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Evaluation of serum ischemia modified albumin (IMA) level and serum uric acid level as a preliminary biomarker of epilepsy

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ABSTRACT

Background: Oxidative stress, hypoxia, and ischemia are considered prime factors in the pathogenesis of epilepsy. Hyperuricemia (HU) is a common incidental finding in epileptic patients, but the importance of HU as a risk factor in epilepsy is debated. Many hypothetical mechanisms that link HU to increased oxidative stress in epilepsy have been put forward. This research aimed to evaluate the role of serum Ischemia Modified Albumin (IMA) level and serum uric acid (UA) level as a preliminary biomarker in the diagnosis of epilepsy.

Materials and Methods: The present cross-sectional research was conducted on 25 individuals having epilepsy and 25 healthy individuals. Serum uric acid level had estimated by the uricase method. Serum IMA was evaluated by albumin cobalt binding assay to assess oxidative stress, hypoxia, and ischemia.

Results: Mean Serum UA and IMA level were found significantly high ($p<0.001$) in epileptic group. In the epileptic group, 76 % (19/25) individuals had HU. Mean serum IMA was higher in epileptic individuals with HU compared to epileptic individuals with normal serum UA levels. Correlation between serum UA level and serum IMA level was found positive among the study population. Serum UA showed a significant odd ratio to predict epilepsy on binary logistic analysis.

Conclusions: The present study revealed that serum UA levels and IMA levels escalated in epileptic patients. Thus, serum UA levels and IMA levels can be deemed as preliminary biomarkers for the diagnosis of epilepsy.

1. Introduction

Epilepsy is the traditional disabling disorder of the nervous system. It shows bimodal distribution according to age group, the highest incidence in younger individuals and older age groups.[1] It hurts the quality of life of the affected individuals apart from other adverse medical outcomes. It is a complex multifactorial disease with a genetic and epigenetic predisposition. There is the need of the hour to find various risk factors associated with this debilitating disease.

Some studies point towards the role of metabolism in the etiopathogenesis of epilepsy. Metabolic alteration and dysfunctions are crucial in various neurological disorders, such as epilepsy.[2]The final consequence of purine metabolism is Uric acid (UA). UA is an established etiological agent in the pathogenesis of certain diseases like gout and acute uric acid nephropathy, but its role in epilepsy

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is questionable. On one side, UA is responsible for the genesis of reactive oxygen species, which frisk a crucial role in the pathophysiological processes of numerous diseases. On another side, UA showed an anti-oxidant role. It helps to prevent various neurological degenerative disorders such as multiple sclerosis. Thus, UA has a biphasic function.[3] UA is involved in the increased expression of various growth factors, hormones, pro-inflammatory cytokines, and interleukin genes, thus involved in the pathogenesis of metabolic diseases. It can penetrate the vascular smooth muscle cell and activate many down signaling pathways, which upregulate inflammatory pathways. It also activates cell senescence, apoptosis, and renin-angiotensin system and triggers oxidative stress and various cell organelle stress.[4]

An unusual high UA level in the blood is known as Hyperuricemia (HU), which is presumptive a risk factor for the development of epilepsy.[5] Oxidative stress, free radicals, and ischemia caused by HU were also responsible for the onset of epilepsy.[6–8] Serum Ischemia Modified Albumin (IMA) may presume as a preliminary biomarker of ischemia, oxidative stress, and free radical production.[9] The association of HU and serum IMA could help in establishing the kinship between serum uric acid and epilepsy. This research aimed to evaluate the role of serum Ischemia Modified Albumin (IMA) level and serum uric acid (UA) level as a preliminary biomarker in the diagnosis of epilepsy.

2. Materials and Methods

The current cross-sectional study was undergone from June 2019 to March 2020 in the Department of Biochemistry at AIIMS, Nagpur. After obtaining ethical clearance by the Institutional Ethical Committee, sampling of the study subjects was done by random selection.

2.1. Sampling and Sample size

The "openEpi" software had used to calculate the sample size. Serum UA value of 6.8 mg/dl with SD 1.2 mg/dl had prophesied as mean serum UA in the epileptic group, although in the control group, the serum UA value of 6 mg/dl with SD 0.7 mg/dl had prophesied as the mean serum UA.[10] The power of study was 80 %, Confidence Interval = 95 %, chance of error = 5 %. After calculating, the overall sample size needed for the present study was 50 individuals (including a 10 % loss of follow-up). The first group encompassed 25 epileptic patients who were presented in the emergency ward within 12 hours of the episode of seizures. The second group encompassed 25 healthy individuals. Individuals with a history of HU, gout, renal disease, liver disease, inflammatory disease, or autoimmune disorders had excluded from the present study.

2.2. Sample collection

Bilingual informed consent had taken from the patient and his relatives. 3 ml of venous sample was taken from the study subject under sterile conditions into plain red cap vials (BD Vacutainer® blood collection tubes, New Jersey, USA) without anticoagulant for routine and special investigations. Vials were permitted to clot for 30 minutes and centrifuged at 2000 RPM for 5 min in Remi Compact Laboratory Centrifuge (Mumbai, India). The serum was separated and aliquot in two different 5 ml Eppendorf tubes (Hamburg, Germany). One serum sample had sent to the central clinical laboratory for estimating UA. The serum sample of the other tube was aliquoted for IMA estimation and stored at -20^0C in a deep refrigerator for batch analysis. Serum UA values of 2.5-5.6 mg/dl and 3.1-7.0 mg/dl had taken as normal serum UA values in females and males, respectively. In males, a serum UA level of more than 7 mg/dl has been defined as HU, although in females, a serum UA level of more than 5.6 mg/dl has been defined as HU.[11] Serum UA had measured by the uricase method on a fully automated random biochemistry analyzer, Cobas 6000 series module with a closed system of reagents from Roche Diagnostics (Basel, Switzerland). Quality control had assured by Bio-Rad internal quality control (QC) materials from Hercules, California, USA.

2.3. Principal and method of measurement of serum IMA

IMA level in blood had estimated by the colorimetric method, which is contingent on Albumin Cobalt Binding Assay.[12] The principle of the aforesaid assay is that ischemia, hypoxia, and oxidative stress alter the metal-binding site of albumin in the serum, displayed by reduced exogenous cobalt binding.

200 μl of patient sera had taken in the glass test tube. 50 μl of 0.1% cobalt chloride was mixed in this serum and left standing for 10 mins for the reaction of cobalt with albumin. 50 μl of dithiothreitol (DTT), a coloring reagent added. The reaction had quenched after 10 minutes by adding 1 ml of 0.9 % NaCl. The absorbance of the colored compound had measured by spectrophotometer, Motras diagnostic, India, which was directly proportional to IMA level, and readings had expressed as absorbance unit (ABSU).

2.4. Statistical analysis

Statistical analysis of data was executed by using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL) and GraphPad Prism (San Diego, California). Results were recorded as mean values ± standard deviation (SD). The significance of differences between group parameters was analyzed by Student's unpaired t-test and differences were considered significant if $p < 0.05$. Pearson correlation analysis was done to manifest the direction

and strength of the association of variables. Receiver operating characteristics (ROC) analysis was performed to find appropriate cut-off for serum UA to distinguish between epileptic individual vs. healthy control. Binary logistic regression analysis was performed to find out the predictability of the independent variable.

3. Results

Mean age of study population was 17.9 ± 0.57 years in epileptic group and 18.2 ± 0.6 years in healthy control group. The sex (male/female) distribution was 15/10 in epileptic group and 14/11 in control group, P = 0.77. In epileptic group, 19 out of 25 individuals had HU and 6 out of 25 individuals had normal serum UA level. In control group, only 1 out of 25 individuals had HU and 24 out of 25 individuals had normal serum UA. Mean serum UA and IMA level were found statistically higher in epileptic group [Table 1, Figures 1 and 2]. The mean serum IMA was higher in HU epileptic individuals as compared to that of normal serum UA epileptic individuals, although the difference was found statistically non-significant [Table 2, Figure 2]. Correlation between serum UA level and serum IMA level was found positive (r = 0.144, P = 0.492) among the study population as shown in Figure 3, although the correlation was statistically non-significant. As shown in Figure 4, ROC curve of serum UA predicts that at cut-off 6.1 mg/dl, the serum UA has 84 % sensitivity and 80 % specificity for diagnosing epilepsy.

On Binary Logistic Regression Analysis, serum UA (p=0.01, Odd ratio=3.2, 95 % CI = 1.6-6.2) showed significant predictability for epilepsy. Odd's ratio value indicates that the risk of developing epilepsy has increased by 3.2 times if serum UA is increased by one unit.

4. Discussion

UA impairs the endothelial function and its integrity. Thus it is responsible for the pathogenesis of kidney disease, cardiovascular diseases, hypertension, metabolic disorders, non-alcoholic fatty liver disease, and many others.[4] Some experiments showed that epilepsy increases the formation of UA, which leads to increased UA in serum. Thyrion L et al[13] conducted a study on the mice and showed that UA level increases in the brain tissue of mice during epilepsy. Jiang M et al reported an increased amount of UA in CSF and brain tissue of epileptic mouse models during the episodes of seizure. After seizures in epilepsy, the serum UA falls rapidly due to the halt of certain inflammatory signaling pathways responsible for seizures.[14] The high concentration of UA forms monosodium crystals. These crystals trigger the inflammatory pathways and increase excitability in the neuronal tissue.[13,14] The present study showed that epileptic patients had increased serum UA levels compared to normal individuals. This finding is in harmony with the study done

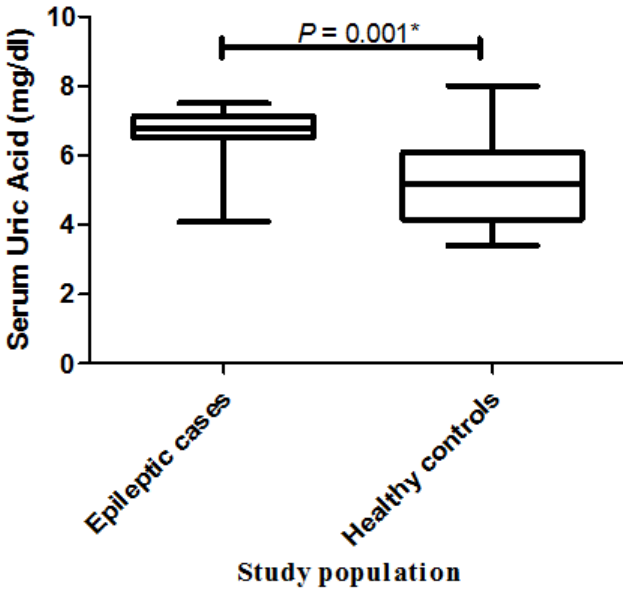

Figure 1: Serum uric acid (UA) level in the study population.

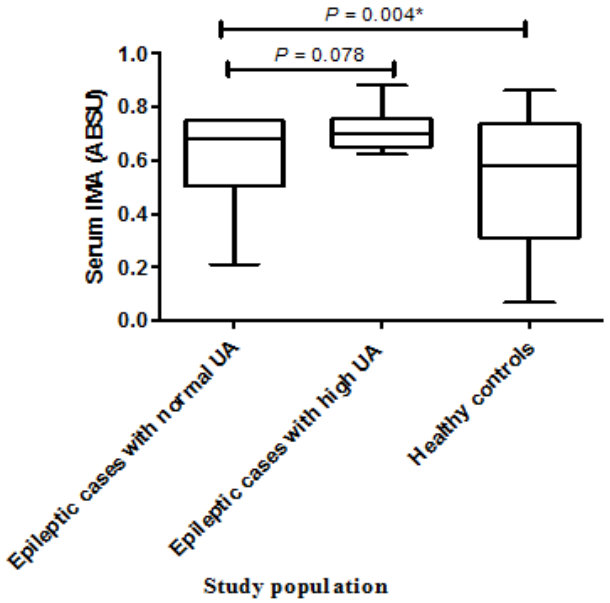

Figure 2: Serum Ischemia modified albumin (IMA)level in the study population

Table 1: Biochemical profile of the study population

Parameters	Epileptic group (n=25) (mean ± SD)	Control group (n=25) (mean ± SD)	P value
Serum Uric Acid (mg/dl)	6.6 ± 0.9	5.2 ± 1.2	0.001*
Serum IMA (ABSU)	0.7± 0.1	0.52± 0.2	0.004*

* Statistically significant.

Table 2: Serum IMA level in epileptic cases.

Parameters	Normal serum UA epileptic individuals (n=6) (mean ± SD)	Hyperuricemia epileptic individuals (n=19) (mean ± SD)	P value
Serum IMA (ABSU)	0.61 ± 0.2	0.71 ± 0.07	0.078
Serum UA (mg/dl)	5.4 ± 1	7 ± 0.2	0.001*

*Statistically significant. ABSU = Absorbance unit

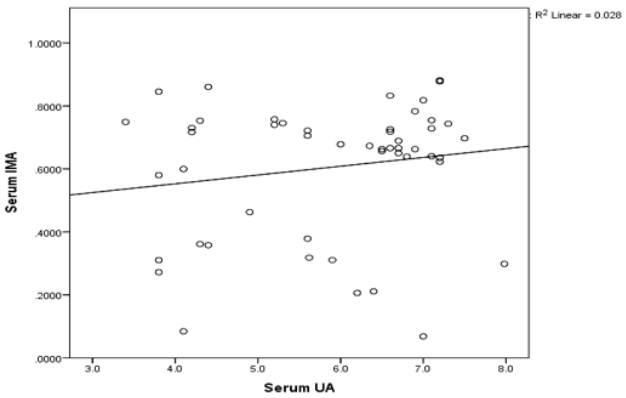

Figure 3: Pearson's correlation analysis of serum UA with serum IMA.

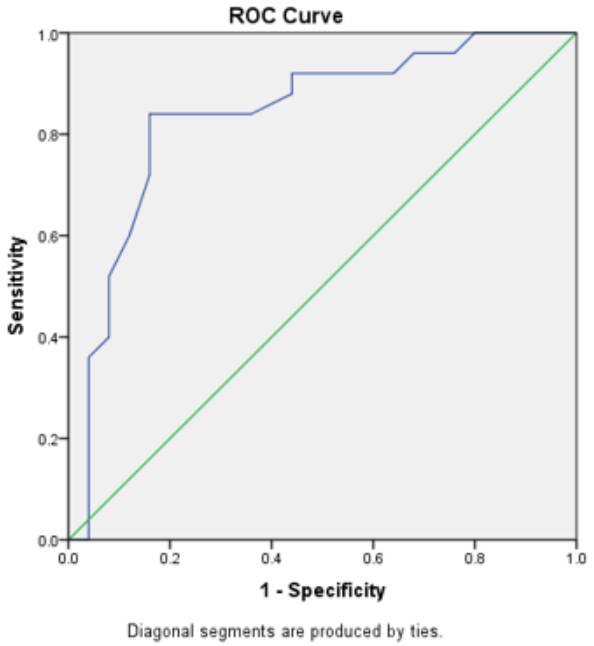

Figure 4: Shows ROC curve of Serum uric acid which predicts that at cut-off 6.1 mg/dl, the serum UA has 84 % sensitivity and 80 % specificity for diagnosing epilepsy.

by earlier researchers.[5,15] Studies reported that allopurinol (a serum UA lowering drug) has effective in epilepsy treatment.[16] All these findings show that increased serum UA is not just a coincidental laboratory finding but might actively effectuate the pathophysiology of epilepsy.

Ischemia, hypoxia, and oxidative stress have also been considered important factors in the pathogenesis of epilepsy.[17] Ischemia modified albumin (IMA) has formed in hypoxic, ischemic, and oxidative stress conditions due to a change in N- terminal of albumin. Serum IMA has been studied in ischemic diseases such as myocardial infarction and found as a sensitive biomarker of ischemic, hypoxic, and oxidative stress conditions.[18] IMA is considered an effective biomarker in the differential diagnosis of seizures.[19] Kamasak T et al reported that serum IMA levels differentiate the non-epileptic and epileptic seizures. Serum IMA level increased significantly with the duration of the seizure.[20] In the present study, mean serum IMA level was found significantly high in epileptic patients. This finding is in-line with the study of Uzel M et al[19] and Kocaoglu C et al.[21]

In the current study, mean serum IMA level was found high in hyperuricemic epileptic patients compared to epileptic individuals with normal serum UA levels. Serum UA has a positive correlation with the serum IMA. In contrast to our finding, Jena I et al[22] found a negative correlation between serum IMA and serum UA level.

In the current study, the ROC curve of serum UA at a cut-off value of 6.1 mg/dl, predicted that serum UA has 84% sensitivity and 80 % specificity for diagnosing epilepsy. Serum UA is a routine biochemical test that can be done in an epileptic patient in an emergency department, which could help in the management of a case where the diagnosis is not confirmed in clinical practice.[14] There

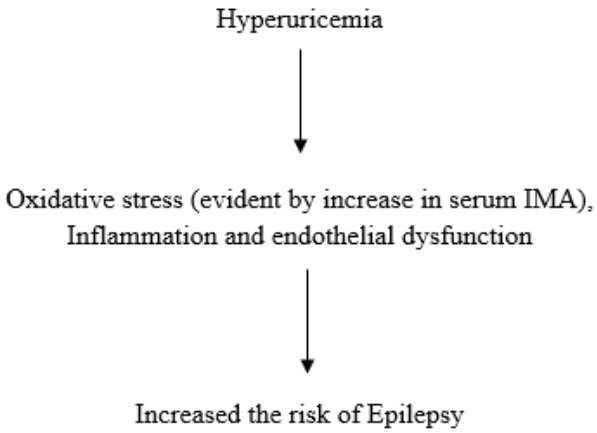

Figure 5: Probable link between the increased serum uric acid and epilepsy according to the present study.

are also certain neurological disorders such as syncope, movement disorders, narcolepsy or cataplexy, etc. which can mimic epilepsy, hence these disorders need to be carefully ruled out in making the diagnosis of epilepsy, which could also be achieved with the help of elevated serum UA. Research around the uric acid in epilepsy can contribute to a better interpretation of the pathophysiology of epilepsy and develop novel potential therapeutic targets in epilepsy.

5. Conclusion

Serum IMA is an ischemic, hypoxic, and oxidative stress biomarker and epilepsy can be caused by the foreknown conditions. The present study revealed serum UA levels and serum IMA levels increased in epileptic patients, and epileptic patients with high serum UA levels also have been high serum IMA levels compared to epileptic patients with normal serum UA levels. Conclusively serum IMA and serum UA levels could be used for the early diagnosis of epilepsy as shown in figure 5. The current study also revealed serum UA levels have a good predictive marker for epilepsy with 84% sensitivity and 80% specificity.

6. Source of Funding

None.

7. Conflict of Interest

None.

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