26}. Serum MDA is estimated by methods of (Benge J.A.1978) \textsuperscript{27}. The product breaks down of polyunsaturated fatty acids. Basically, this was the end product of lipid peroxidation which can be added to the thiobarbituric acid (TBA) to get rid of chromophore which is useful in the determination of lipid peroxides at 535 nm. The NSMCE2 assay employs the quantitative sandwich enzyme immunoassay. The color development is stopped and the intensity of the color is measured.

**RESULTS**

The present study included sixty male patients with atherosclerosis and thirty males matched apparently healthy individuals as control group. Table (1) and Figure (1) show Serum lipid profile of patients and control groups. A significant increase (p <0.01) in serum cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol in patients group compared to control group, while HDL-cholesterol shows a significant decrease in patients group compared to control group. LDL/HDL ratio showed highly significant increase in patients group compared to control group.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum lipid profile in patients and control groups</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients group [n=60]</th>
<th>Control group [n=30]</th>
<th>Comparison of Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.cholesterol[mmol/l] Mean ± SD Range</td>
<td>4.96±0.94 4.00-8.30</td>
<td>4.29±0.25 4.00-4.80</td>
<td>0.008 Significant [p&lt;0.01]</td>
</tr>
<tr>
<td>S.TG[mmol/l] Mean ± SD Range</td>
<td>1.61±0.76 0.70-4.60</td>
<td>1.11±0.23 0.80-1.50</td>
<td>0.009 Significant [p&lt;0.01]</td>
</tr>
<tr>
<td>HDL-cholesterol[mmol/l] Mean ± SD Range</td>
<td>1.05±0.24 0.75-1.78</td>
<td>1.46±0.18 1.20-1.87</td>
<td>0.007 Significant [p&lt;0.01]</td>
</tr>
<tr>
<td>LDL-cholesterol[mmol/l] Mean ± SD Range</td>
<td>3.17±0.89 1.77-6.15</td>
<td>2.32±0.31 1.69-2.87</td>
<td>0.008 Significant [p&lt;0.01]</td>
</tr>
<tr>
<td>VLDL-cholesterol[mmol/l] Mean ± SD Range</td>
<td>0.77±0.35 0.41-2.12</td>
<td>0.52±0.10 0.40-0.69</td>
<td>0.009 Significant [p&lt;0.01]</td>
</tr>
<tr>
<td>LDL:HDL ratio Mean ± SD Range</td>
<td>3.24±1.41 1.22-7.98</td>
<td>1.61±0.33 0.99-2.35</td>
<td>0.001 Highly Significant [p&lt;0.001]</td>
</tr>
</tbody>
</table>
The mean levels of serum MDA showed a significant increase ($p < 0.001$) in patients group (3.01±0.96 $\mu$mol/l) when compared to control group (1.77±0.62 $\mu$mol/l) as shown in Table (2) and Figure (2).

**Table 2**

*The mean and standard deviation of serum MDA [μmol/l] in patients and control groups.*

<table>
<thead>
<tr>
<th>Studied group</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Comparison of Significant p-Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>60</td>
<td>3.01±0.96</td>
<td>0.95-4.28</td>
<td>0.001</td>
<td>Significant $[p&lt;0.001]$</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>1.77±0.62</td>
<td>0.80-2.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td></td>
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</table>
The present study showed that mean levels of sera NSMCE2 have a highly significantly increase \((p<0.0001)\) in patients group compared to control group as shown in Table (3) and Figure (3).

**Table (3)**

<table>
<thead>
<tr>
<th>Studied group</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Comparison of Significant</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>60</td>
<td>219.25±52.04</td>
<td>98.52-337.98</td>
<td>p-Value: 0.0001</td>
<td>Highly Significant ([p&lt;0.0001])</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>101.82±23.20</td>
<td>62.02-155.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td></td>
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</table>

**DISCUSSIONS**

The small ubiquitin-related modifier (SUMO) system has been implicated in numerous physiological and pathological processes through altering the functions of its target proteins. The SUMO covalent linkage is usually through the lysine residue(s). Some sumoylation assays revealed that in the presence of E1 and E2, the E3 ligase was dispensable to accomplish SUMO conjugation. However, SUMO E3 ligases...
contributed to the efficiency and specificity of SUMO conjugation and were attributed to the RING domain, which is similar to the corresponding structure in E3 ligases involved in the ubiquitination. Several reports refer that SUMO modification activated several cardiac muscle-restricted genes. Increasing pieces of evidence support the important role of ROS and cytokine production in the process of atherosclerosis via regulating various signaling pathways leading to vascular inflammation. Another review focused on ROS-mediated SUMOylation, which is one of the posttranslational modifications, and discussed its possible implications on vascular inflammation. It becomes apparent that ROS production can regulate the process of SUMOylation in both vasculature and heart and mediate a number of biological processes, such as apoptosis and inflammation. The SUMO modification of proteins has been suggested to regulate many physiological processes, such as transcriptional regulation, stress responses and protein localization. Recent studies indicate a role for sumoylation in the regulation of inflammation that is initiated in response to tissue damage and infectious agents. Inflammatory responses must be regulated properly, and unrestricted inflammation can lead to inflammatory disorders. Atherosclerosis is considered to be a chronic inflammatory disease. The transcriptional induction of genes involved in inflammatory responses is controlled by various transcription factors, including nuclear factor κB (NF-κB), signal transducer and activator of transcription (STAT) and activator protein-1 (AP-1). Sumoylation can regulate inflammation through the direct modulation of the activity of key transcription factors involved in inflammatory responses. A member of the protein inhibitor of activated STAT (PIAS) family, PIAS1, which possesses SUMO E3 ligase activity, is a transcriptional repressor of NF-κB and STAT1. PIAS1 functions by blocking the DNA-binding activity of NF-κB and STAT1 on gene promoters. Recent studies indicate that PIAS1 is activated by phosphorylation in response to pro-inflammatory stimuli, a process that requires the SUMO ligase activity of PIAS1. Activated PIAS1 is then recruited to inflammatory gene promoters to repress NF-κB and STAT1-mediated transcription. These findings support a hypothesis that targeting the PIAS1 sumoylation pathway might represent a novel therapeutic strategy for the treatment of inflammatory disorders such as atherosclerosis. The association between high serum cholesterol levels, especially high LDL-cholesterol, and CAD is caused and independent of other risk factors. The Jellinger study found a strong and progressive relationship between elevated total cholesterol levels and death of CAD. Since multiple studies have demonstrated that lowering LDL-cholesterol results in decreased CAD risk, the focus of risk prediction and reduction has shifted toward LDL-cholesterol management in CAD and primary prevention in people with multiple risk factors. Low HDL-cholesterol is associated with hypertriglyceridemia and can act synergistically with other lipid risk factors to increase CAD risk. For example, the ratio of total cholesterol or LDL-cholesterol to HDL-cholesterol may be a clinically valuable and potentially sensitive marker of CAD risk and highly predictive of major cardiovascular event risk. Triglyceride levels are an important component of risk evaluation in both men and women. Abundant clinical evidence indicates that elevated triglyceride levels may be an independent risk factor. Triglyceride levels 2.28 mmol/l or higher may indicate a substantial increase in CAD risk. Hypertriglyceridemia is also commonly associated with a pro-coagulant state and hypertension. As triglyceride levels increase with age, the importance of hypertriglyceridemia as a CAD risk factor also appears to increase. Furthermore, research suggests that low HDL-cholesterol, high serum triglyceride levels may act synergistically with other lipid abnormalities to increase CAD risk. Atherosclerosis is a chronic inflammatory disease and infections potentially contribute to its pathogenesis. Oxidation of LDL-cholesterol is a key event in atherogenesis. Lipid peroxidation produces reactive aldehydes such as MDA and MDA acetaldehyde that forms immunogenic adducts on for example LDL-cholesterol.
particles. Malondialdehyde, which is one of the most popular markers, was designed to indicate lipid peroxidation\textsuperscript{39}. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of atherosclerosis and progression of atherosclerotic lesions. One of the initial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall. In addition, it has been demonstrated that increased intracellular generation of reactive oxygen species plays an important role in chronic inflammatory responses to atherosclerosis. The ROS are generated in aerobic organisms during physiological or physiopathological oxidative metabolism of mitochondria. The ROS may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, thereby interfering with cell function\textsuperscript{39,40}. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems. Impairment in the oxidant/antioxidant equilibrium provokes a situation of oxidative stress and generally results from hyperproduction of ROS. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases. A lot of oxygenated compounds, particularly aldehydes such as MDA and conjugated dienes, are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids\textsuperscript{39,40}. In present study, the result of atherosclerosis patients shows that the increase in NSMCE2, and MDA may play a role in developments of change DNA damage in the patients with atherosclerosis. Increased in NSMCE2 and lipid peroxidation, through increase MDA levels, patients with atherosclerosis recorded in this study, in a result of elevated ROS production. The antioxidant state is reduced and the oxidative stress is increased in patients with atherosclerosis group. An increase in serum NSMCE2 concentration may resulted from the effect of elevation in lipid peroxidation. As our knowledge, no previous studies have shown these results in atherosclerosis patients.

REFERENCES

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