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Comparative Proteomics of Lipid Transport Proteins and Assessment of Oxidative Stress Parameters in Acute Ischemic Stroke and Healthy Control

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Abstract Background: Stroke is one of the major causes of mortality worldwide. Ischemic stroke occurs due to blood vessel blockage that limits blood supply to the brain. The present study aims to identify the serum proteins in terms of its lipid transportation function and the level of oxidative stress parameters. The study was done in human blood samples of ischemic stroke patients and healthy control participants. The sample size used was 26 in each group for oxidative stress study and 3 per group for mass spectrometric protein analysis. The study was designed as a comparative analysis of oxidative stress parameters between control and ischemic stroke. The in gel tryptic digestion followed by Liquid chromatography – mass spectrometry (LC-MS-MS) analysis was done to identify the differentially expressed serum proteins between control and stroke. Students t test was used for comparison between groups and p value less than 0.05 ($p<.05$) considered as significant. The study observed that the antioxidant parameters such as catalase and glutathione had lowered activity in ischemic stroke group compared to healthy control and malondialdehyde levels were higher in ischemic stroke compared to control. From the in gel tryptic digestion, we observed that proteins linked to lipid transport and inflammation had differential expression between stroke and control. The serum proteins which were differentially altered in 25 KDa region in electrophoretogram between control and stroke had functional association with lipid transportation process and stress associated inflammatory roles.

Keywords Serum Amyloid P, Dyslipidemia, Apolipoprotein, Inflammation

1. Introduction

Stroke stands in second position as a common cause of mortality and third position in terms of disability worldwide [1]. Globally, it is reported that 68% of strokes are ischemic stroke and 32% are hemorrhagic stroke [2]. Ischemic stroke occurs mainly in acute form featured by blockage of blood vessel which limits blood supply to the brain. It has been widely reported that dyslipidemia is closely associated with artery occlusions contributing to both cerebrovascular and cardiovascular diseases. Serum levels of total cholesterol, LDL-cholesterol and HDL-cholesterol are positively correlated with the outcome in patients with acute ischemic stroke [3]. Henceforth, it is worth investigating the lipid transport protein levels in blood during ischemic stroke and exploring the possibility as a reliable biomarker. Altered lipid transport is an important event during stroke manifested with hypercholesterolemia and increased LDL-cholesterol. However, HDL-cholesterol and its apolipoprotein components tend to be varied in stroke [4].

HDL-cholesterol being an antioxidant inhibits oxidation of phospholipids in cell membrane. It is well established that improved HDL-cholesterol and its subcomponents are good in protecting major blood vessels against injury [5]. HDL particles possess pro inflammatory and pro atherogenic characteristics especially during acute inflammatory response [6]. Steroid biosynthesis and its metabolism are also found to be important in cerebral blood flow. In fact, preservation of progesterone is effective in cerebro protection at early acute phase after stroke. Neuronal oxidative stress is an important event associated with reperfusion during stroke [7]. Oxidative stress contributes to neuronal and cerebral artery injury contributing to pathophysiology of acute ischemic stroke [8]. Stroke events are reported to be associated with severe imbalance in pro oxidants and antioxidants resulting in generation of free radicals resulting in activation of systemic inflammation [9]. Recent studies have been directed towards understanding the sources of oxidative stress during stroke and to inhibit free radical generation. The present study aims to analyse the differentially expressed serum proteins in electrophoresis between control and ischemic stroke along with assessment of oxidative stress. Most of the identified differentially expressed proteins in stroke samples in 25 KDa region in electrophoretogram were unique and novel and are connected with lipid transportation. Hence the study has the potential to explore these proteins as promising evaluative biomarker for ischemic stroke.

2. Materials and Methods

The study was conducted in accordance with institutional ethics and research guidelines. The study groups were categorised into two, group 1 as healthy control, group 2 as acute ischemic stroke in the age between 45 and 65 years. The sample size was 26 per each group for oxidative stress parameters study. For electrophoresis three samples (n=4) each from control and stroke was used to study comparative expression of albumin globulin depleted serum proteins.

All the samples selected were males. Stroke samples had hyperlipidemia with high total cholesterol (Mean ± SD of 158± 28 [contro]) versus 267 ± 54 [stroke] and low HDL-cholesterol (Mean ± SD of 42± 13 [control] versus 30 ± 12 [stroke]. 2 mL blood was collected in clot activator tube and centrifuged and serum was separated. The serum was further subjected to antioxidant assays which include catalase activity and glutathione estimation according to standard protocol. The proxidant parameter, malondialdehyde (MDA) estimation was done by thiobarbituric acid method using colorimetric assay. The remaining serum was subjected to albumin globulin depletion according to kit based protocol (GE health care) followed by salt depletion using 5 KDa cut off filter device (Millipore). The protein content in the samples were estimated by BCA assay (Pierce) and normalised to 1 mg/mL concentration. The samples were then subjected to electrophoresis using 10 % SDS-PAGE. The bands were analysed and altered band intensity observed in the region of 25 KDa between control and stroke samples were excised from the gel. The sliced gels from representative control and stroke samples were subjected to in gel tryptic digestion followed by Liquid chromatography-mass spectrometry (LC-MS-MS) analysis. The proteins in the sliced gel portion were identified by uniprot analysis followed by MASCOT data base search. Uniprot whole human database was used for protein identification.

Statistics

SPSS version 20 was used for statistical analysis. The average values of oxidative stress parameters were represented as Mean ± SD. For comparison between groups Students t test was done with P value less than 0.05 ($p<.05$) considered as significant. The study was designed as a comparative analysis of oxidative stress parameters between control and acute ischemic stroke.

3. Results

The study compared the oxidative stress parameters such as glutathione (GSH), catalase and malondialdehyde (MDA) in serum between control and acute ischemic stroke. The serum proteins was further subjected to albumin globulin depletion and subjected to SDS-PAGE. Based on the differential expression in band intensity in electrophoretogram the gel portion in 25 KDa region was subjected to in gel tryptic digestion followed by mass spectrometric analysis. The result of electrophoretogram was compiled in table 1.

The table 1 is a representation of mass spectrometric analysis of proteins obtained from in gel tryptic digestion in the 25 KDa region in the electrophoretogram. The comparative analysis showed that proteins such as serum amyloid P component and alkaline phosphatase had increased expression in stroke compared to control and apolipoprotein-A1 had decreased expression in stroke compared to control. The study also identified other stroke specific lipid transport proteins such as apolipoprotein-D, apolipoprotein E, and epididymal sperm binding protein. The protein hydroxy steroid dehydrogenase and ATPase family AAA domain-containing protein 3B were yet other unconventional stroke specific proteins identified.

Table 1. In gel tryptic digestion data describing normalised abundance and expression of Control vs. stroke proteins in 25 KDa region in SDS-PAGE

Accession	Peptide count	Unique peptide	Description	Normalised abundance Control	Normalised abundance Stroke
A0A024R4A2;P09923	2	2	Alkaline phosphatase	6363.725763	7542.623999
P02647;A0A024R3E3 Control	46	46	Apolipoprotein A-I	171695.8427	
P02647;A0A024R3E3 Stroke	55	54			112750.5493
P02743;V9HWP0 Control	7	7	Serum amyloid P-component	5614.057014	
P02743;V9HWP0 Stroke	8	8			9169.402137
A0A0K0K1H8;B4DEX9	1	1	Epididymis secretory sperm binding protein Li 71p		2998.822619
C9JF17;P05090	4	3	Apolipoprotein D (Fragment)		2380.791698
P02649;A0A0S2Z3D5	7	7	Apolipoprotein E		7174.179912
Q5T9A4;H0Y2W2	2	1	ATPase family AAA domain-containing protein 3B		2078.27261
Q53AI5	2	2	HSD-41		24801.73893

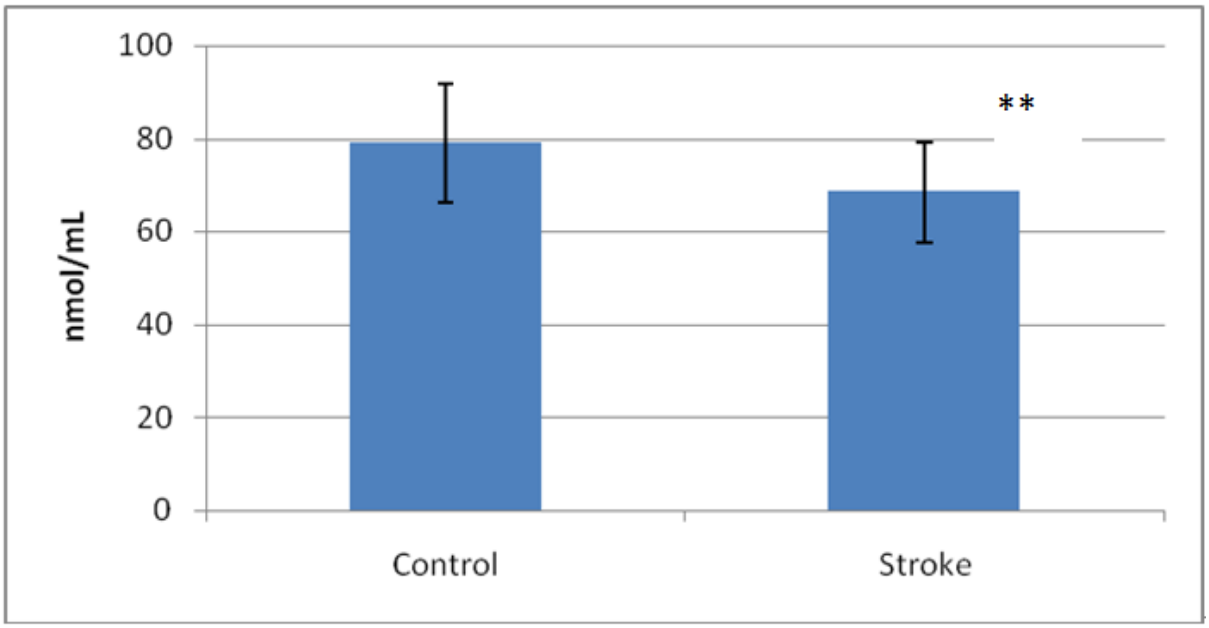

Figure 1. Estimation of glutathione

It was observed that the antioxidant parameters glutathione (GSH) and catalase had decreased levels in stroke samples compared to control. The graphical representation of GSH and catalase were given in figure 1 and 2. Besides, the malondialdehyde estimated by Thiobarbituric acid reactive substances (TBARS) had increased level in stroke compared to control as represented in figure 3. These findings indicated that oxidative stress is increased in stroke adding to the pathophysiology.

(Figure 1) It was observed that GSH concentration in serum was significantly decreased in stroke plasma samples compared to control with $p < 0.01$ (**). The Mean ± SD between control and stroke was 78.97 ± 12.64 Versus 68.54 ± 10.77 nmol/mL. GSH being an endogenous antioxidant to neutralise H_2O_2 is important in maintaining the redox balance in body.

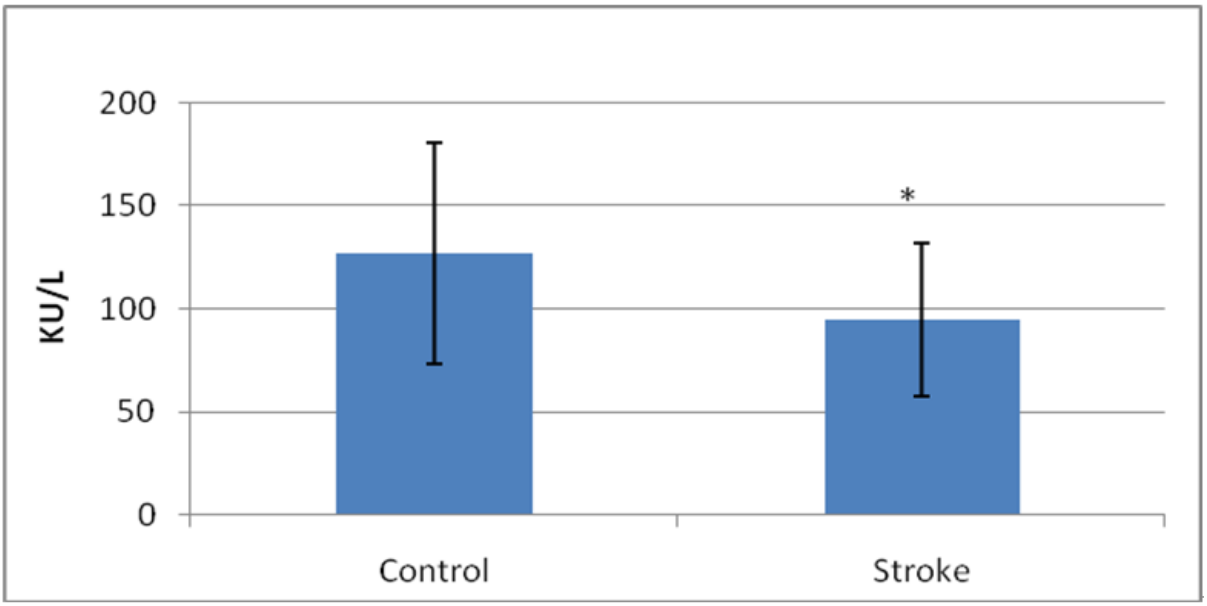

Figure 2. Catalase assay

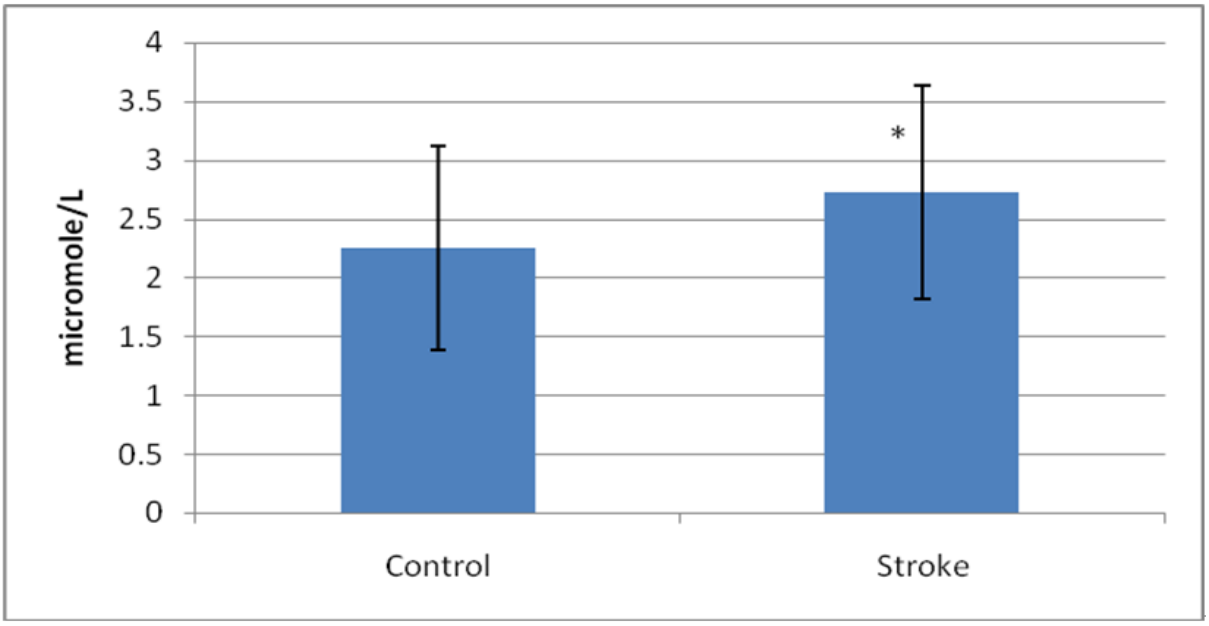

Figure 3. Estimation of Malondialdehyde

(Figure 2) It was observed that catalase activity in stroke plasma was significantly decreased in comparison to control with p value < 0.05 (*). The Mean ± SD between control and stroke was 127.21 ± 53.72 Versus 94.92 ± 37.29 KU/L. Catalase is an important antioxidant enzyme responsible for neutralising H_2O_2 as part of free radical scavenging mechanism.

(Figure 3) It was observed that MDA concentration was significantly increased in stroke samples compared to control with p value < 0.05 (*). The Mean ± SD between control and stroke was 2.25 ± 0.86 Versus 2.72 ± 0.9 micromole/L. MDA is known to react with thiobarbituric acid and is the terminal product of lipid peroxidation. The level of MDA is an indicator of severity of oxidative stress in the body.

The electrophoretic analysis by SDS-PAGE revealed that there was differential band intensity between control and stroke in the 25 KDa region corresponding to stroke and control lanes represented in figure 4. The in gel tryptic digestion followed by mass spectrometric analysis revealed that majority of altered proteins in 25 Kda region were connected with cholesterol transport. The band specific to stroke showed high peptide sequence with apolipoprotein-D, E, epididymal sperm binding protein and hydroxy steroid dehydrogenase.

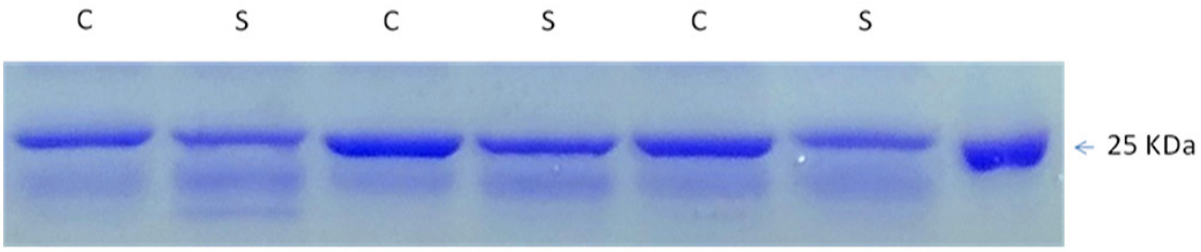

Figure 4. Electrophoretogram of albumin globulin depleted blood plasma

Figure 4 was the electrophoretogram of albumin globulin depleted blood plasma samples of control and stroke patients in the 25 KDa region based on marker position. The bands in 25 KDa region had consistently decreased band intensity in stroke compared to control. It was observed from in gel tryptic digestion of the respective gel slice from representative samples of control and stroke followed by LC-MS-MS that the proteins which showed upregulation in stroke samples were alkaline phosphatase and serum amyloid P component and the downregulated protein was apolipoprotein A-1. The other proteins with unique peptide sequence one or above and which were stroke specific included were epididymis secretory sperm binding protein, Apolipoprotein D (Fragment), Apolipoprotein E, ATPase family AAA domain-containing protein 3B and hydroxy steroid dehydrogenase (HSD-41). These proteins were identified by uniprot analysis for their common functionality related by its role in cholesterol transportation across the blood stream.

4. Discussion

The present study analysed the blood plasma proteins obtained from stroke and control samples along with comparative analysis of oxidative stress parameters. The first part of study comprised of assessment of oxidative stress parameters in blood plasma of control and acute ischemia stroke (AIS) patients. It was observed that there was decrease in antioxidant parameters, catalase activity and glutathione (GSH) concentration in stroke plasma samples compared to control. Besides, malondialdehyde (MDA), a lipid peroxide product was increased in stroke compared to plasma. Henceforth, it can be inferred that there was compromise in redox balance during acute ischemic stroke.

In the second part plasma proteomics of albumin globulin depleted plasma samples was done and revealed that there was obvious difference in band intensity pattern between control and stroke groups in 25 KDa region based on protein marker position in electrophoretogram. From the electrophoretogram followed by SDS PAGE it was observed that the band intensity in the region of 25 KDa was decreased in stroke groups compared to control. The gel bands representing both control and stroke in the 25 KDa was subjected to in gel tryptic digestion followed by LC-MS-MS. Based on uniprot analysis the proteins which showed minimum one unique peptide was considered for comparative analysis of proteins. Based on peptide sequences and MASCOT search the proteins identified were apolipoprotein A-1 (Apo A-1), serum amyloid P component (SAP) and alkaline phosphatase (AP). Among the above proteins, both serum amyloid P and and AP showed increased expression and Apo A-1 showed decreased expression in stroke compared to control plasma based on its normalised abundance. Apo A-1, a high density lipoprotein particle binding protein is involved in cholesterol transport from peripheral tissues to liver. In a study it was observed that Apo A-1 unique peptide was inversely correlated with acute ischemic stroke and the same was observed in the present study [10]. Serum amyloid P which belongs to pentraxin family is an acute phase protein and may regulate complement activation as well and found to have increased expression in any neuronal injury [11]. Alkaline phosphatase belongs to ectophosphatase enzyme family is known as a putative biomarker for neuronal injury. AP levels have been shown to enhance in relation to large volume cerebral white matter hyperintensities and may be associated with multi cerebral microbleeds during AIS [12]. Another study showed that AIS patients with highest serum AP quartile had the highest incidence of early mortality in stroke [13].

Apart from these proteins, there were a few other proteins identified in stroke which was absent in control based on its unique peptide count and matches. These proteins are mostly involved in cholesterol transport or steroidogenesis and some of the proteins are closely associated with oxidative stress as well. The proteins which were found to be specific to stroke in 25 KDa regions were apolipoprotein D fragment, ATPase AAA domain, hydroxy steroid dehydrogenase and epidydimis sperm hbinding protein. Apo D is a 29 KDa protein mainly localised in extracellular secretory sites is a non-conventional apoliporotein based on its site of synthesis. Apo D is mainly synthesised in brain and testes unlike other apolipoproteins which are synthesised in liver. Aberrant Apo D expression is associated with altered lipid metabolism and risk of coronary artery disease. Increased Apo D expression occurred during traumatic brain injury [14]. Apo D can directly influence triglyceride metabolism as well [15]. Apolipoprotein E and its polymorphism are

closely associated with neuronal inflammation and have been found to increase the chance of post stroke depression [16].

ATPase family AAA domain is a mitochondrial localised protein controlling localised metabolism involved in enhanced channelling of cholesterol for hormone dependent steroidogenesis [17] Based on uniprot analysis, epidydimis sperm binding protein is involved in MAPK signalling and respiratory burst during inflammatory response and is known to augment peroxidase activity. HSD-42, the steroid dehydrogenase enzyme is involved in steroid metabolic pathways and involved in inactivation of bioactive steroid hormones [18]. The stroke specific proteins connected with cholesterol transport and steroidogenesis was compared and functionally analysed and can be extrapolated to be biomarker for acute ischemic stroke.

5. Conclusion

Overall, the study pointed out that most proteins which are specific to stroke which were differentially altered in band intensity in 25 KDa region are connected with lipid metabolism, oxidative stress associated inflammation and steroidogenesis. This accounts for the relationship between stroke and dyslipidemia from proteomics perspective. The study has a future potential in exploring further these proteins as reliable diagnostic or prognostic biomarkers from blood for acute ischemic stroke.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] M. Giroud.,A. Jacquin.,Y. Béjot, "The worldwide landscape of stroke in the 21st century," *The Lancet,* vol. 383, pp. 195-197, 2014.

[2] C. Chugh, "Acute ischemic stroke: management approach," *Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine,* vol. 23, p. S140, 2019.

[3] X. Tian.,J. T. Zhang.,Y. Mei.,H. Zhang.,W. Q. Liu.,K. Yan.,X. Tan.,Y. H. Zhang, "Dyslipidemia and outcome in patients with acute ischemic stroke," *Biomedical and environmental sciences,* vol. 27, pp. 106-110, 2014.

[4] K. Tziomalos.,V. G. Athyros.,A. Karagiannis.,D. P. Mikhailidis, "Dyslipidemia as a risk factor for ischemic stroke," *Current topics in medicinal chemistry,* vol. 9, pp. 1291-1297, 2009.

[5] R. Jin.,L. Liu.,S. Zhang.,A. Nanda.,G. Li, "Role of inflammation and its mediators in acute ischemic stroke," *Journal of cardiovascular translational research,* vol. 6, pp. 834-851, 2013.

[6] B. Menon.,K. Ramalingam.,R. Kumar, "Evaluating the role of oxidative stress in acute ischemic stroke," *Journal of neurosciences in rural practice,* vol. 11, pp. 156-159, 2020.

[7] S. Manzanero.,T. Santro.,T. V. Arumugam, "Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury," *Neurochemistry international,* vol. 62, pp. 712-718, 2013.

[8] I. M. Cojocaru.,M. Cojocaru.,V. Sapira.,A. Ionescu, "Evaluation of oxidative stress in patients with acute ischemic stroke," *Romanian journal of internal medicine= Revue roumaine de medecine interne,* vol. 51, pp. 97-106, 2013.

[9] B. McColl.,S. Allan.,N. Rothwell, "Systemic infection, inflammation and acute ischemic stroke," *Neuroscience,* vol. 158, pp. 1049-1061, 2009.

[10] X. Zhao.,Y. Yu.,W. Xu.,L. Dong.,Y. Wang.,B. Gao.,G. Li.,W. Zhang, "Apolipoprotein A1-unique peptide as a diagnostic biomarker for acute ischemic stroke," *International journal of molecular sciences,* vol. 17, p. 458, 2016.

[11] K. Bodin.,S. Ellmerich.,M. C. Kahan.,G. A. Tennent.,A. Loesch.,J. A. Gilbertson.,W. L. Hutchinson.,P. P. Mangione.,J. R. Gallimore.,D. J. Millar, "Antibodies to human serum amyloid P component eliminate visceral amyloid deposits," *Nature,* vol. 468, pp. 93-97, 2010.

[12] J. Liu.,D. Wang.,J. Li.,Y. Xiong.,B. Liu.,C. Wei.,S. Wu.,M. Liu, "High serum alkaline phosphatase levels in relation to multi-cerebral microbleeds in acute ischemic stroke patients with atrial fibrillation and/or rheumatic heart disease," *Current neurovascular research,* vol. 13, pp. 303-308, 2016.

[13] C. Zhong.,S. You.,J. Chen.,G. Zhai.,H. Du.,Y. Luo.,X. Dong.,Y. Cao.,C.-F. Liu.,Y. Zhang, "Serum alkaline phosphatase, phosphate, and in-hospital mortality in acute ischemic stroke patients," *Journal of Stroke and Cerebrovascular Diseases,* vol. 27, pp. 257-266, 2018.

[14] G. Perdomo.,H. H. Dong, "Apolipoprotein D in lipid metabolism and its functional implication in atherosclerosis and aging," *Aging (Albany NY),* vol. 1, p. 17, 2009.

[15] G. Perdomo.,D. H. Kim.,T. Zhang.,S. Qu.,E. A. Thomas.,F. G. Toledo.,S. Slusher.,Y. Fan.,D. E. Kelley.,H. H. Dong, "A role of apolipoprotein D in triglyceride metabolism [S]," *Journal of lipid research,* vol. 51, pp. 1298-1311, 2010.

[16] X.-b. Li.,J. Wang.,A.-d. Xu.,J.-m. Huang.,L.-q. Meng.,R.-y.

Huang.,J.-l. Wang, "Apolipoprotein E polymorphisms increase the risk of post-stroke depression," *Neural regeneration research,* vol. 11, p. 1790, 2016.

[17] X. Du.,A. S. Kazim.,I. W. Dawes.,A. J. Brown.,H. Yang, "The AAA ATPase VPS4/SKD1 regulates endosomal cholesterol trafficking independently of ESCRT‐III," *Traffic,* vol. 14, pp. 107-119, 2013.

[18] Z. Krozowski.,K. Li.,K. Koyama.,R. Smith.,V. Obeyesekere.,A. Stein-Oakley.,H. Sasano.,C. Coulter.,T. Cole.,K. Sheppard, "The type I and type II 11β-hydroxysteroid dehydrogenase enzymes," *The Journal of steroid biochemistry and molecular biology,* vol. 69, pp. 391-401, 1999.