

## THE STUDY OF PREDICTION OF BODY MASS INDEX THROUGH GENETIC POLYMORPHISM OF SEROTONIN TRANSPORTER GENE (SLC6A4) 5-HTTLPR IN RAKMHSU STUDENTS

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### Abstract

**Introduction:** In recent years obesity has become a major health problem and life style changes, habits aggravated the prevalence of obesity in young children. Many studies have suggested that serotonin has been linked with the occurrence of obesity along with various other factors. Based on these facts we aimed to study the relation of serotonin allele's expression with BMI of university students.

**Materials and Methods:** The study was a case control involving 66 participants done in Biochemistry department, RAK Medical and Health Sciences University, Ras Al Khamiah. A standardized diet history questionnaire used to calculate the calorie intake. The saliva was collected and using the manufactures guidelines the DNA has been extracted using DNA Genotek kit. The polymerase chain reaction used to detect the polymorphism in the subjects. Finally DNA gel electrophoresis done to detect the alleles in different subjects.

**Results:** The study results showed that BMI, Waist circumference and body weight percentage was significant in overweight and obese subjects. The most common alleles in normal subjects was SS when compared with overweight and obese showing SL, LL.

**Discussion and Conclusion:** Our study was in accordance with other studies which showed L allele associate with obesity. We suggest that, L allele associate is a risk and can be used to assess/predict the obesity.

**Key words:** Obesity, Polymorphism, Overweight, Body mass index, Polymerase chain reaction

### Introduction:

The industrialization, urbanization has led to lot of changes in people life including their eating habits. Adding to this the physical inactivity and dietary changes in terms of eating unhealthy food has contributed to lot of problems such as obesity, heart problems, joint problems etc.

According to a study childhood obesity is a growing concerns in developed countries along with developing countries. (1) Another study found that female are more prone for obesity than males due to inherent hormonal variations. (2) The obesity serves as co factor for diabetes mellitus and coronary artery disease early in their life. (3)

The tackling of childhood obesity is a medical emergency now days and children in schools, colleges exhibit high body mass index which is a worrying sign.

Globally in 2010, the number of overweight children under the age of five is estimated to be over 42 million. Close to 35 million of these are living in developing countries.

In the 20<sup>th</sup> century the rates of overweight, obesity have increased worldwide according to many studies. (4, 5) The various studies were done to know the etiological aspects of

obesity in recent years and many factors have been found. One such factor of genetic aspect that makes the person susceptible and researchers found the several genes suggesting their role. (6,7) One such gene was of serotonergic system with their effect on brain and gastrointestinal system. The serotonin (5-hydroxytryptamine or 5-HT) plays a major role in the regulation of body energy balance through the effect on various downstream neuropeptide systems along with autonomic pathways. (8)

According to the researchers, various effects of 5-HT include mood control, urine storage, voiding, sleep regulation, body temperature, circadian functions, feeding behavior, body weight, and intestinal motility (9,10,11) and its regulation is through the 5-HT transporter (5-HTT). (12)

The study by Lesch et al (1996) showed that 5-HTT is encoded by the serotonin transporter gene (*SLC6A4*) on chromosome 17, at 17q11.2- 17q12. This is located at 1,400 bp upstream of the transcription start site of the gene [9]. The studies also showed the functional polymorphism, the *SLC6A4*-linked polymorphic region or 5-HTTLPR. (13,14) It mainly consists of two common alleles of short (S) and long variant (L). (9,15)

The study by Iordanidou *et al.*, 2010 shown that the S allele (SS or SL genotypes) is associated with lower *SLC6A4* expression, thereby resulting in reduced 5-HT reuptake and release capability, whereas the L variant is associated with an almost threefold increase in gene transcription. (8)

The purpose of intake of food is to restore the calorie homeostasis and is controlled by both peripheral and central mechanisms. (16) The later mechanism control involves various parts of brain including neurotransmitters and neuropeptides once such example of neurotransmitter is serotonin. (17)

The studies have suggested that CNS serotonin involved in appetite and subsequent nutrient intake. The peripheral serotonin has a role in various aspects of digestion, insulin production, and liver repair process. (18)

The studies have stated that reducing peripheral serotonin synthesis and signaling in adipose tissue can prevent obesity, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) due to increased energy expenditure. (19-24)

Based on these findings the study was done to assess the use of the genetic polymorphism of serotonin transporter gene (*SLC6A4*) 5-HTTLPR in the prediction of body mass index (BMI).

### Materials and Methods:

The study was a case control study which consisted of 66 individuals with different BMI (36 normal, 16 overweight, and 14 obese). The study conducted in department of Biochemistry, RAKCOMS, RAK Medical and Health Sciences University (RAKMHSU). After obtaining the university ethical committee clearance. The participants were approached and full details about the study was given. The total duration of the study was 4 months. A set of pre designed and pre validated questionnaire was given to students to gather personal information, family, medical and dietary history. Based on their response from the food sheet the average calories intake per day was calculated.

In the Biochemistry lab all participants weight, height, waist and hip circumferences were measured using the standard instruments. The body mass index (BMI) was calculated according to the classification given by world health organization (WHO). (25)

The saliva sample collected from each participants for analysis of DNA.

### DNA extraction from saliva

The procedure was explained clearly to all participants. The saliva sample collected and mixed in the DNA Genotek kit by inversion and gentle shaking for a few seconds. The sample was incubated at 50°C in the water bath for 1 hour. A 500µl of the sample transferred into micro centrifuge tube and 20µl of prep IT.L2P(PT-L2P) was added and mixed by vortex for few seconds. The sample then

incubated on ice for 10 minutes and centrifuged at room temperature (RT) for 5 minutes at 15,000xg.

The clear supernatant was carefully transferred with a pipette to fresh micro centrifuge tube and 600µl of room temperature 100% ethanol was added and mixed gently by inversion 10 times.

The sample was left to stand at room temperature for 10 minutes so that DNA gets fully precipitated. The sample was centrifuged again at 15,000xg for 2 minutes and supernatant was discarded. A 250µl of 70% ethanol added to the DNA pellets and allowed to stand for 1 minute at room temperature. The ethanol removed carefully without disturbing the pellets and 100µl of TE solution (10mM Tris-HCL, 1mM EDTA,pH (8.0) added and vortexed for 5 seconds.

The sample was incubated overnight at RT and then stored at -20 °C.

The sample was analyzed for DNA purity using spectrophotometric assay with measurement of absorbance at 260 and 280nm after adding RNase to digest contaminating RNA. (26)

### Polymorphism detection using polymerase chain reaction

The gene was amplified by polymerase chain reaction on 96 well Amp PCR system 2720 Thermo cycler (Applied Bio systems). Primer sequences was synthesized by Promega as follows:

Forward 5'-TGAATGCCAGCACCTAACCC-3', reverse as 5'- TTCTGGTGCCACCTAGACGC-3'. The PCR procedure was performed with 25ul reaction mixture (100ng of DNA, 4nm of each primer, 2.5mM of each dNTP, 2.5mM of MgCL2,0.025U Taq polymerase, and 1XPCR buffer, promega) and had initial step of 2 min at 95 °C, followed by 40 cycles of 30s at 95 °C, 30s at 61 °C and 1 min at 71 °C and final elongation step involved 10mins at 72 °C. The product obtained was separated by electrophoresis on 2% agarose gel stained with ethidium bromide 0.01µg. The final products analyzed for the S and L allele respectively. (27)

The genotype and allele frequency done using allele counting [28]. The SPSS 24 version used for analysis. The chi square test done to find the genotype prevalence and associate between case and control groups. The describe strength of associate the odds ratio and 95% confidence interval used. The student T test used for finding the mean value of calories intake, waist and hip circumference, weight and BMI. A p value less than 0.05 was considered as significant.

### Results:

The study demographic data is shown in the table 1 and showed BMI, waist circumference, percentage of body fat and weight was significant in overweight and obese when compare with normal subjects.

**Table 1: Demographic data of the studied groups**

<b>PARAMETER \ BMI</b>	<b>NORMAL BMI</b>	<b>OVERWEIGHT AND OBESE</b>	<b>P</b>
<b>AGE</b>	19.1	18.7	>0.05
<b>BMI</b>	22.1	27.8	<0.001**
<b>WAIST CIRCUMFERENCE</b>	79.6	88	<0.05*
<b>% OF BODY FAT</b>	21	26.3	<0.001**
<b>WEIGHT</b>	59.5	80.7	<0.001**

The study also showed that, the most common genotype in normal was SS (64%) as compared to obese who had predominately LL (79%) genotype as shown in table 2.

**Table 2: Frequency of different genotypes of 5-HTTLPR among individuals with different BMI.**

<b>BMI \ GENOTYPE</b>	<b>SS</b>	<b>SL</b>	<b>LL</b>
<b>NORMAL (N=36)</b>	23(64%)	10(28%)	3(8%)
<b>OVERWEIGHT (N=16)</b>	4(25%)	5(31%)	7(44%)
<b>OBESE (N=14)</b>	3(21%)	0(0%)	11(79%)

The table 3 shows the association of different polymorphic form of 5HTTLPR genes with BMI. It showed that SS was major in normal and SL, LL were in overweight/obese subjects. The study also found that a high risk of obesity was seen among carrier of L allele of serotonin transporter gene compared to S allele.

**Table 3: Association of different polymorphic form of 5HTTLPR with BMI**

<b>BMI \ GENOTYPE</b>	<b>SS</b>	<b>SL AND LL</b>
<b>NORMAL (N=36)</b>	23(64%)	13(36%)
<b>OVERWEIGHT AND OBESE (N=30)</b>	7 (23%)	23(77%)

On comparison of phenotype with genotypes we could not find the any significance as shown in Table 4.

**Table 4: Comparing the mean standard deviation of obesity phenotype among carriers of different genotype of 5HTTLPR**

<b>Phenotype \ Genotype</b>	<b>SS</b>	<b>SL &amp; LL</b>	<b>P</b>
<b>WEIGHT(Kg)</b>	64.9±13.1	67.7±15.8	NS
<b>BMI(kg/m<sup>2</sup>)</b>	23.9±2.9	24.0±4.0	NS
<b>CALORIES(kcal)</b>	1550.7±451	1613.4±441	NS
<b>% OF BODY FAT</b>	24.0±6.7	25.7±5.4	NS

NS- No significance

#### Discussion:

Our study found that SL and LL genotypes were associated with the overweight and obese subjects when compared to normal with SS alleles.

A study of Bah *et al.* (2010) in a Swedish population showed that the 5-HTTLPR SS genotype is more frequent in underweight subjects (29) but our subjects with SS genotype were normal.

Our study was correlating with the finding of Peralta *et al.* (2012), in a Mexican population sample, reported the L allele associated with overweight/obesity. (30) Our study also showed L genotype was associated with overweight and obese subjects. A study by Heils *et al.*, 1996 suggested 5-HTTLPR S allele is associated with a lower transcriptional activity resulting in reduced reuptake of serotonin in synaptic cleft. (14) The expression of SS alleles in our study in normal subjects may be the reason for them not to be overweight.

There were some studies which reported contrary results showing S allele associated with higher BMI (31,32), which was not the case in our study.

In terms of body weight we couldn't find a significant variation in SS and SL, LL genotypes, although multiple studies found an increased body weight by direct effect of carrying an S-allele. (30, 31-34) But we found that SL and LL subjects were having higher body weight when compared to SS genotype.

The studies done on patients with SS or SL genotypes had great availability of serotonin in their central serotonergic synapses, which increase the satiety and reduce food intake. The overall effect is lowering the body mass and thereby affecting the BMI. (35) The same effect may have contributed in the subjects with SS genotypes having normal BMI, it should be kept in mind that various single-nucleotide polymorphisms of serotonergic genes, including some serotonin receptors, have been linked to greater adiposity or metabolic disease. (36, 37) These all factors should be assessed while attributing to the genotype predicting the obesity development.

### Conclusion:

Serotonin involved in range of central and peripheral nervous system functions. The single nucleotide polymorphism has been associated with obesity. In our study we conclude that, carrier of L allele of serotonin transporter gene polymorphism are at risk of obesity.

### Limitations:

- Small sample size and done on particular age groups in one institution students.
- Gender wise variation was not done.

### Future Research:

- To increase sample size for better results
- Expand to wide age group
- Focus on a particular ethnic group
- To study the interactions of 5-HTTLPR with other genes and how they lead to obesity

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### Competing Interests:

The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists. All the authors have contributed for the work.

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