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Original Article

Can Nesfatin-1 Predict Hypertension in Obese Children?

Short title: The relation between hypertension and Nesfatin1

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What is already known on this topic?
The childhood obesity increasing over the years and it brings some morbidities like hypertension. The mechanism of hypertension in obesity can be explained for various reasons.

What this study adds?
Obesity causes hypertension but why all obese individuals have not got hypertension is controversial. In this study, we tried to clarify this issue by comparing obese peers in terms of blood pressure and found that Nesfatin-1 independently predicts hypertension in obese children.

Abstract
Objective: The prevalence of childhood obesity is increasing and, it brings co-morbidities like hypertension. However, it is still not clear whether some obese individuals are hypertensive and others do not. Nesfatin-1 is a recently discovered anorexigenic peptide which also has effects on blood pressure. Our aim is to evaluate the relationship between obesity-related hypertension and Nesfatin-1.
Methods: For this cross-sectional study 87 obese children were included. The patients were divided into two groups as hypertensive (n=30) and normotensive (n=57) obese. We used the American Academy of Pediatrics guidelines to diagnose hypertension. Blood samples were collected after 12 hours of fasting to examine the levels of Nesfatin-1. We also evaluated the serum trace elements of the patients besides the routine blood tests.
Results: Body mass index (BMI), weight and serum Nesfatin-1 were higher in the hypertensive group (p=0.002, p=0.001, and p=0.007 respectively). There was no difference between serum zinc levels but copper levels were significantly lower in the hypertensive group (p=0.248, p=0.007, respectively). There was a positive correlation between blood pressure and BMI and weight z scores and a negative correlation with Cu. The optimal cut-off value of Nesfatin-1 to predict hypertension was found to be >1.8 ng/ml, with specificity of 71.9% and sensitivity of 96.7% (AUC=0.703; 95% CI, 0.577-0.809; p=0.002). In the multiple logistic regression analysis Nesfatin-1 (OR=1.103, 95% CI: 1.039-1.171, p=0.001), Cu (OR=0.947, 95% CI: 0.915-0.979, p=0.001) and BMI for age z score (OR=56.277, 95% CI: 5.791-546.907, p=0.001) still remained significant predictors of hypertension.
Conclusion: We found Nesfatin-1 was higher and Nesfatin-1 levels independently predict hypertension in obese subjects.
Keywords: Obesity, hypertension, Nesfatin-1, children

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Introduction

The prevalence of childhood obesity is increasing over the years. The estimated prevalence among the world's children population is 6.7% and is expected to be 9% in 2020 (1). In a meta-analysis study, the prevalence is increasing by 0.7% to 7.1% in Turkey between 1990 to 2015 (2), and the estimated prevalence is over 10% (3). This increased prevalence also poses a more serious problem by increasing the incidence of co-morbid conditions in a number of obesity-related conditions. In addition to metabolic diseases such as diabetes, insulin resistance, it is prone to various cardiovascular diseases such as hypertension and dyslipidemia. This increased disease burden starting from childhood deserves detailed research in terms of affecting adult health (4). Obesity-related hypertension is a serious problem in childhood. The underlying etiology is complex and multiple factors are associated with this issue such as; activation of the renin-angiotensin-aldosterone system, stimulation

of the sympathetic nervous system, hyperinsulinemia, peripheral fat tissue compression in the renal parenchyma, a number of cytokines affecting the vascular endothelium, and the abnormalities of some adipokines such as leptin (1). Obesity also related some multi-nutrient and trace element deficiencies. For example, zinc deficiency contributes to leptin reduction in rats and humans (5). Most of the trace elements have antioxidant roles on the vascular endothelium which results in its damage contribute to hypertension. Because these elements are the components of the antioxidant enzymes such as cytoplasmic Cu–Zn superoxide dismutase (6-8).
Nesfatin-1 is recently discovered anorexigenic peptide which originates from its precursor protein nucleobindin-2(NUCB2) (9, 10). It has been associated with appetite, food intake and weight loss (11). In addition to regulating food intake, Nesfatin-1 has been shown to regulate energy hemostasis, to achieve water balance, gastrointestinal motility and cardiovascular effects (12-14). NUCB2 / nesfatin-1 have been shown to be distributed in areas of cardiovascular areas such as the hypothalamus, nucleus tractus solitarius and dorsal vagal complex (9, 15). Central administration of Nesfatin-1 increases blood pressure and heart rate by the pressor effects of increased vasopressin, renin and catecholamine levels (16-18). Also, peripheral injection of Nesfatin-1 increases blood pressure (19,20). The mRNA expression of NUCB2 has been shown to be increased in the media of the aorta of hypertensive rats so it may be contributed to hypertension mechanism (21). In a study, serum Nesfatin-1 levels were higher in essential hypertension than the control group, and it was correlated with systolic blood pressure (22). Some studies demonstrated elevated Nesfatin-1 levels in hypertensive patients' especially obese subjects than the control (23). In accordance with this issue, Nesfatin-1 has been a risk factor for obesity-related hypertension.
Although it has been shown that weight gain contributes to hypertension, it is unclear why there is no hypertension in some obese individuals. From this point on, we want to investigate if there is an effect of Nesfatin-1 on this issue. The aim of our study is to demonstrate and compare the levels of Nesfatin-1 in obese hypertensive children and non-hypertensive or normotensive obese to identify the role of this peptide on obesity related-hypertension. In addition to this, we wanted to examine the relationship of zinc and copper which is one of the serum trace elements.

Materials and Methods

For this cross-sectional study, 87 obese children (41 male, 46 female) who admitted to our hospital's pediatric endocrinology and metabolism outpatient clinic, aged between 8 to 18 years were included. We divided the patients into two groups with similar age and sex, as a hypertensive obese group (n=30) and non-hypertensive or normotensive obese group (n=57), as control. Patient's weight and height measurements were applied by a pediatric endocrinology nurse. The patients who have primary hypertension, hormonal abnormalities such as Cushing syndrome, hyperthyroidism, diabetes mellitus, medication-related hypertension, renal disease, heart disease, and other chronic diseases were excluded from the study.

The weights of the patients were measured only when they had underwear and height was measured by using Harpenden stadiometer. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m2). Obesity is defined as the BMI index is above the 95th percentile according to specific percentile curves determined with age and gender. An individual was considered as morbidly obese if on the 99th percentile (24).

Blood pressure (BP) was measured by an experienced nurse with an appropriately sized cuff at least 10 minutes after resting by the auscultatory method. The measurements were repeated three times in different clinical visits and the average value was recorded. Hypertension was defined as average clinic measured systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) ≥95th percentile on the basis of age, sex, and height percentiles (25). Ambulatory blood pressure measurements were performed to patients with normal out-of-hospital blood pressure and the diagnosis of white coat hypertension was excluded and these patients were excluded from the study (Figure 1). Both SBP, DBP, BMI, weight and height z scores for age of the patients were calculated from the program which is available on the internet (www.quesgen.com/BMIPedsCalc.php).

Blood samples were collected from all study groups following a fasting period of 12 hours between 08:00 a.m. and 10 a.m. Venous blood samples collected were centrifuged at 4,000 rpm for 10 minutes. The serum samples obtained were frozen at -80°C until time of analysis. Routine biochemical analyses [serum glucose, insulin, high density lipoprotein(HDL), low density lipoprotein(LDL), total cholesterol, triglyceride, other biochemical parameters and complete blood count(CBC)] were run through autoanalyser whereas serum levels of Nesfatin-1 was detected by ELIZA (enzyme-linked immunosorbent assay) method through commercial Eliza kit (Bioassay Technology Laboratory, China and Wuhan Fine Biotech Co. Ltd., China), automated ELISA reader (Thermo Scientific, Finland) and a computer program (Scanlt for Multiscan FC 2.5.1) according to directives of the manufacturer company. For Nesfatin: Sensitivity was 0.15 ng/ml and assay range was 0.30 ng/ml – 90 ng/ml., intra-assay CV% was <8% whereas inter-assay CV% was <10% and the results were expressed in ng/ml. Serum samples for serum Zinc (Zn) and Copper (Cu) levels were determined by flame spectrophotometry method in Perkin Elmer Analyst 800 model atomic absorption spectrometer device after a 1/4 dilution with 5% glycerol for serum zinc and a 1/2 dilution with 10% glycerol for serum copper level determination. It was calculated as µg / dL.

The study was conducted in accordance with the Declaration of Helsinki. Before starting, Sütçüimam University Ethics Committee approval under protocol number 333 and informed consent form were obtained from the patients and their parents.

Statistical Analysis

Data management and analysis were performed by using SPSS program v.14 (SPSS Inc., Chicago, IL) and a two-sided p-value ≤0.05 was considered as statistically significant. Continuous data were expressed as mean ± standard deviation or median and categorical data were expressed as percentages. A mean was compared by using an independent sample t-test, and in the case of an abnormal distribution, Mann–Whitney U test with median was used. Chi-square test was used for the categorical data. A stepwise multiple regression analysis was used for the definition of the significant determinants of hypertension, and incorporating variables that correlated with a P value of less than 0.1 in the correlation analysis. A value of $p< 0.05$ was considered statistically significant.

Results

The demographic and laboratory characteristics of study groups were shown in Table 1. Age and gender were not statistically different from each other in two groups (p=0.135, p=607, respectively), whereas BMI and weight z scores were high in obese hypertension group (p<0.001, p=0.002, respectively). There was a significant difference between each group according to SBP and DBP z scores (p<0.001, both). When laboratory data were compared between to each group, no statistical difference was found, except creatinin. The creatinin levels were higher in hypertensive group, but in the normal range in both groups (Table 1). Serum Nesfatin-1 levels were higher in the obese group with hypertension than the group with no hypertension (p=0.007) (Figure 2). When we compared both groups in terms of trace element levels, there was no difference in serum Zn levels, whereas serum Cu levels were significantly lower in the hypertensive group (p=0.248, p=0.007, respectively).

When correlation analysis performed, there were positive correlations between BMI and weight z scores besides negative correlations between Cu with SBP and DBP (Table 2).

Receiver operating characteristics curve (ROC) shows that; the optimal cut-off value of Nesfatin-1 to predict hypertension was found to be >1.8 ng/ml, with specificity of 71.9% and sensitivity of 96.7% (AUC=0.703; 95% CI, 0.577-0.809; p=0.002) (Figure 3).

In the multiple logistic regression model using a backward stepwise method, Nesfatin-1 (OR=1.103, 95% CI: 1.039-1.171, p=0.001), Cu (OR=0.947, 95% CI: 0.915-0.979, p=0.001) and BMI for age z score (OR=56.277, 95% CI: 5.791-546.907, p=0.001) still remained significant predictors of hypertension after adjusting for the confounding variables, which were either found to be statistically significant in the univariate analysis and for the variables which were also statistically significant in the t-test(Table 3).

Discussion

In this study, we showed Nesfatin-1 levels were independently related to hypertension and, higher in obese hypertensive children than the obese normotensive counterparts. BMI z scores were higher in hypertensive group and positively correlated with blood pressure. We also found serum Cu levels were low in the hypertensive group. Our study is original comparing obese children with their peers.

Weight gain causes hypertension in some individuals but not in others. This may be related to how long the individual is obese and the long-term effects of over-adiposity (26). Although there was no difference in age between the groups, the mean age of the hypertensive group was higher in our study. Our results revealed that serum Nesfatin-1 levels were higher in the obese hypertensive group. This is consistent with to the study of Zhao et al. who investigated the levels of Nesfatin-1 in 40 hypertensive adults and 40 healthy controls, and they found significantly higher levels of Nesfatin-1 in the hypertensive group especially in obese individuals (23). In the study of Anwar et al. the Nesfatin-1 levels were higher in obese adolescents than healthy peers and correlated with BMI (27). Their results were similar in the study of Tan et al. who compared the levels of Nesfatin-1 in 38 adult subjects (28). Sahin et al. found higher values of Nesfatin-1 in polycystic ovary syndrome than the healthy control and also positively correlated with SBP and DBP (29). It was demonstrated that Nesfatin can cross the blood-brain barrier in both directions and this can explain the effect of this peptide on the central control cardiovascular effects (30). The i.c.v. administration of Nesfatin-1 increased plasma renin, catecholamine, and vasopressin which cause hypertension (17, 18, 31). The central melanocortin system is responsible for hypertensive effects of Nesfatin-1 in normotensive animals and also mentioned as above in obesity-related hypertension (17, 26, 31-33). In our study Nesfatin-1 independently associated with hypertension and, predicts hypertension in obese subjects. Zhao et al. and Sahin et al. found a positive correlation between Nesfatin-1 level and BP in their studies (23, 29). But, we cannot find any correlation between Nesfatin-1 and BP. This may be contributed to the low number of cases in the hypertensive group.

We also evaluated the serum trace elements between the groups and find a significant difference in terms of Cu levels. The Cu levels were lower in the hypertensive group and negatively correlated with BP, and independently associated with hypertension. But there was no correlation between BP and Zn levels and there was not a significant difference in groups in terms of Zn. There were several studies on trace elements about the

association of hypertension in the literature. It has been considered that Zn and Cu may take a role in the pathogenesis of hypertension because of the necessity of these electrolytes in the regulatory enzymes of the vascular system (34). Low serum Cu levels were detected in terms of hypertension in human and animal studies and negative correlations were found (35-38). This can be related to the inhibitory effect of Cu on angiotensin converting enzyme activity (39, 40). Also, the Cu deficiency causes hypercholesterolemia and increased oxidative stress which can lead to hypertension (41).

Study Limitations and Strengths

There were some limitations of our study. Firstly, the number of cases is low, especially in the hypertensive group. Maybe we can compare the parameters by a healthy control group but we wanted to investigate the effect of Nesfatin-1. Additionally the serum Nesfatin levels were both in normal commercial kit range among the groups. This result may have changed if we had taken an additional healthy control group. Another limitation is the heterogeneity of the groups by means of BMI and weight. This may be related to cross-sectional study design, perhaps a longer-term study may help to overcome this heterogeneity, or the increase in weight increases the tendency to hypertension can cause this heterogeneity. There were also some strengths of our study. Firstly, it is important comparing obese children with each other to determine the effect of Nesfatin-1 on obesity-related hypertension.

Conclusion

To the best of our knowledge, this is the first study evaluating serum Nesfatin-1 in obese hypertensive children. Nesfatin-1 levels independently predict hypertension in obese subjects. This study may show a way to explain why some obese patients have hypertension while others do not. Of course, more randomized controlled trials are needed to claim this.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Sütçüimam University (decision no: 333, date: 29.08.2018).

Informed Consent: Informed consent was obtained from all students and their parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Hatice Güneş, Hakan Güneş, Filiz Alkan Baylan, Fatih Temiz Concept: Hatice Güneş, Hakan Güneş, Filiz Alkan Baylan, Fatih Temiz Design: Hatice Güneş, Hakan Güneş, Fatih Temiz Data Collection or Processing: Hatice Güneş, Hakan Güneş, Fatih Temiz Analysis or Interpretation: Hatice Güneş, Hakan Güneş, Filiz Alkan Baylan, Fatih Temiz Literature Search: Hatice Güneş, Hakan Güneş, Filiz Alkan Baylan, Fatih Temiz Writing: Hatice Güneş, Hakan Güneş, Filiz Alkan Baylan, Fatih Temiz

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Table 1: Demographic and laboratory data of the study groups

	Obese hypertensive group (n=30)	**Obese normotensive group (n=57)**	**p**
Age, years[a]	13.5 (9-15)	11 (9-14)	0.135
Gender, male/female, n	13/17	28/29	0.607*
Height, m[b]	1.53±0.18	1.51±0.15	0.502
HAZ[a]	1.25 (-0.001-1.69)	1.17 (0.13-1.72)	0.724
Weight, kg[b]	83.7±26.3	65.3±18.9	0.001
WAZ[a]	2.56 (2.30-2.95)	2.19 (1.82-2.54)	0.002
BMI, kg/m^2[a]	32.8 (27.4-40.3)	27.2 (24.5-31.8)	0.002
BAZ[a]	2.49 (2.18-2.61)	2.02 (1.87-2.25)	<0.001
SBP, mmHg[a]	130 (123.7-140)	100 (100-110)	<0.001
SAZ[a]	1.96 (1.53-2.58)	-0.16 (-0.97-0.48)	<0.001
DBP, mmHg[a]	90 (77.5-90)	70 (60-70)	<0.001
DAZ[a]	2.1 (0.80-2.30)	0.28 (-0.20-0.66)	<0.001
Fasting blood glucose, mg/dl[a]	89 (83.2-98)	90 (85-94)	0.849
Insulin, µIU/mL[a]	18.8 (14.2-39.4)	16.1(10.6-23)	0.057
HbA1c,%[b]	5.4±0.33	5.4±0.32	0.962
ALT, U/L[a]	22 (16.5-32.5)	20 (16.7-29)	0.429
AST, U/L[a]	22 (20.1-28)	23.9 (20-28.5)	0.209
Total protein, g/dL[a]	7.6 (7.4-7.9)	7.6 (7.2-8)	0.859
Albumin, g/dL[b]	4.7±0.2	4.7±0.4	0.733
Urea, mg/dl[b]	9.5±2.4	8.8±2.8	0,375
Creatinin, mg/dL[b]	0.6±0.2	0.4±0.2	0.002
Triglyceride, mg/dL[a]	122 (90-161)	114 (85-157)	0.151
Total cholesterol, mg/dL[b]	171.2±35.9	161.6±29.7	0.199
HDL cholesterol, mg/dL[b]	40.9±7.9	43.8±10.4	0.191

LDL cholesterol, mg/dL[b]	100.4±27.2	99.5±30.5	0.895
WBC, X10^3 mm^{3a}	8.8 (7.1-10.3)	8 (6.8-9.8)	0.273
Hemoglobin, g/dL[b]	13.56±1.18	13.54±1.11	0.925
Platelet count, X10^3 mm^{3b}	358±63.6	354±58.3	0.789
Cu, µ/dL[a]	85.2 (75.7-103.0)	110.5 (89.9-125.4)	0.002
Zn, µ/dL[b]	102.7±46.7	112.9±34.2	0.248
Nesfatin, ng/ml[a]	11.9 (5-22.6)	4.8 (1.7-10.4)	0.007

[a]median (interquartile range), [b]mean±SD, [*]Chi-Square $p \leq 0.05$ was considered statistically significant
ALT: Alanin aminotransferase, AST: Aspartate aminotransferase, BMI: Body mass index, BAZ: Body mass index for age z scores, Cu: Copper, DBP: Diastolic blood pressure, DAZ: Diastolic blood pressure for age z scores, HDL: High density lipoprotein, HAZ: Height for age z scores LDL: Low density lipoprotein, SBP: Systolic blood pressure, SAZ: Systolic blood pressure for age z scores, WAZ: Weight for age z scores, WBC: White blood cell count, Zn: Zinc.

Table 2: Correlation coefficients of systolic and diastolic blood pressures

	Systolic blood pressure		**Diastolic blood pressure**	
	r	**p**	**r**	**p**
BAZ	0.404	<0.001	0.271	0.012
WAZ	0.350	0.001	0.324	0.002
Cu	-0.303	0.005	-0.249	0.023
Creatinin	0.248	0.021	0.094	0.389

BAZ: Body mass index for age z scores, WAZ: Weight for age z scores

Table 3: Univariate and multivariate analyses of study group

Univariate Analysis							**Multivariate Analysis**					
Variable	**B**	**S.E.**	**Wald**	***p***	**OR**	**%95 CI**	**B**	**S.E.**	**Wald**	***p***	**OR**	**%95CI**
Statistically Significant Variables												
Nesfatin	0.058	0.022	7.355	0.007	1.060	1.016-1.106	-5.463	2.769	3.892	0.001	1.103	1.039-1.171
Cu	-0.033	0.011	8.371	0.004	0.968	0.946-0.989	-0.055	0.017	10.228	0.001	0.947	0.915-0.979
BAZ	3.416	0.913	14.007	0.000	30.450	5.089-182.199	4.030	1.160	12.067	0.001	56.277	5.791-546.907
WAZ*	1.389	0.507	7.516	0.006	4.011	1.486-10.829						
Creatinin*	2.778	0.978	8.068	0.005	16.085	2.366-109.361						

All the variables from Table 1 were examined and only those significant at $p < 0.05$ level are shown in univariate analysis. Multivariate logistic regression analyses including all the variables in univariate analysis with enter method. $p < 0.05$ was considered statistically significant. *Non-significant variables in multivariate logistic regression analysis were not indicated in the table.
B: Beta coefficients; BAZ: Body mass index for age z scores, WAZ: Weight for age z scores; CI: Confidence interval; Cu: Copper; OR: Odds ratio; S.E.: Standart Error; Wald: Wald test.

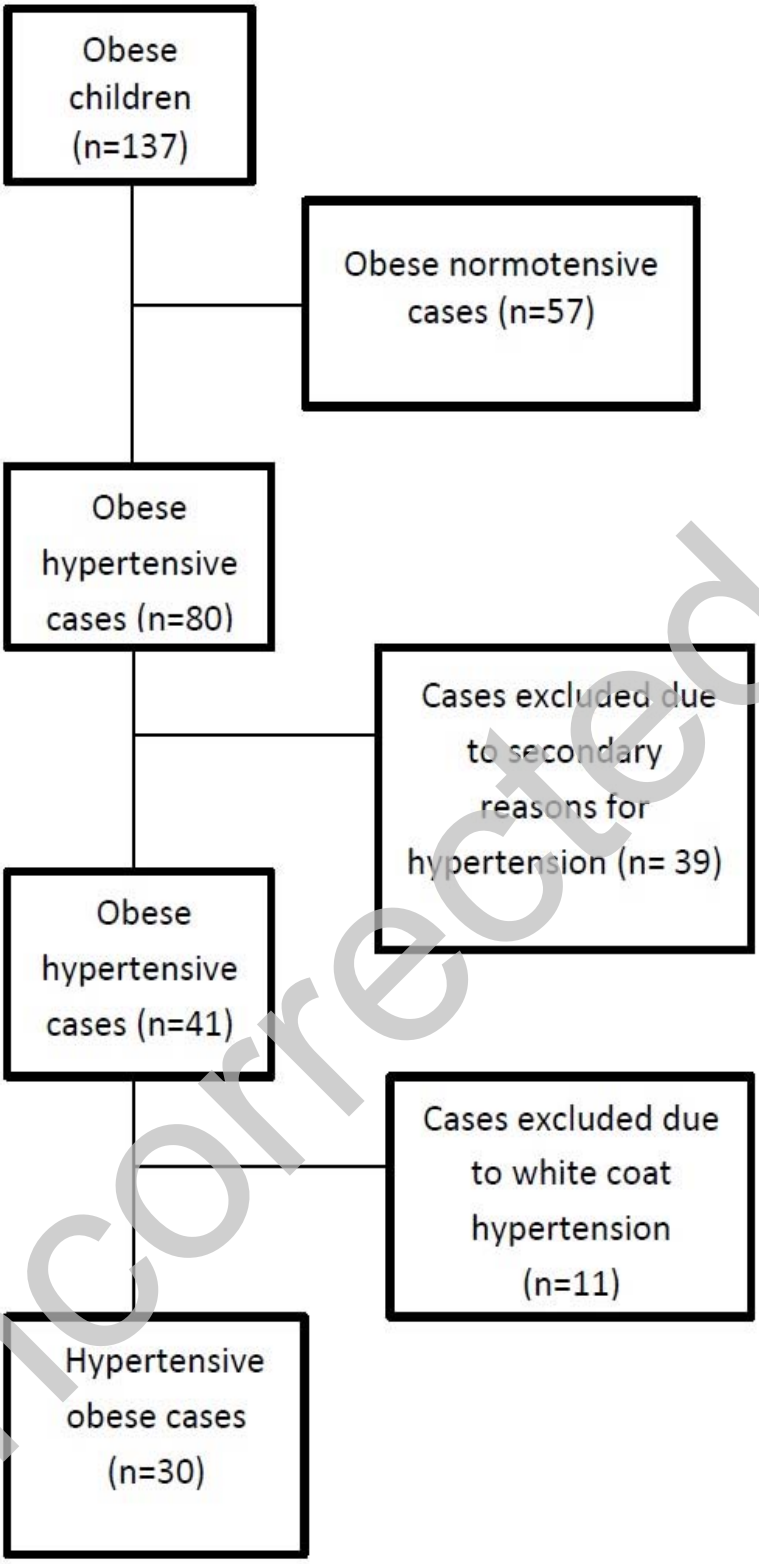

Figure 1: Study flow-chart

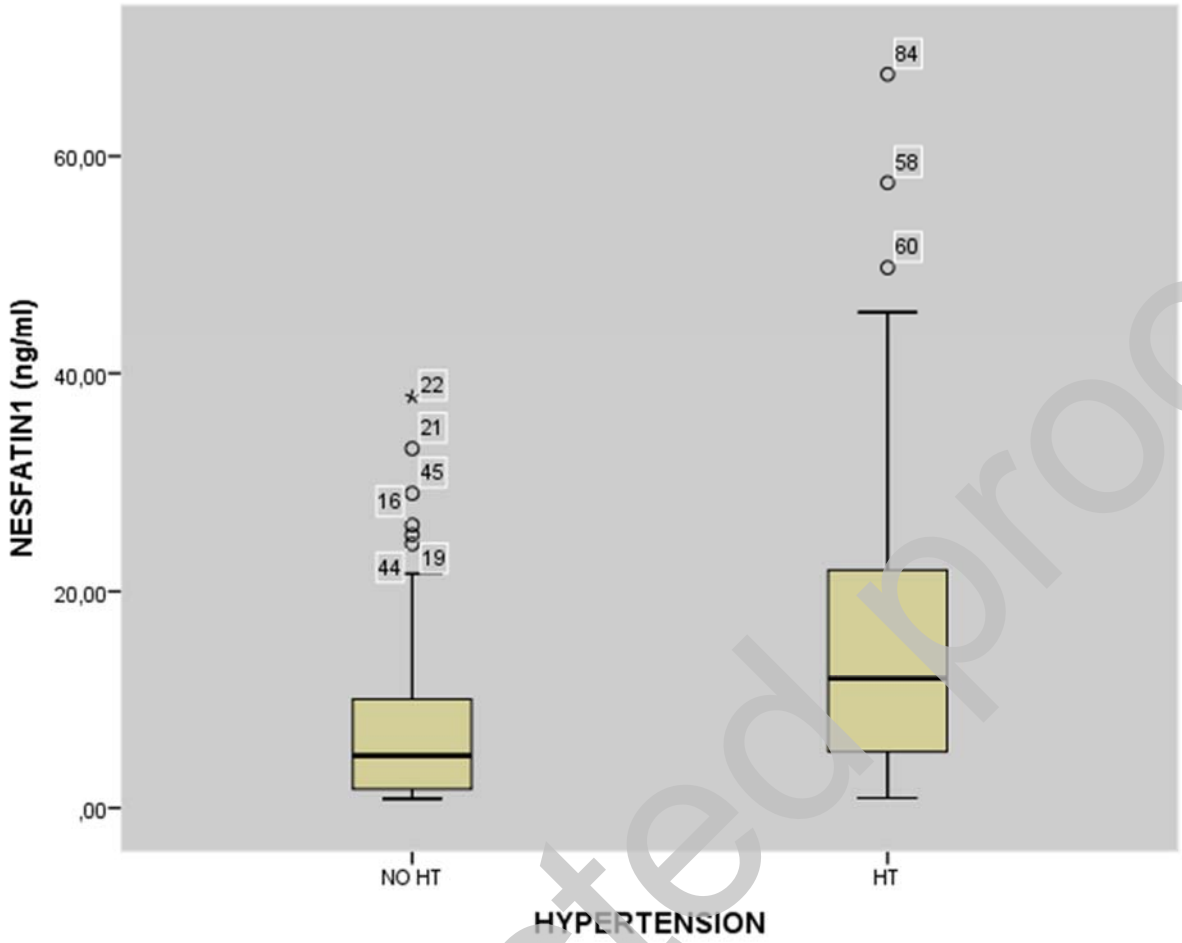

Figure 2: Distribution of Nesfatin1 levels between the obese subjects with and without hypertension

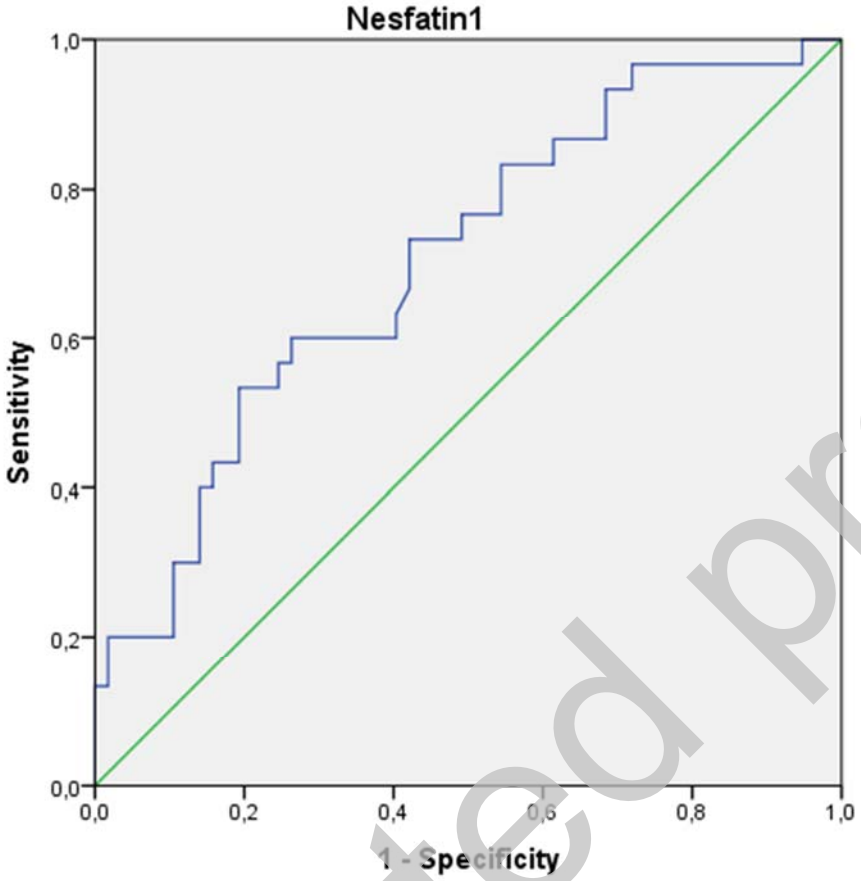

Figure 3: Receiver operator characteristic Curve (ROC) of Nesfatin1 to predict hypertension