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Comparative Evaluation of Serum Copeptin in Obesity with Non Alcoholic Fatty Liver Disease

Shubham Majumdar, Manisha Bais Thakur, Anita Rani

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BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming a major cause of liver disease in India. NAFLD is regarded as the metabolic syndrome's hepatic component. AVP has a significant impact on glucose and lipid metabolism by stimulating hepatic glycogenolysis, gluconeogenesis, and fat production by modulating insulin and glucagon release from the pancreatic Langerhans' islets. AVP has a relatively short half-life of about 20 minutes, and more than 90% of AVP in circulation is bound to platelets. As a result, AVP is ineffective as a biomarker in clinical practice. Copeptin is a cleavage product of the AVP precursor, pre-pro-vasopressin, which is secreted by the posterior pituitary gland in response to hypotension and hyperosmolality. We aimed to compare the serum copeptin level in obese with NAFLD patients with obese without NAFLD patients.

METHODS: we collected data from 80 obese patients which were divided into two groups based on the presence and absence of NAFLD. Serum copeptin levels were measured by a Human Copeptin ELISA kit (Shanghai Coon Koon). The level of serum Copeptin was compared between two groups and also in three different grades of NAFLD.

RESULTS: Out of 80 subjects in our study, the mean value of serum copeptin in the group of obese with NAFLD was 24.96 pmol/L and in the group of obese without NAFLD was 15.38 pmol/L. The median (IQR) of serum copeptin in obese with NAFLD group was 24.5 pmol/L and in obese without NAFLD group was 12.25 pmol/L. The highest median S. Copeptin in pmol/L was seen in the obese with NAFLD group with a significant difference between 2 groups (W = 1388.500, p = <0.001). Strength of Association (Point-Biserial Correlation) = 0.44 (Large Effect Size). The mean value of serum copeptin was 20.00, 24.14, and 31.77 pmol/L in NAFLD grade 1, grade 2, and grade 3 respectively. The highest median S. Copeptin in pmol/L is seen in NAFLD Grade 3 group with a significant difference between 3 groups ($\chi 2$ = 23.446, p = <0.001). Strength of Association (Kendall's Tau) = 0.63 (Large Effect Size).

CONCLUSIONS: Serum copeptin can be used as an additional tool to predict the severity of the nonalcoholic fatty liver disease. Measurement of serum copeptin levels can be used as an additional, simple, non-invasive, easily accessible, and cost-effective parameter to predict the severity of the nonalcoholic fatty liver disease.

KEYWORDS: AVP, NAFLD.

I. BACKGROUND

NAFLD is defined by (1) the presence of hepatic steatosis (HS), as determined by imaging or histology, and (2) the absence of secondary causes of hepatic fat accumulation, such as excessive alcohol consumption, long-term use of a steatogenic medication, or monogenic hereditary disorders.[1]

The prevalence of NAFLD is comparable to the obesity epidemic.[2] Hyperinsulinemia and insulin resistance are both important in the pathophysiology of NAFLD.[3] Pancreatic beta cells secrete insulin primarily in response to circulating glucose levels under normal conditions. Insulin stimulates esterification of fatty acids and storage in lipid droplets while inhibiting the opposing process of lipolysis in several metabolic tissues, including adipose tissue.[4] AVP, also known as antidiuretic hormone, is a nonapeptide produced in the hypothalamus. The neurohypophysis releases AVP to promote renal water conservation, which affects osmoregulation and cardiovascular homeostasis. However, because of its instability and short half-life, plasma AVP levels are difficult to measure and unreliable. The C-terminal part of the AVP precursor peptide, plasma C-terminal provasopressin (copeptin), is stable in serum or plasma, making it a more convenient biomarker.[5] Copeptin has been established in clinical practice as a reliable marker of circulating AVP concentration.[5] Copeptin has been found to be strongly and positively associated with insulin resistance, obesity, and metabolic abnormalities, all of which are major risk factors for the development of diabetes in several cross-sectional population studies.AVP has a significant impact on glucose and lipid metabolism by stimulating hepatic

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glycogenolysis, gluconeogenesis, and fat production by modulating insulin and glucagon release from the pancreatic Langerhans' islets.[2] AVP stimulates pancreatic cell glucagon release via the V1b receptor.[3] This effect occurs in the absence of any discernible change in insulin secretion. AVP stimulates the production of glucose in the liver. Through the V1a receptor, AVP regulates gluconeogenesis and glycogenolysis. AVP's actions in the liver differ from those of glucagon and are mediated by a calcium-dependent pathway, possibly through the V1 receptor. AVP stimulates glucocorticoid levels in plasma by mediating ACTH release via the V1b receptor in the anterior pituitary. This release is not affected by glucocorticoid feedback. AVP also increases epinephrine by activating V1b receptors on chromaffin cells in the adrenal medulla, which contributes to the development of hyperglycemia.[6]

II. METHODS

Study Design

It was single center observational cross-sectional comparative study. The study included 80 obese patients, admitted in medicine wards , came to medicine OPD of Safdarjung Hospital. The inclusion criteria was -1) Age 18 years and above. 2) Cases-Body Mass Index (BMI) >25 kg/m^2. The exclusion criteria included-1) Persons taking significant alcohol consumption defined as > 21 standard drinks per week in men and > 14 standard drinks per week in women.(A standard alcoholic drink is any drink that contains about14 g of pure alcohol) 2) Patients of Acute fulminant hepatitis, Hepatitis B and Hepatitis C or any other coexisting chronic liver disorder. 3) Patients on drugs that cause fatty Liver (amiodarone, methotrexate, Tamoxifen, steroids, valproate, antiretroviral medications). 4) Patients with known malignancy. 5) Patients on parenteral nutrition. 6) Pregnancy. The study protocol was approved by the ethics committee of Safdarjung Hospital, New Delhi,India.

Clinical data collection

All the patients as per inclusion criteria were taken and enrolled and a proper history and clinical examination were done. Serum copeptin was measured with the help of Human Copeptin ELISA Kit (Shanghai Coon Koon).

Table 1: Baseline variables and clinical outome in study subjects

Parameters	NAFLD		p value
	Yes (n = 40)	No (n = 40)	
Age (Years)	42.48 ± 13.62	40.17 ± 14.54	0.468[1]
Age			0.828[2]
18-30 Years	8 (20.0%)	12 (30.0%)	
31-40 Years	10 (25.0%)	10 (25.0%)	
41-50 Years	10 (25.0%)	8 (20.0%)	
51-60 Years	7 (17.5%)	6 (15.0%)	
61-70 Years	5 (12.5%)	3 (7.5%)	
71-80 Years	0 (0.0%)	1 (2.5%)	
Gender			0.263[3]
Male	23 (57.5%)	18 (45.0%)	
Female	17 (42.5%)	22 (55.0%)	
Alcohol Intake* **			0.026[2]
No	40 (100.0%)	34 (85.0%)	
Not Significant	0 (0.0%)	6 (15.0%)	
Systolic BP (mmHg)* **	133.98 ± 13.21	128.25 ± 10.62	0.018[4]
Diastolic BP (mmHg)	82.55 ± 7.59	79.20 ± 9.79	0.062[4]

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Parameters	NAFLD		p value
	Yes (n = 40)	No (n = 40)	
Pulse Rate (BPM)* **	81.15 ± 7.20	87.17 ± 11.36	0.006[1]
Respiratory Rate (CPM)	17.45 ± 1.28	17.58 ± 2.05	0.523[4]
Nutritional Status (Obese)	40 (100.0%)	40 (100.0%)	1.000[3]
GPE* **			0.028[2]
NAD	34 (85.0%)	25 (62.5%)	
Pallor	6 (15.0%)	11 (27.5%)	
Ichterus	0 (0.0%)	4 (10.0%)	
Weight (Kg)	68.67 ± 6.53	66.53 ± 2.75	0.337[4]
Height (m)	1.56 ± 0.06	1.55 ± 0.03	0.764[4]
BMI (Kg/m^2)	28.13 ± 1.49	27.65 ± 1.08	0.214[4]
Examination: Abdominal* **			<0.001[2]
NAD	40 (100.0%)	29 (72.5%)	
Tender	0 (0.0%)	6 (15.0%)	
Distended	0 (0.0%)	2 (5.0%)	
Hepatomegaly	0 (0.0%)	2 (5.0%)	
Scar Mark	0 (0.0%)	1 (2.5%)	
Examination: Respiratory			0.116[2]
NAD	40 (100.0%)	36 (90.0%)	
Crepitations	0 (0.0%)	4 (10.0%)	
Examination: CNS (NAD)	40 (100.0%)	40 (100.0%)	1.000[3]
Examination: CVS			0.494[2]
S1/S2 Normal	40 (100.0%)	38 (95.0%)	
Muffled Heart Sound	0 (0.0%)	1 (2.5%)	
Msm @ Mitral Area	0 (0.0%)	1 (2.5%)	
Hemoglobin (g/dL)	10.38 ± 1.55	10.60 ± 2.06	0.595[1]
TLC (/mm^3)	7.06 ± 1.51	9.14 ± 4.25	0.050[4]
Platelet Count (x10^3/mm^3)* **	306.32 ± 104.76	251.02 ± 118.94	0.030[1]
FBS (mg/dL)	106.65 ± 21.97	106.60 ± 29.32	0.580[4]
PPBS (mg/dL)* **	189.90 ± 42.98	165.22 ± 43.05	0.007[4]
S. Sodium (mEq/L)* **	138.00 ± 4.25	140.22 ± 2.43	0.003[4]
S. Potassium (mEq/L)	3.76 ± 0.32	3.71 ± 0.25	0.114[4]
Urea (mg/dL)* **	31.82 ± 11.74	39.17 ± 13.91	0.013[1]

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Parameters	NAFLD		p value
	Yes (n = 40)	No (n = 40)	
S. Creatinine (g/dL)	0.82 ± 0.38	0.85 ± 0.31	0.371[4]
Total Bilirubin (mg/dL)* **	0.81 ± 0.31	1.04 ± 0.29	<0.001[4]
Direct Bilirubin (mg/dL)	0.19 ± 0.10	0.22 ± 0.12	0.202[4]
Indirect Bilirubin (mg/dL)* **	0.62 ± 0.24	0.82 ± 0.23	<0.001[4]
SGOT (U/L)* **	28.20 ± 12.28	54.02 ± 27.03	<0.001[4]
SGPT (U/L)* **	28.27 ± 13.52	61.77 ± 28.66	<0.001[4]
ALP (U/L)* **	98.58 ± 42.89	185.93 ± 97.83	<0.001[4]
PT- INR	1.02 ± 0.32	1.14 ± 0.27	0.072[1]
S.Protein (g/dL)	6.68 ± 0.67	6.46 ± 0.90	0.233[1]
S.Albumin (g/dL)	3.44 ± 0.60	3.27 ± 0.59	0.226[1]
VLDL (mg/dL)	20.65 ± 6.05	19.38 ± 7.75	0.415[1]
LDL (mg/dL)* **	128.98 ± 36.20	101.40 ± 22.76	<0.001[4]
HDL (mg/dL)	53.77 ± 15.37	52.33 ± 12.25	0.642[1]
TG (mg/dL)	168.70 ± 40.10	162.52 ± 46.42	0.433[4]
HbA1c (%)	5.35 ± 0.79	5.71 ± 1.17	0.158[4]
S. Copeptin (pmol/L)* **	24.96 ± 6.93	15.38 ± 12.07	<0.001[4]

***Significant at p<0.05, 1: t-test, 2: Fisher's Exact Test, 3: Chi-Squared Test, 4: Wilcoxon-Mann-Whitney U Test

III. STATISTICAL ANALYSIS

Categorical variables will be presented in number and percentage (%) and continuous variables will be presented as mean ± SD and median. Normality of data will be tested by Kolmogorov-Smirnov test. If the normality is rejected then non parametric test will be used. Statistical tests will be applied as follows- 1.Quantitative variables will be compared using Unpaired t-test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups. 2.Qualitative variables will be compared using Chi-Square test /Fisher's exact test. A p value of <0.05 will be considered statistically significant.The data will be entered in MS EXCEL spreadsheet and analysis will be done using Statistical Package for Social Sciences (SPSS) version 21.0.

IV. RESULTS

The mean (SD) of Age (Years) in the NAFLD: Yes group was 42.48 (13.62). The mean (SD) of Age (Years) in the NAFLD: No group was 40.17 (14.54). The median (IQR) of Age (Years) in the NAFLD: Yes group was 42 (31-54). The median (IQR) of Age (Years) in the NAFLD: No group was 35.5 (28.75-49). The Age (Years) in the NAFLD: Yes ranged from 19 - 67. The Age (Years) in the NAFLD: No ranged from 19 - 76. The mean (SD) of S. Copeptin in the NAFLD: Yes group was 24.96 (6.93) pmol/L. The mean (SD) of S. Copeptin in the NAFLD: No group was 15.38 (12.07) pmol/L. The median (IQR) of S. Copeptin in the NAFLD: Yes group was 24.5 (21.12-27.5) pmol/L. The median (IQR) of S. Copeptin in the NAFLD: No group was 12.25 (9.48-13.93) pmol/L. The S. Copeptin in the NAFLD: Yes ranged from 11.5 - 43 pmol/L. The S. Copeptin in the NAFLD: No ranged from 6 - 60 pmol/L. There was a significant difference between the 2 groups in terms of S. Copeptin (W = 1388.500, p = <0.001), with the median S. Copeptin being highest in the NAFLD: Yes group. The mean (SD) of S. Copeptin in the NAFLD Grade 1 group was 20.00 (4.32) pmol/L. The mean (SD) of S. Copeptin in the NAFLD Grade 2 group was 24.14 (3.67) pmol/L.

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The mean (SD) of S. Copeptin in the NAFLD Grade 3 group was 31.77 (6.17) pmol/L. The median (IQR) of S. Copeptin in the NAFLD Grade 1 group was 20 (16.38-23.62) pmol/L. The median (IQR) of S. Copeptin in the NAFLD Grade 2 group was 23.5 (22.25-26) pmol/L. The median (IQR) of S. Copeptin in the NAFLD Grade 3 group was 28 (27.5-36) pmol/L. The S. Copeptin in the NAFLD Grade 1 ranged from 11.5 - 27 pmol/L. The S. Copeptin in the NAFLD Grade 2 ranged from 17.5 - 31.5 pmol/L. The S. Copeptin in the NAFLD Grade 3 ranged from 24.5 - 43 pmol/L. There was a significant difference between the 3 groups in terms of S. Copeptin (pmol/L) ($\chi 2$ = 23.446, p = <0.001), with the median S. Copeptin (pmol/L) being highest in the NAFLD Grade 3 group.

Figure1: The bar graph below depicts the means of S. Copeptin (pmol/L) in the 2 different groups.

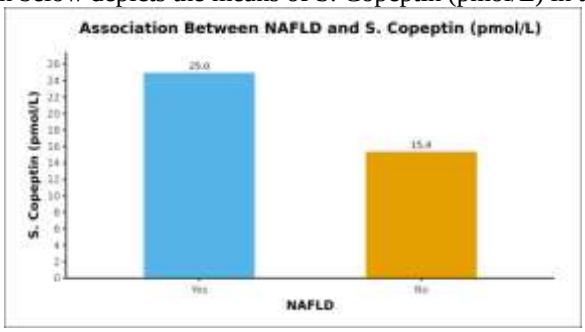
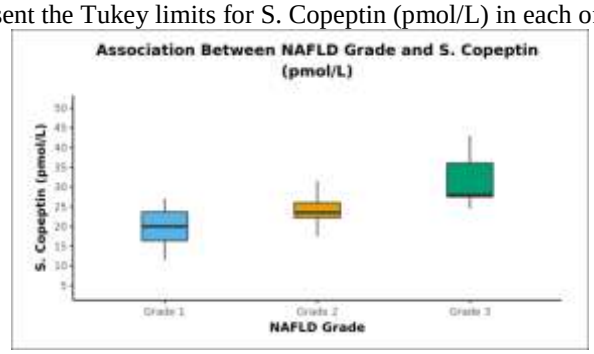


Figure2: The Box-and-Whisker plot below depicts the distribution of S. Copeptin (pmol/L) in the 3 groups. The middle horizontal line represents the median S. Copeptin (pmol/L), the upper and lower bounds of the box represent the 75th and the 25th centile of S. Copeptin (pmol/L) respectively, and the upper and lower extent of the whiskers represent the Tukey limits for S. Copeptin (pmol/L) in each of the groups.



V. DISCUSSION

The findings in our study clearly show that serum copeptin increases in cases of NAFLD, which shows that there is a pathophysiological role of arginine vasopressin in the pathogenesis of fatty liver disease.In our study after comparison of serum copeptin in NAFLD grades shows, the mean value of serum copeptin is 20.00, 24.14, and 31.77 pmol/L in NAFLD grade 1, grade 2, and grade 3 respectively. The value of serum copeptin is highest in grade 3 NAFLD.Our study shows compared to the group of obesity without NAFLD. It also shows that as the severity of NAFLD increases the serum copeptin levels also increase. So our study has found the role of AVP in the pathogenesis of NAFLD and we can assess the severity by measuring copeptin level and further many pharmacological interventions can be studied for the treatment aspect of NAFLD.

VI. LIMITATIONS

Our study is a single centered, cross sectional and with small sample size of 80. We recommend more studies with big sample size and in multi centers for further exploring the pathological, prognostic and diagnostic accuracy of serum copeptin in NAFLD. Studies with longitudinal design are needed for exploring the possible involvement of arginine vasopressin system in the development and prognosis of NAFLD.

VII. CONCLUSIONS

The findings from our study shows that serum copeptin can be used as an additional tool to

predict the severity of nonalcoholic fatty liver disease. As serum copeptin is not done as a routine investigation in India to predict the severity of nonalcoholic fatty liver disease. On the basis of our study we recommend that measurement of serum copeptin levels can be used as an additional, simple, non-invasive, easily accessible and cost effective parameter to predict the severity of nonalcoholic fatty liver disease

Abbreviations

NAFLD : Nonalcoholic fatty liver disease; AVP : Arginine vasopressin; SD : standard deviation; IQR : interquartile range; TLC : total leucocyte count; FBS : fasting blood sugar; PPBS : postprandial blood sugar; SGOT : serum glutamic oxaloacetic transaminase; SGPT : serum glutamic pyruvic transaminase; ALP : alkaline phosphatase; PT-INR : prothrombin time –international normalized ratio; VLDL : very low density lipoprotein; LDL : low density lipoprotein; HDL : high density lipoprotein; TG : triglycerides; S. Copeptin : serum copeptin

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