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Original Research Paper

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# A STUDY OF PLASMA NESFATIN-1 IN OBESE AND NON OBESE IN RELATION TO TYPE 2 DIABETES MELLITUS

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## ABSTRACT

The prevalence of metabolic disease is reaching pandemic proportions worldwide, of which obesity and diabetes are the primary diseases of concern. In major cases of diabetes and obesity, the defects lie in glucose metabolism and energy homeostasis.

**AIMS AND OBJECTIVES:**

a) Estimation and comparison of Plasma Nesfatin-1 in patients with Obesity and DM with Normal individuals
b) Estimation and comparison of plasma Nesfatin-1 levels in Obese and Non-obese individuals.
c) Estimation and comparison of plasma Nesfatin-1 levels in Diabetic and Normal individuals

**METHODS:** It is a hospital based prospective study of 80 patients, which is conducted at SREE BALAJI MEDICAL COLLEGE. The material for study is formed by adult patients admitted to General wards in our hospital between October 2017 to August 2018 fulfilling the inclusion and exclusion criteria.

**CONCLUSION:** In summary, our study demonstrated a significant lower values of plasma nesfatin-1 in obese individuals when compared to normal individuals and higher values in type2 diabetic individuals when compared to normal individuals

**KEYWORDS** : Obesity, Diabetes, Nesfatin-1

## INTRODUCTION

Nesfatin-1 (NEFA/nucleobindin-2 Encoded Satiety and FAT Influencing proteIN-1) is a recently identified 82 amino acid anorexigenic (food intake inhibitory) peptide that is derived from the post-translational cleavage of its secreted precursor peptide nucleobindin-2 (NUCB2) at its N-terminal by pro hormone convertases.[9] Plasma nesfatin-1 concentrations are inversely correlated with glucose levels in rats and diabetic (T1DM and T2DM)humans.[10] Since its discovery in 2006, nesfatin-1 has emerged as a multi functional peptide having a variety of tissue-specific functions. Nesfatin-1 similar to the intestinal hormones, has a crucial role in modulating insulin secretion.[4,10] It regulates a number of physiological processes including food intake glucose homeostasis and insulin secretion.

Nesfatin-1 has an inhibitory effect on food intake and thus alleviates obesity in a dose- and time-dependent manner upon intra cerebroventricular and intra peritoneal injection, as well as after intranasal administration.[14] It is also known that there is a slightly positive but inconsistent correlation of nesfatin-1 with BMI in humans, since variables as sex and stress seem to confound this relationship. Aim of our study is mainly to establish the role of nesfatin in energy metabolism and its role about regulation of insulin secretion in diabetic patients.

## OBJECTIVES OF THE STUDY

- Estimation and comparison of Plasma Nesfatin-1 in patients with Obesity and DM with Normal individuals.
- Estimation and comparison of plasma Nesfatin-1 levels in Obese and Non-obese individuals.
- Estimation and comparison of plasma Nesfatin-1 levels in Diabetic and Normal individuals

## MATERIALS AND METHODS

**Source of data:**

It is a hospital based prospective study of 80 patients, which is conducted at sree balaji medical college and hospital. The material for study is formed by adult patients admitted to General wards in SBMCH.

**DURATION:** FROM OCTOBER 2017 – AUGUST 2018.

**INCLUSION CRITERIA:**

- Obese individuals with or without Type-2 Diabetes mellitus
- Non-Obese individuals with or without Type-2 Diabetes mellitus

**EXCLUSION CRITERIA:**

- Type1 DM
- Patients with pre-diabetic status
- Patients having other co-morbid diseases

**SELECTION OF CASES**

- The study cases are selected from adult patients more than 18 years of age admitted for fever and other general illnesses.
- After meticulous screening, those patients who satisfy inclusion criteria were enrolled for this study.
- Consent was taken from each participant in this study.

Clinical charts were reviewed for a history of DM, type of DM, duration of the disease and the medications the patients were on for the same.

Detailed physical examination of the patients done including the Height, weight, BMI, and detailed systemic examination was done. Laboratory data like complete blood count, RFT, FBS, PPBS, HbA1C were measured as a part of routine patient care after the admission before discharge. Blood samples for Plasma Nesfatin-1 were collected from the patients in the fasting state.

**DESCRIPTION OF TESTS**

Plasma Nesfatin-1 was measured using the RayBio® Nesfatin Enzyme Immunoassay (EIA) Kit. It is an in vitro quantitative assay for detecting Nesfatin peptide based on the competitive enzyme immunoassay principle in whole blood or plasma specimens. We measured plasma Nesfatin-1 in 80 blood samples.

**Sampling and Measurement of NESFATIN-1**

Blood samples for Nesfatin-1 were collected in EDTA tubes. Then the samples were centrifuged @5000 rpm for 10min and the plasma is collected from each sample and stored at -80 degree temperature. Plasma Nesfatin-1 was measured using the RayBio® Nesfatin Enzyme Immunoassay (EIA) Kit. It is an in vitro quantitative assay for detecting Nesfatin peptide based on the competitive enzyme immunoassay principle in whole blood or plasma specimens. The inter-assay and the intra-assay coefficients of variation were <15% and <10% respectively. We measured plasma Nesfatin-1 in 80 blood samples.

## 5. RESULTS

**TABLE-1 Descriptive Statistics**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| AGE | 80 | 20 | 68 | 41.93 | 12.661 |
| FBS | 80 | 72 | 254 | 114.14 | 36.791 |
| PPBS | 80 | 102 | 442 | 175.05 | 63.242 |
| HbA1C | 80 | 5.1 | 11.0 | 6.545 | 1.1785 |

| | | | | | |
|---|---|---|---|---|---|
| HEIGHT | 80 | 145 | 186 | 164.40 | 8.774 |
| WEIGHT | 80 | 50 | 98 | 72.14 | 12.452 |
| BMI | 80 | 19 | 35 | 26.51 | 5.375 |
| PLASMA NESFATIN-1 | 80 | .08 | 3.24 | 1.3935 | .73356 |
| Valid N (List wise) | 80 | | | | |

**TABLE 2:** Nefatin-1 values among all the four groups

| | | | CATEGORY | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | D |
| Nesfatin cat | <2 | Count | 16 | 20 | 13 | 18 |
| | | % within nesfatin cat | 23.9% | 29.9% | 19.4% | 26.9% |
| | >2.1 | Count | 3 | 0 | 6 | 2 |
| | | % within nesfatin cat | 27.3% | 0.0% | 54.5% | 18.2% |
| Total | | Count | 19 | 20 | 19 | 20 |
| | | % within nesfatin cat | 24.4% | 25.6% | 24.4% | 25.6% |

Table-2 shows the Nesfatin – 1 levels among various groups and it shows that total 67 people have the value of <2ng/dl while 13 people had value of >2ng/dl. The X2 value is 8.379 with df of 3.

**TABLE-3: Comparison of Plasma Nesfatin-1 among patients of T2DM with obesity and Normal individuals:**

**Group Statistics**

| | Category | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| PLASMA NESFATIN-1 | A | 20 | 1.6710 | .47615 | .10647 |
| | D | 20 | 1.5850 | .39333 | .08795 |

**Independent Samples Test**

| PLASMA NESFATIN-1 | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | .501 | .484 | .623 | 38 | .537 | .08600 | .13810 | -.19357 | .36557 |
| Equal variances not assumed | | | .623 | 36.692 | .537 | .08600 | .13810 | -.19389 | .36589 |

Table -3 shows that the mean level of plasma nesfatin-1 was slightly higher in patients with T2DM and Obesity when compared to normal individuals. The P value being 0.537 this difference is not statistically significant.

**Table-4: Comparison of Plasma Nesfatin-1 among patients with obesity and Normal individuals**
**Group Statistics**

| | Category | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| PLASMA NESFATIN-1 | B | 20 | .4065 | .27114 | .06063 |
| | D | 20 | 1.5850 | .39333 | .08795 |

**Independent Samples Test**

| PLASMA NESFATIN-1 | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 2.580 | .116 | -11.032 | 38 | .000 | -1.17850 | .10682 | -1.39475 | -.96225 |
| Equal variances not assumed | | | -11.032 | 33.731 | .000 | -1.17850 | .10682 | -1.39566 | -.96134 |

Table 4 shows that the patients with obesity have significantly lower levels of Nesfatin-1 when compared to normal individuals. The p value is <0.001 suggests that the difference is very much significant and not by chance.

**TABLE 5: Comparison of Plasma Nesfatin-1 among patients with T2DM and Normal individuals:**
**Group Statistics**

| | Category | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| PLASMA NESFATIN-1 | C | 20 | 1.9115 | .59577 | .13322 |
| | D | 20 | 1.5850 | .39333 | .08795 |

**Independent Samples Test**

| PLASMA NESFATIN-1 | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | T | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.950 | .171 | 2.045 | 38 | .048 | .32650 | .15963 | .00334 | .64966 |
| Equal variances not assumed | | | 2.045 | 32.919 | .049 | .32650 | .15963 | .00169 | .65131 |

Table 5 shows that there is increased levels of Nesfatin-1 among patients with T2DM when compared with normal individuals. The p value is <0.005(0.048) suggests that the difference which we found is significant and not by chance.

**TABLE 6: Descriptive statistics of Plasma Nesfatin-1 among all four groups:**
**Descriptive Statistics**

| CATEGORY | | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|---|
| A | PLASMA NESFATIN-1 | 20 | .82 | 2.60 | 1.6710 | .47615 |
| | Valid N (list wise) | 20 | | | | |
| B | PLASMA NESFATIN-1 | 20 | .08 | .98 | .4065 | .27114 |
| | Valid N (list wise) | 20 | | | | |
| C | PLASMA NESFATIN-1 | 20 | .92 | 3.24 | 1.9115 | .59577 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Valid N (list wise) | 20 | | | | |
| D | PLASMA NESFATIN-1 | 20 | 1.12 | 2.60 | 1.5850 | .39333 |
| | Valid N (list wise) | 20 | | | | |

Table 6 shows that the mean plasma Nesfatin-1 levels were lower among obese individuals and higher among diabetics when compared to normal individuals while there is no significant difference among individuals with both T2DM and Obesity when compared with normal individuals.

**TABLE 7 :Correlation of Plasma Nesfatin-1 with other parameters:**

| | | FBS | PPBS | HbA1C | Height | Weight |
|---|---|---|---|---|---|---|
| FBS | Pearson Correlation | 1 | .921** | .734** | -.328** | .047 |
| | Sig. (2-tailed) | | .000 | .000 | .003 | .680 |
| | N | 80 | 80 | 80 | 80 | 80 |
| PPBS | Pearson Correlation | .921** | 1 | .780** | -.193 | -.045 |
| | Sig. (2-tailed) | .000 | | .000 | .087 | .694 |
| | N | 80 | 80 | 80 | 80 | 80 |
| HbA1C | Pearson Correlation | .734** | .780** | 1 | -.160 | -.107 |
| | Sig. (2-tailed) | .000 | .000 | | .155 | .347 |
| | N | 80 | 80 | 80 | 80 | 80 |
| HEIGHT | Pearson Correlation | -.328** | -.193 | -.160 | 1 | -.003 |
| | Sig. (2-tailed) | .003 | .087 | .155 | | .977 |
| | N | 80 | 80 | 80 | 80 | 80 |
| WEIGHT | Pearson Correlation | .047 | -.045 | -.107 | -.003 | 1 |
| | Sig. (2-tailed) | .680 | .694 | .347 | .977 | |
| | N | 80 | 80 | 80 | 80 | 80 |
| BMI | Pearson Correlation | .227* | .082 | .002 | -.500** | .854** |
| | Sig. (2-tailed) | .043 | .470 | .989 | .000 | .000 |
| | N | 80 | 80 | 80 | 80 | 80 |
| PLASMA NESFATIN-1 | Pearson Correlation | .377** | .438** | .441** | .053 | -.583** |
| | Sig. (2-tailed) | .001 | .000 | .000 | .640 | .000 |
| | N | 80 | 80 | 80 | 80 | 80 |

| | | BMI | PLASMA NESFATIN-1 |
|---|---|---|---|
| FBS | Pearson Correlation | .227 | .377** |
| | Sig. (2-tailed) | .043 | .001 |
| | N | 80 | 80 |
| PPBS | Pearson Correlation | .082** | .438 |
| | Sig. (2-tailed) | .470 | .000 |
| | N | 80 | 80 |
| HbA1C | Pearson Correlation | .002** | .441** |
| | Sig. (2-tailed) | .989 | .000 |
| | N | 80 | 80 |
| HEIGHT | Pearson Correlation | -.500** | .053 |
| | Sig. (2-tailed) | .000 | .640 |
| | N | 80 | 80 |
| WEIGHT | Pearson Correlation | .854 | -.583 |
| | Sig. (2-tailed) | .000 | .000 |
| | N | 80 | 80 |
| BMI | Pearson Correlation | 1* | -.529 |
| | Sig. (2-tailed) | | .000 |
| | N | 80 | 80 |
| PLASMA NESFATIN-1 | Pearson Correlation | -.529** | 1** |
| | Sig. (2-tailed) | .000 | |
| | N | 80 | 80 |

Table-7 shows Plasma Nesfatin-1 is positively correlated with FBS, PPBS, HbA1c with P-value being <0.05 which is statistically significant. Plasma Nesfatin-1 is negatively correlated with BMI with p-value being <0.05 which is statistically significant.

## DISCUSSION

It is shown that there is some amount of positive correlation between Nesfatin-1 and Type2 DM in humans. There are very few studies available regarding the pattern of Nesfatin-1 in the patients with both diabetes and obesity. So, this study makes an important contribution to the body of literature on Nesfatin-1 by establishing the relation between Nesfatin -1 and obesity and its relation with diabetic status of the individual. This study also correlates the pattern of Nesfatin-1 in patients with both diabetes and obesity.

This study included total of 80 patients, out of which 53 were males and 27 were females (Male patients being more than female patients).91.3% of people were of less than 60 yrs of age. Patients are diagnosed by using ADA guidelines for DM and WHO guidelines for Obesity and enrolled in the study and plasma Nesfatin-1 levels were measured in these individuals .The mean value of Nesfatin-1 in our study is 1.393 with SD of 0.733.

In this study the Plasma Nesfatin-1 level were found to be negatively correlated with BMI with pearson correlation of -0.529 with p value of <0.001 which is highly significant. When we compare the fasting plasma nesfatin-1 values among people with obesity and normal individuals the values were significantly low in the group with obese people($p<0.001$).

The low levels of Nesfatin-1 in obese patients in our study can be speculated that the low levels of this satiety peptide in obese subjects may be one of the reasons of in adequately controlled food intake. There will be some amount of insulin resistance in cases of obese people with more adipose tissue so necessity to investigate other factors, such as insulin resistance, that may affect synthesis of nesfatin-1 in obese subjects. The contrary results in other studies might be due to different ethnicity, type of ELISA kit used and some studies have included even the patients with overweight in the category of obesity.

In our study the Plasma Nesfatin-1 level were found to be positively correlated with the FBS,PPBS,HbA1C of the patients with P value of <0.05. The Plasma Nesfatin-1 levels were slightly higher in the group of diabetics when compared to normal individuals with p value of <0.05 which shows that the difference observed is statistically significant.

The probable explaination for the elevated levels of Nesfatin-1 in the diabetic individuals is mainly due to the insulin resistance. This peripheral insulin resistance results in the resting hyper insulinaemic state in the body in the early stages of diabetes when there is adequate beta cell reserve. As we already mentioned that the NUCB2 molecules were co-localized in the islets along with insulin and as we hypothesized that nesfatin-1 helps in differentiation of pre proinsulin to insulin and helps in releasing of insulin into the blood stream and at the same time decreasing the peripheral resistance of the insulin, in this process the precursors of nesfatin-1 molecules were also differentiate to produce more and more nesfatin-1 molecules to facilitate the above mentioned processes and this results in increased plasma nesfatin-1 levels in diabetic individuals. This possible explanation might not hold good in all diabetic patients because along with duration of the disease there will be decrease in the beta cell reserve and once the beta cell reserve is reduced there are no healthy islet cells remaining the body

to secrete nesfatin-1 along with the insulin, so in those cases the expected findings might be different.

In a study conducted by Zhang et al. they have found increased levels of plasma Nesfatin-1 in patients with newly detected T2 DM.66 In their study Plasma nesfatin-1 levels were significantly increased in both the nT2DM and IGT groups when compared with the controls (1.91 ± 0.79 and 1.80 ± 0.80 vs. 1.41 ± 0.58 μ g/L, both $P < 0.01$.

Although the results of this study are correlating with the above study, there are many studies with contradictory results with our observations.There are very few studies available regarding estimation of Nesfatin-1 in people with both obesity and DM. Although the study conducted by Qing – chung Li et al. showed negative correlation between the nesfatin-1 levels between this group of people and normal individuals. The probable explanation for this discrepancy of the results is that when more than one factor is influencing the variable in the same individual in different ways, the net result of that variable will depend on the severity and duration of the each factor with the dominant one influencing the result. In the same way in these patients according to our study and observations obesity will be lowering the nesfatin-1 levels in the blood while DM will increase the nesfatin-1 level in the blood. So, if both of these factors were present in the same individual the net effect on nesfatin-1 level will depend on the severity of obesity, degree of sugar control, duration of diabetes and many other factors. The difference of the results in both these studies can be attributed to all these factors and the different ethnicity and experimental conditions involved in both these studies.

In our study we also found that plasma Nesfatin-1 is positively correlated with FBS, PPBS, HbA1c with P-value being <0.05 which is statistically significant and plasma Nesfatin-1 is negatively correlated with BMI with p-value being <0.05 which is statistically significant.

Furthermore plasma nesfatin-1 is associated with many biochemical and anthropometric parameters in patients with DM and obesity. Association of these metabolic indices suggests the role of elevated nesfatin-1 levels as an anorexigenic molecule to induce satiety and improve insulin resistance to maintain better metabolic homeostasis of the body.

## SUMMARY & CONCLUSION

In summary, our study demonstrated a significant lower values of plasma nesfatin-1 in obese individuals when compared to normal individuals and higher values in type2 diabetic individuals when compared to normal individuals. There is no statistically significant correlation that could be established among the patients who are both obese and diabetic when compared to normal individuals.

We have also noticed positive correlation of plasma nesfatin-1 with FBS, PPBS, HbA1c with P-value being <0.05.

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