Advances in Research
4(2): 79-86, 2015, Article no.AIR.2015.063
ISSN: 2348-0394

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Relationship between E3 SUMO-protein Ligase NSE2 (NSMCE2) with Ecto-5'-nucleotidase, ADA and AMPDA Enzymes in Patients with Atherosclerosis

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Authors' contributions

This work was carried out in collaboration between all authors. Author WAM designed the study, wrote the protocol. Author NAJ did the field work (samples collection), laboratory work (data collection), data editing, and wrote the first draft of the manuscript. Author WAM managed the literature searches. Author WAM supervised the entire work and corrected the manuscript. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/AIR/2015/14224
Editor(s):
(1) Francisco Marquez-Linares, Nanomaterials Research Group, School of Science and Technology, University of Turabo, USA.
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Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=869&id=31&aid=7493

Original Research Article

Received 23rd September 2014
Accepted 25th November 2014
Published 26th December 2014

ABSTRACT

Objective: The aim of this study was to determine the level of the E3 SUMO-Protein Ligase (NSMCE2) and their relationship to Ecto-5'-nucleotidase, ADA and AMPDA enzymes in patients with atherosclerosis compared to control group.
Method: Five ml of venous blood was drawn from sixty patients of atherosclerosis ranging between (40-75) years old and patients with diabetes mellitus and kidney diseases were excluded .The activity in U/L for Ecto-5'-nucleotidase, AMPDA and level of NSMCE2 (pg/ml) were measured in serum from control and patients with atherosclerosis.
Results: The present study included 60 males patients with atherosclerosis and 30 matched males

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healthy individuals as control group. The present study showed that mean levels of sera Ecto-5'-nucleotidase, ADA and AMPDA activities and NSMCE2 have a highly significantly increase (p<0.0001) in patients compared to control group.
Conclusion: The measurement of E3 SUMO-Protein Ligase (NSMCE2) and the activates of Ecto-5'-nucleotidase, ADA and AMPDA enzymes U/L in patients with atherosclerosis was highly significant elevated, compared to control group. This is normally expressed in vascular endothelial cells and in a broad range of immune cells. Therefore, adenosine formed by extracellular nucleotide catabolism on endothelial cells and immune cells appears to be an important endogenous modulator of arteriogenesis and key transcription factor involved in inflammatory responses.

Keywords: Atherosclerosis; E3-sumo-protein ligase; NSMCE2; ecto-5'-nucleotidase; ADA; AMPDA.

1. INTRODUCTION

Atherosclerosis is a slowly progressing and multifactorial disease, in which endothelial dysfunction and damage play an initial role. Many risk factors for atherosclerosis can lead to endothelial damage of the vessel, especially in the areas where blood flow is disturbed. In the presence of hyperlipidemia, disturbed blood flow results in increased endothelial turnover in the arterial wall. It was demonstrated that disturbed blood flow activates endoplasmic reticulum stress initiating a signal pathway leading to endothelial apoptosis. Following endothelial death, the neighboring mature endothelial cells actively proliferate and migrate to heal the wound [1].

Small Ubiquitin-like Modifier (SUMO) proteins are a family of small proteins that belongs to the ubiquitin (Ub) and ubiquitin-like (Ubl) protein family [2,3]. The SUMO proteins are small; most are around 100 amino acids in length and 12 kDa in mass. The exact length and mass varies between SUMO family members and depends on which organism the protein comes from. Sumo shares only 18% sequence homology with ubiquitin [3]. Although SUMO has very little sequence identity with ubiquitin at the amino acid level, it has a nearly identical structural fold (structurally similar to ubiquitin [2-5]. The SUMO proteins are covalently attached to certain lysine residues of specific target proteins in cells and alters a number of different functions depending on the substrates [2]. The SUMO ylation is a active and reversible progression controlled by several enzymes via a three step process and three enzyme reactions, E1 to E3. SUMOylation is an main of part of controlling mechanisms that adjust proteins in the nucleus and regulate multiple cellular processes such as nucleo-cytoplasmic signal transduction, protein stability, stress responses, apoptosis, subcellular localization of proteins, protein-protein interactions, protein-DNA interactions, and progression through the cell cycle [5,6].No previous study showed to any correlation between E3 SUMO-Protein Ligase (NSMCE2) with atherosclerosis.

Ecto-5'-nucleotidase is an enzyme that hydrolyzes extracellular AMP to adenosine and represents the major control point for extracellular adenosine levels and is a regulator of the adenosine signaling pathway [7]. Ecto-5'-nucleotidase is an intrinsic membrane glycoprotein, present as an ectoenzyme in a wide variety of mammalian cells and it belongs to a conserved superfamily of metallo phospho-diesterases. The Ecto-5'-nucleotidase Extracellular and intracellular activities regulate the quantity of nucleotides generated from both de novo salvage pathways ,and participate in purine salvage to support balanced synthesis of nucleotides, which is critical for maintaining high fidelity of DNA replication [7,8].

Adenosine aminohydrolase (ADA) is a polymorphic enzyme involved in purine metabolism and it is essential in the purine salvage pathway [9]. The ADA catalyzes the irreversible hydrolytic cleavage (deamination) of adenosine to inosine and ammonia. The enzyme is widely distributed in animal and human tissues. It is present in the cytoplasmic fraction and a certain amount is located in the nucleus [10,11] Although found in most tissue, ADA activity is greatest in lymphoid tissue . Its activity is 10-20 times more active in T lymphocytes than in B lymphocytes and it is necessary for the proliferation, maturation and function of lymphocytes, specifically for T lymphocytes and the maturation of monocytes to macrophages [11,12].

The AMP-aminohydrolase (AMPDA) is a key enzyme of nucleotide breakdown involved in

regulation of adenine nucleotide pool in the liver and energetic metabolism in mammalian cells. Also, it plays a crucial role in the synthesis of guanine nucleotides and in the provision of anaplerotic substrates for the Krebs cycle. The AMPDA is an enzyme that converts AMP to IMP, freeing an ammonia molecule in the process. It has several unique cellular functions and its activity and expression pattern are highly tissue specific. It plays an important role in the purine nucleotide cycle, which is designed to preserve adenylate's energy charge and phosphorylation potential under conditions of insufficient energy supply [13].

The aim of the study is to detect of E3 SUMO-Protein Ligase NSE2 (NSMCE2) and several enzymes in patients with atherosclerosis patients in comparison with healthy individuals, also to prove that these parameters may be a good other indication to diagnosis the development the atherosclerosis disease

2. MATERIALS AND METHODS

Blood samples were collected from sixty patients of males diagnosed to atherosclerosis as they were submitted to the Ibn Al-Bitar Hospital for Cardiac Surgery in Baghdad, Iraq and thirty healthy persons to be used as control ranging between (40-75) years. The samples were collected within three months starting from August until the end of November. The diagnosis for atherosclerosis based by angiographic catheter. The resultant serum were separated and stored at [-20]ºC until used. The NSMCE2 was measured by Enzyme Linked Immunosorbent Assay (ELISA)(CUSABIO BIOTECH COM.). Ecto-5'-nucleotidase activity was measured in serum according to Wood and Williams's method (1981) [14]. Adenosine Aminohydrolase (ADA) activity was determined by Giusti and Galanti method (1970). The ADA hydrolyze adenosine to inosine and ammonia, which is determined by Berthelot reaction, where ammonia forms an intensely blue indophenol with sodium hypochlorite and in alkaline solution. [15].

Determination of AMPDA activity was carried out using the same procedure that was used for ADA activity except changing the buffer and substrate used, since AMPDA activity has an absolute requirement for K^+ ions.

All statistical analyses in this study was performed using SPSS version 22.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $p<0.05$=significant, $p>0.05$=non-significant.

3. RESULTS AND DISCUSSIONS

The present study included sixty male patients with atherosclerosis and thirty males matched apparently healthy individuals as control group. 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 1 and Fig. 1.

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). Several sumoylation evaluates shown that in the occurrence of E1 and E2, while the E3 ligase was unnecessary to complete SUMO conjugation. The SUMO E3 ligases may be used to the efficiency and specificity of SUMO conjugation in addition were credited to the RING domain. Several reports described that SUMO modification activated several cardiac muscle-restricted genes [16,17].

Other study showed a role for sumoylation in the regulation of inflammation that is started in response to tissue damage and communicable agents. Inflammatory responses essential to regulated properly, and unlimited inflammation can lead to inflammatory diseases [18].

Table 1. The mean and standard deviation of serum NSMCE2 [pg/ml] in patients and control groups

Studied group	No.	Mean ± SD	Range	*p*-Value
Patients group (n=60)	60	219.25±52.04	98.52-337.98	
Control group (n=30)	30	101.82±23.20	62.02-155.22	0.0001

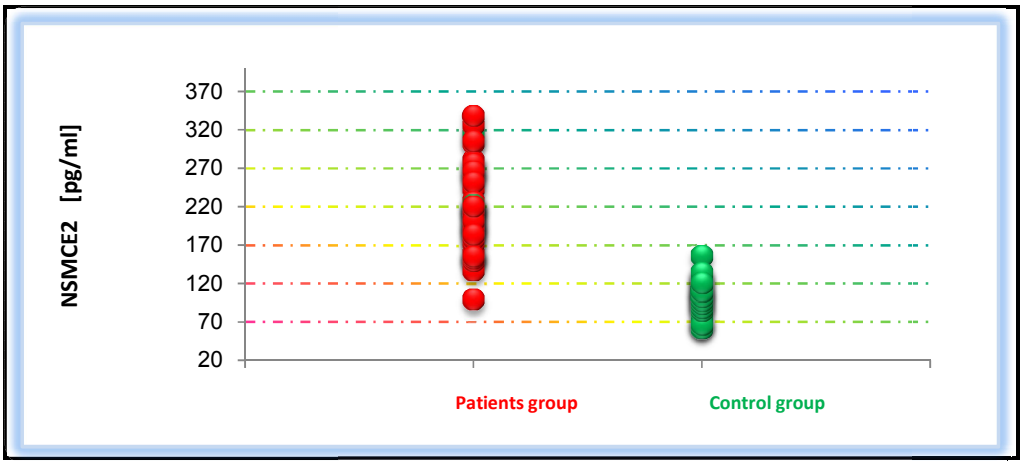

Fig. 1. Values forNSMCE2 [pg/ml] in patients and control group

Atherosclerosis is mirrored to the chronic inflammatory disease [19]. The transcriptional initiation of genes complex in inflammatory reactions is organized via several transcription features, such as signal transducer and activator of transcription (STAT) and activator protein-1 (AP-1). Sumoylation may be control inflammation by variation of activity of key transcription features complicated in the responses of inflammatory [20,21].

PIAS1 is associate of protein inhibitor of activated STAT (PIAS) family, which possesses SUMO E3 ligase activity [22]. PIAS1 functions via obstructive the DNA-binding activity of NF-κB and STAT1 on gene organizers. Other study designate that PIAS1 is activated by phosphorylation in response to pro-inflammatory stimuli, a process that requires the SUMO ligase activity of PIAS1. These conclusions support the hypothesis that targeting the PIAS1 sumoylation pathway might represent a novel therapeutic strategy for the treatment of inflammatory disorders such as atherosclerosis [23].

The activity and specific activity of serum ecto-5'-nucleotidase showed a highly significant increase (p<0.001) in patients group compared to control group as shown in Table 2 and Fig. 2.

Table 3 and Fig. 2 illustrates the activity and specific activity of serum ADA showed a highly significant increase (p<0.001) in patients group in comparison to control group. Activity and specific activity of serum AMPDA in patients group are compared to control group as shown in Table 4 and Fig. 2. In general, the activity of AMPDA in patient group showed highly significant (p<0.001) increased compared to control group.

Ecto-5'-nucleotidase is important membrane-bound enzyme involved in the metabolism of extracellular nucleotides [24,25]. It catalyzes the hydrolysis of AMP generating adenosine that is a potent vasodilator and anti-inflammatory molecule and participates in numerous important biological functions and physiological effects, such as mediation of tubuloglomerular feedback, playing a crucial role in hypoxia-induced vascular leakage. It also acts as an endogenous modulator protecting against vascular inflammation and monocyte recruitment to limit the progression of atherosclerosis, and enable the efficient entry of lymphocytes into the central nervous system during autoimmune encephalitis [25,26]. It is becoming increasingly apparent that adenosine plays a central role in the regulation of the inflammatory response. Ecto-5'-nucleotidase is an enzyme normally expressed on vascular endothelial cells and a broad range of immune cells [27]. Therefore, adenosine formed by extracellular nucleotide catabolism on endothelial cells and immune cells appears to be an important endogenous modulator of arteriogenesis, so it is obvious that the role of ecto-5'-nucleotidase-derived adenosine in a model of chronic vascular inflammation such as atherogenesis. This establishes Ecto-5'-nucleotidase-derived adenosine as a direct or indirect regulator of atherogenesis [27,28].

In the present study, a highly significant increase in ecto-5'-nucleotidase levels in atherosclerosis patients explains that its adenosine can convey protection against atherosclerosis, an

interference with intrinsic physiological pathways involved in the adenosine metabolism and ecto-5'-nucleotidase is crucially involved in the finely tuned constitutive regulation balancing proinflammatory in the microvasculature.

Adenosine aminohydrolase also has effect on the activation of complement system by the deamination of adenosine. The ADA catalyzes the conversion of adenosine to inosine, so adenosine aminohydrolase associates with and alter local concentrations of adenosine. The major source of serum ADA may be lymphocytes or the monocyte-macrophage cell system. It was reported by that elevated levels of ADA reflect the changes in the immune response in the pathogenesis of atherosclerosis and coronary heart disease. Hence ADA can be considered as important marker in assessing atherosclerosis and coronary heart disease [29]. Also, ADA is considered as an inflammatory marker [30]. And also affecting in inflammation process. Several effects created by ADA are initiated by the metabolism of adenosine. Adenosine can increase coronary artery blood flow during active stress and hypoxia to balance the oxygen supply and demand. If adenosine is rapidly metabolized by the high level of ADA, the benefits of adenosine will gone. As a final fact, adenosine is catalyzed to inosine, which may products superoxide radicals and exaggerate the ischemic/reperfusion injury [31]. AMP-aminohydrolase is the rate-limiting step for entry into the purine nucleotide cycle and catalyzes the conversion of adenosine monophosphate (AMP) to inosine monophosphate (IMP) [32,33]. A genetic background to the diversity seen in the clinical progression of heart disease is well documented. Genetic diversity in pathways involving nucleotide metabolism are particularly important due to the latter's direct links to myocardial function and metabolic regulation. Several polymorphisms of the AMPDA gene may be associated with progression of the heart disease [13,34,35].

Table 2. Themean and standard deviation of serum Ecto-5'-nucleotidase [U/L] in patients and control groups

Characteristics	Patients group [n=60]	Control group [n=30]	*p* Value
Activities [U / L]			
Mean±SD	49.05±18.08	12.15 ±3.97	0.0007
Range	25.19-119.84	5.34-21.37	
Specific Activities [U/mg]			
Mean±SD	0.72±0.29	0.17±0.05	0.0004
Range	0.35-2.02	0.08-0.30	

Table 3. The mean and standard deviation of serum ADA [U/L] in patients and control groups

Characteristics	Patients group [n=60]	Control group [n=30]	*p* Value
Activities [U / L]			
Mean±SD	41.74±16.89	14.35 ±3.01	0.0008
Range	16.44-78.00	10.00-21.00	
Specific Activities [U/mg]			
Mean±SD	0.61±0.26	0.20±0.04	0.0009
Range	0.22-1.22	0.13-0.28	

Table 4. The mean and standard deviation of serum AMPDA [U/L] in patients and control groups

Characteristics	Patients group[n=60]	Control group [n=30]	*p* Value
Activities [U /L]			
Mean±SD	39.06±15.03	12.53 ±4.20	0.0005
Range	23.00-77.56	4.03-19.23	
Specific Activities [U/mg]			
Mean±SD	0.57±0.23	0.17±0.06	0.0006
Range	0.30-1.13	0.06-0.27	

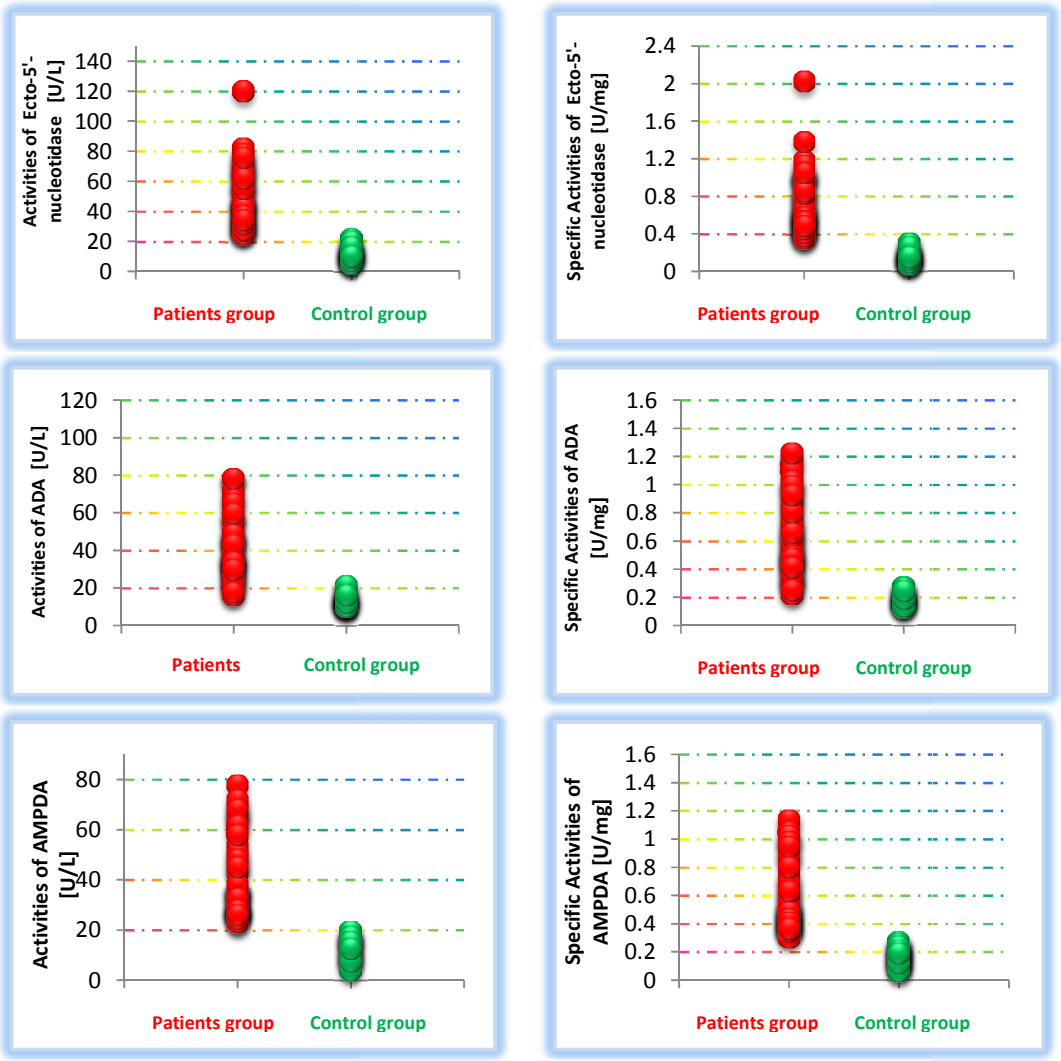

Fig. 2. Values of ecto-5'-nucleotidase, ADA and AMPDA activities [U/L] and specific activities [U/mg] in patients and control group

A study by Safranow [36] showed that no different in the AMPDA polymorphism genotype distributions in patients with heart failure and coronary artery disease and in a random control group. It could be interpreted as a protective effect of the T allele against cardiovascular diseases, but such hypothesis should be treated with caution due to the low moderate statistical significance [35,37]. The precise mechanism of the beneficial clinical effect of the AMPDA mutation is uncertain. In spite of the increased production of adenosine, a product of the alternative pathway of AMP catabolism has been suggested [35,36]. Adenosine exerts numerous effects which can attenuate the progression of heart and ischemic heart disease, such as vasodilatation, inhibition of platelet adherence, anti-adrenergic regulation of the inflammatory and the immune system [38]. The elevation in the activity of AMPDA might be associated with intima-media thickness of the carotid and brachial artery, endothelial function of the brachial artery, glucose metabolism, haemostatic variables and cardiac hypertrophy in patients with coronary heart disease, which need further study.

In conclusion, according to this study, the increase in NSMCE2 may play a role in DNA damage in the patients with atherosclerosis. Increased in ecto-5'-nucleotidase activity also recorded in this study, could be a resulted from

the elevated in lipid peroxidation effect on the permeability of cell membrane since ecto-5'-nucleotidase is a membrane bound protein

4. CONCLUSION

The present study conclude that ADA may serve as an indicator of underlying inflammation, and ADA can have an important role in coronary artery disease. This observation has been detected for the first time to the best of our knowledge. Further studies are needed to certify ADA, AMPDA, ecto-5'-nucleotidase, and NSMCE2 in other populations to evaluate that these factors may be used as a diagnostic tool for the development of the atherosclerosis disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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