

Relationship of Serum Leptin with Menarche and Pubertal Stages in young girls

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ABSTRACT

Background: Leptin hormone is considered to play an important role in the regulation of puberty in adolescent girls. It varies widely during different stages of puberty and has some relationship with menarche and pubertal stages.

Aim: To have a visual clarification for effect of Serum leptin level on pubertal stages in Pakistani girls.

Methodology: It was a cross-sectional analytic study. One hundred and fifty two girls were selected from different schools and colleges of Lahore. The sample was divided into 10 groups, comprising approximately 15 girls in each age group with age range of 8.0 to 17.99 years. Their demographic data was recorded. Age of menarche was obtained through a questionnaire. Stages of puberty were determined by inspection and palpation according to Tanner's staging system. Blood samples were collected to determine the serum leptin level by ELISA method.

Data was entered into SPSS (Statistical package for social sciences) version 16 and analyzed. Spearman's correlation test was applied to observe the correlation between the serum leptin and stages of puberty.

Results: The mean age at menarche was 12.76±1.24 years. Mean serum leptin value was 6.653±7.11 ng/ml in over all groups. Serum leptin concentration progressively increased with advancement of mean pubertal stages ($P < 0.000$). Leptin concentration showed a positive correlation with mean pubertal stages ($\rho = 0.349$, and $P < 0.000$) and pubertal levels ($\rho = 0.395$, and $p < 0.000$). The serum leptin concentration in pre menarcheal and post menarcheal girls were 4.63±4.74 and 8.62±8.41 ng/ml respectively ($P < 0.000$).

Conclusion: Higher serum leptin level leads to an early onset of puberty and menarche.

Key words: Puberty, Pubertal stages, Menarche, Serum leptin.

INTRODUCTION

Physical and psychological growth at which an individual is able to reproduce sexually is called as Puberty^{1,2,3}. 'Female Tanner Staging' described by Tanner in (1962) is a 5 stage system which describes different physical changes taking place during the course of puberty in girls¹. Menarche (first menstrual bleeding) is symbolic start of womanhood. It usually starts two years after thelarche. It occurs during 9 to 18 years of age^{4,5,6}. There is strong evidence that age of onset of puberty and age at menarche is continuously decreasing^{7,8,9,10}. Different factors are considered to affect time for start of puberty these are environment, obesity, genetics, chemical and hormones in diet, physical exercise, and presence of disease^{8,11,12}.

Leptin, discovered in 1994, produced by adipocytes and encoded by ob gene is protein in nature, and is considered one of the important

factors in this regard⁽¹³⁾. The major function of this hormone is to govern body weight and metabolism, but also has an important effect on reproduction^{14,15,16}. Leptin exhibits its effect by binding on receptors, which belongs to cytokine family and is the product of db gene. These receptors are present in ovaries, testes, hypothalamus, and anterior pituitary^{16,15,17}. The depots of body fat send signal to brain to regulate the reproduction by secreting leptin, it acts on hypothalamus to enhance the release of gonadotropin releasing hormone (GnRH) which in turn stimulates production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from anterior pituitary thus governing reproduction^{18,17}.

At the onset of puberty along with other sex hormones, an effective amount of leptin is also secreted^{19,20}. Leptin effect on reproduction is substantiated by the reality that puberty and age at menarche is decreased by increase in serum leptin concentration²¹. A level of 12.2ng/ml of serum leptin level may be associated with reduction of age at menarche. Age at menarche is declined by 1 month with an increase of serum leptin level of 1 ng/ml²².

The puberty onset is mysterious process and its relationship with serum leptin concentration appears

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to be intriguing, enthralling and controversial. In this study serum leptin concentration is measured and its relationship with stages of puberty in adolescent Pakistani girls is tried to be established. The objective of this study was to measure the amount of serum leptin level in various stages of puberty and to determine whether raised serum leptin concentration leads to earlier onset of puberty.

MATERIALS AND METHODS

A cross-sectional analytic study was planned to establish the effect of serum leptin level in regulation of adolescence growth. Approval from ethical committee of Postgraduate Medical Institute of Lahore (PGMI) and Advanced Studies and Research Board (ASRB) of University of Health Sciences Lahore was taken.

The calculated sample size was 152 school and college girls. Out of 9 administrative towns of Lahore city, one town was randomly selected, and there are 77 total schools and colleges in these town, 4 schools and 2 colleges were selected randomly. Using balloting method simple random sampling was used. The subjects were divided into 10 groups, comprising approximately 15 girls in each age group. Age range was from 8.0 to 18 years. Permission was taken from concerned institutional administration. Informed voluntary consent was taken from parents and girls to record the data by doing physical examination and for collection of blood and to use this data in research for publication.

The participating girls in the study represented all pubertal stages according to female Tanner staging, and vary between 8 to 18 years. The laboratory work was conducted in the Postgraduate Medical Institute, Lahore and University of Health Sciences, Lahore.

The girls having acute or chronic organic illness, endocrinological upset, congenital anomalies, metabolic disorders and disorders of sexual differentiation, leading to early or delayed onset of menarche were excluded from the study. Confidentiality of the data was ensured.

Complete demographic data including name, age, weight and height were recorded. The data was entered in a proforma. Date of birth was asked from students and confirmed from school register. Then decimal age of each child was calculated from decimal chart⁽²³⁾. The girls were examined for stages of puberty as per Tanner's staging⁽¹⁾. Breast development and pubic hair development was evaluated and recorded according to photographic norms of stages of puberty by inspection and palpation as well as standardized drawing and description based on Tanner criteria^{1,3,24}. Age of

menarche was inquired from girls. Most of the girls remembered exact year and month of their first menstrual period. The age was cross checked from elders. Age of menarche was also calculated in decimals.

Five ml of fasting blood was collected from each girl by using aseptic measures between 10 am to 12 noon to minimize the effect of diurnal variation in serum leptin concentration⁽²⁵⁾⁽²⁶⁾. Blood was allowed to clot at - 4°C. The serum was separated by centrifugation at 2000xg for 10 min. The samples were stored at -70°C in serum cups. ELISA (enzyme linked immunosorbant assay) was used to measure serum leptin concentration⁽²⁷⁾⁽²⁵⁾ by using commercially available ELISA Kit (AviBion Research ELISA, Orgenium Laboratories Finland). Procedure was performed as described in manual of the kit.

Mean pubertal stage was calculated by taking mean of two ((Tanner staging for breast and Tanner staging for pubic hair). Puberty development was simplified by dividing girls into three groups based on level of puberty, i.e., stage I, as pre-pubertal(P I), stage II-IV as puberty(P II), and stage V as post puberty (P III)⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾. Data was analyzed by SPSS version 16. To establish the relationship between serum leptin level, and pubertal stages spearman correlation test was applied. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Data of pubertal development: Data of pubertal development is shown in table 1 below. Majority of girls (58%) were in stage II, III, and IV. Second major group comprising of 25.7% of the girls were still at pre-pubertal stage (Tanner stage 1). Other (15.8 %) were at stage V (post pubertal stage). Mean pubertal stage II and breast development (BII) started at the age of 11.36 ± 1.84 years. Seventy seven (50.7%) girls crossed the land mark of menarche while 75 (49.3%) were still at pre-menarche stage. The mean age at menarche was 12.76 ± 1.24 years.

Relationship of serum leptin with mean pubertal stages: Table 2 shows the serum leptin levels in different pubertal stages (mean pubertal stages). The serum leptin concentration differs significantly in different pubertal stages. The Spearman's correlation test was applied to observe the relationship between serum leptin and mean pubertal stages. A positive correlation between serum leptin and mean pubertal stages was observed. Figure 1 showing that leptin level increases with increase in pubertal stage, ($\rho = 0.349$, and $p < 0.000$). The effect becomes more evident when relationship was evaluated with simplified pubertal levels (Table 2 and figure 2). The Spearman's correlation test was applied to see the

relationship between serum leptin and different pubertal levels. Significant positive correlation between serum leptin and pubertal levels was observed showing that with advancement of pubertal levels serum leptin concentration were also raised, ($\rho = 0.395$, and $p < 0.000$).

Relationship of serum leptin with menarche: A total of 77 girls passed the mile stone of menarche and 75 were still at pre-menarcheal stage. The values of serum leptin in pre-menarcheal girls was

4.63 ± 4.74 ng/ml and it was 8.62 ± 8.40 ng/ml in post-menarcheal girls, revealing a statistically significant difference of serum leptin concentration between two groups ($P < 0.000$). It is evident that serum leptin was higher in post menarcheal subjects as compared to pre menarcheal subjects. Very weak, non significant, inverse correlation ($r = -0.095$ and $P < 0.411$) was seen between serum leptin concentration and age at menarche. This also indicated that as serum leptin level increased, as age at menarche decreased.

Table 1: Frequencies and percentages of subjects at different pubertal development (Mean pubertal stages and pubertal levels). This table shows mean ages of girls at different pubertal stages.

Mean pubertal stage	Mean Age in years (SD)	Frequency	%age	Pubertal levels	Frequency	%age
Mean pubertal stage I	10.13±1.67	39	25.7	Pre-puberty P I	39	25.7
Mean pubertal stage II	11.36±1.84	43	28.3	Puberty P II	89	58.6
Mean pubertal stage III	14.53±1.45	20	13.2			
Mean pubertal stage IV	15.32±1.85	26	17.1			
Mean pubertal stage V	16.11±1.30	24	15.8	Post puberty P III	24	15.8
Total	12.89±2.89	152	100		152	100

Table 2: Values of serum leptin (ng/ml) in mean pubertal stages.

Mean pubertal stage	Number of girls	Serum leptin (ng/ml) mean±SD	Pubertal levels	Number of girls.	Serum leptin (ng/ml) mean±SD
Mean pubertal stage I	39	2.39±2.35	Pre-puberty P I	39	2.39±2.35
Mean pubertal stage II	43	6.66±5.34	Puberty P II	89	7.25±6.58
Mean pubertal stage III	20	6.72±6.37			
Mean pubertal stage IV	26	8.63±8.43			
Mean pubertal stage V	24	11.38±10.07	Post puberty P III	24	11.38±10.07
Total	152	6.65±7.11		152	6.65±7.11
ANOVA		$P < 0.000$			$P < 0.000$

Table 3: Serum leptin concentration in mean pubertal stages, comparison with other studies

Mean Pubertal stage	Our study ng/dl	Wang et al 2004 ng/dl	Hui-juan et al 2010 ng/dl	Blum et al 1997 ng/dl	Ahmad et al 1999 ng/dl	Rutter et al 2009 ng/dl	Li et al 2005 ng/dl	Horlick et al 2000 ng/dl
Stage I	2.38±2.35	3.9±4.5	1.84± 0.15	2.51	2.47	6.2±4.9	5.80 ± 0.43	3.9±1.4
Stage II	6.66±5.34	5.6±6.2	2.14± 0.23	2.86	3.00	8.5±6.0	12.30 ± 1.87	14.4±4.4
Stage III	6.72±6.37	7.8±5.6	5.59 ±0.87	3.81	3.30	7.25±5.0	17.43 ± 1.55	11.6±3.4
Stage IV	8.63±8.43	8.1±7.8	6.90± 0.84	4.39	3.15	6.2±3.3	24.37 ± 3.09	14.8±5.3
Stage V	11.38±10.08	7.9±8.0	9.42 ±0.52	6.24	4.99	10.4±6.6	31.04 ± 5.53	30.3±7.9

Table 4: Comparison of leptin concentration in pubertal levels with other studies.

Pubertal levels	Our series Serum leptin (ng/ml) mean±SD	Garcia-Mayer et al 1997 (ng/ml) mean±SD	Brandao et al 2003 (ng/ml) mean±SD	Kies et al 1998 (ng/ml) mean±SD
Pre-puberty P-1 (Pubertal stage 1)	2.39±2.35	5.4±0.2	7.15± 7.03	2.8±2.3
Puberty P-2 (Pubertal stage 2-4)	7.25±6.58	7.1±0.7	7.91± 9.18	4.77±2.88
Post puberty P-3 (Pubertal stage 5)	11.38±10.07	8.1±0.5	9.78± 5.97	12.64±6.8
P value	<0.0000	< 0.05	< 0.068	<0.005

Fig. 1: Relationship of serum leptin level with mean pubertal stages. $\rho = 0.349$, and $P < 0.000$.

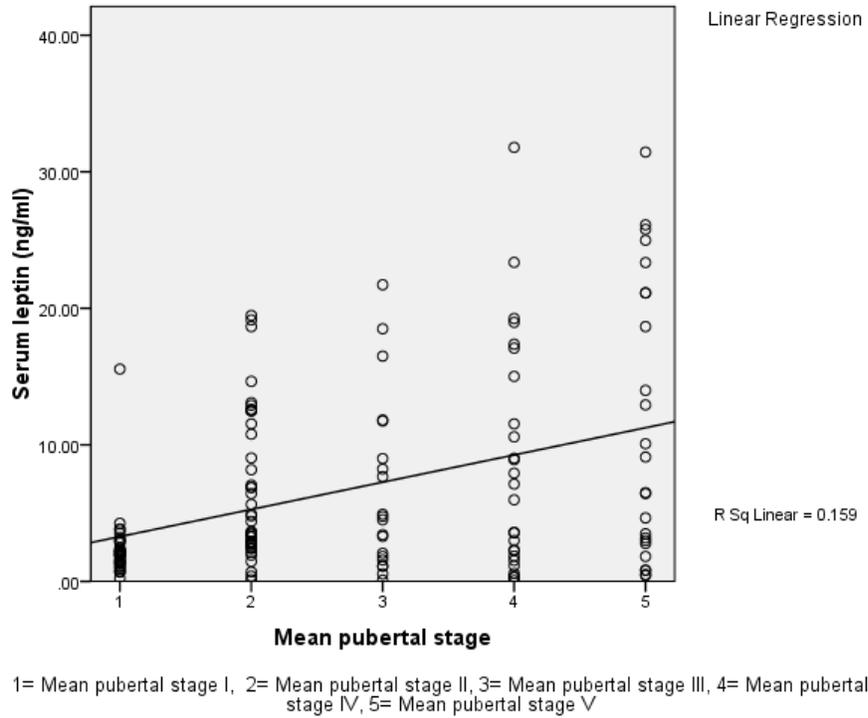


Fig. 2: Relationship of serum leptin level with pubertal levels ($P < 0.000$, $\rho = 0.395$).

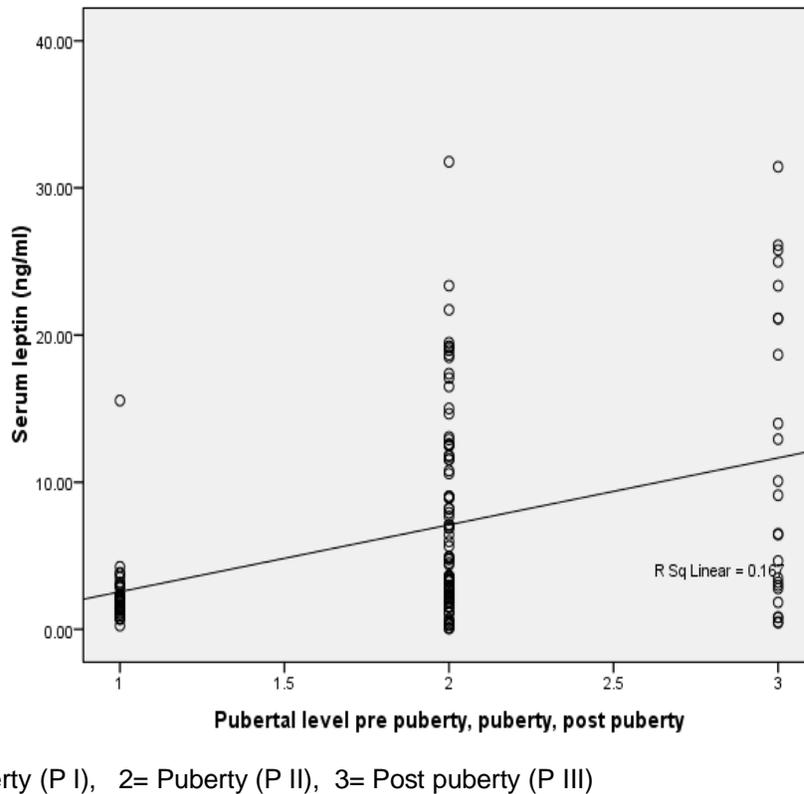


Fig 3: Graph serum leptin with menarche

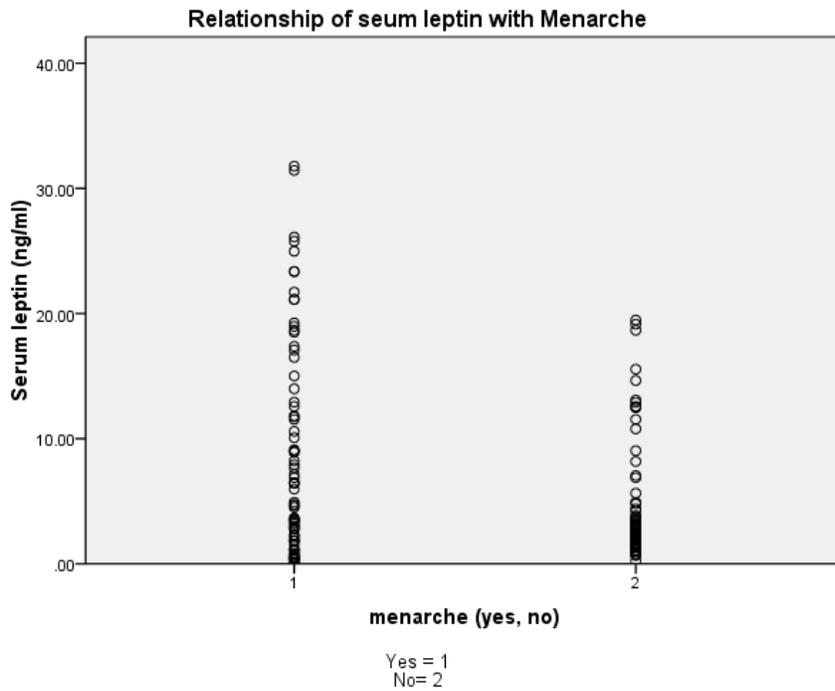
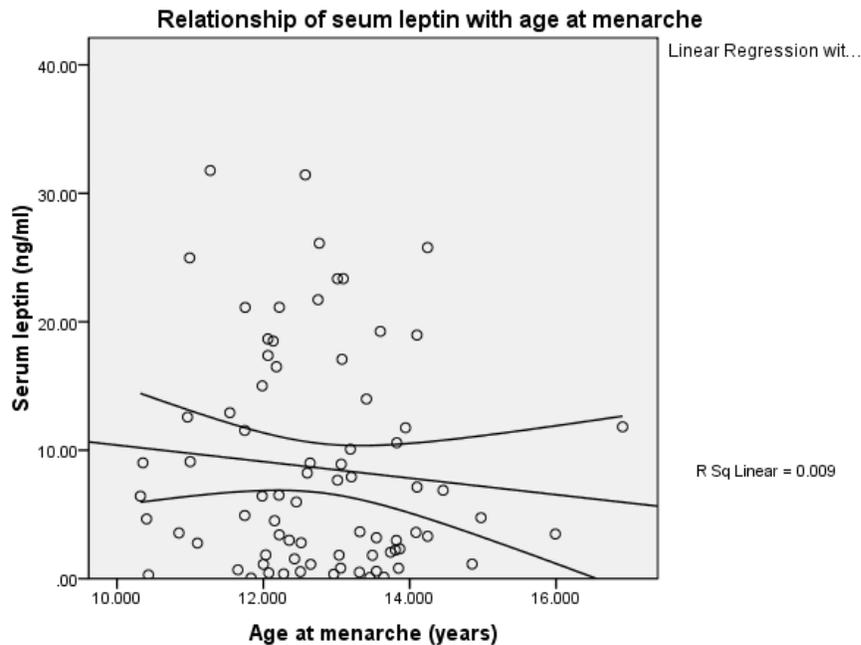


Fig. 3. Relationship of serum leptin with age at menarche.



DISCUSSION

Pubertal growth in adolescent girls is responsible for great variations in height, weight and hence BMI of individual. All these factors are considered to affect the concentration of serum leptin. Leptin

concentration varies widely in children in accordance to age, sex and pubertal growth, as evident in several series⁽³¹⁾⁽³²⁾⁽³⁰⁾⁽³³⁾⁽³⁴⁾⁽³⁵⁾⁽³⁶⁾. The factors affecting the concentration of leptin in adolescence girls are not only the chronological age but the stage of sexual

maturation also affect its, as the girls with same age range may be passing through different pubertal stages. To assess these factors, we determined the relationship of serum leptin concentration with menarche and pubertal stages in young girls.

In our series, data collected from girls in different pubertal stages provides visual interpretation of relationship between pubertal growth and serum leptin level. The results of our co-relational study revealed that mean pubertal stages positively related to the concentration of leptin in girls ($\rho=0.349$, $P<0.000$). Serum leptin progressively increased through puberty as also reported by other studies³¹⁻³⁶ (Table 3).

In our study the overall serum leptin concentration throughout pubertal stages were approximately similar to that of studies carried in Brazil³⁰, Japan³³, Netherland⁽³⁵⁾ and China³⁶ but the serum leptin concentration was significantly higher than our study in the series conducted by American³², China³⁴ and low in the studies carried by Germany⁽³¹⁾; and United Kingdom³⁷ (Table 3). It may be probably because in addition to puberty other factors for example living styles, height, weight³, mass of adipose tissue³⁸. BMI²⁹ waist- hip ratio⁽²⁷⁾ lipid profile³⁹ estrogen level⁴⁰ and percentage of body fat²⁰ affect the serum leptin concentration level.

In our study, the increase in serum leptin along with pubertal stages was not smooth. There was prominent rise in serum leptin concentration between stage I and stage II ($p<0.000$), but minor difference between stage II and stage III (non significant). Again, there was prominent rise in serum leptin after stage III, through IV, and stage V, ($P<0.000$). Almost same findings were observed by other studies⁽³⁷⁾⁽³⁵⁾. To counter this problem we divided the pubertal stages into three levels, pre-pubertal (stage 1), pubertal (stage II, III, IV) and post-puberty (stage V) as practiced by other authors also⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾. The relation of these results were relatively smooth showing progressive and positive relation of serum leptin between three pubertal levels as in our study ($P<0.000$ $\rho=0.395$), which coincides well with all above three studies²⁸⁻³⁰ (Table 4).

We have noted that leptin concentrations were stabilized at 15 years of age and onward in our series and this is also supported by Bandini et al, 2008²⁰. Leptin is secreted in pulsatile manner. Number and amplitude of leptin pulses are not recorded in our study. It is reported in literature that there may be difference in clearance rate and metabolism of leptin, it may also play a role in making a difference in circulating leptin concentration³².

A study carried in USA, by Li et al., 2005 which showed that increase in serum leptin level throughout female pubertal development is associated with

increase in the body fat. He also determined soluble leptin receptors (SOB-R) in serum. They bind the leptin so decreasing the free serum leptin level. There is decline in soluble leptin receptors from Tanner stage I to II, it results in sustained increase in serum leptin level. This increase in serum leptin level from stage I to II, might be involved in onset of puberty and the thelarche. Rise in leptin level especially in latter stages of pubertal development may be that it forecast the upcoming menarche and pregnancy³⁴.

Menarcheal age is main aim and an indicator for scoring the pubertal development in adolescent girls. The mean age at menarche in ours series is 12.76 ± 1.24 years. World Health Organization reported that the average age at menarche is different in different countries; In Bangladesh, it is 15.8 years; in India, it is 14.31 years⁴; in British teenagers, it is 13.1 years⁴² in Iran, it is 12.5 years¹¹; in Kawait it is 12.14 years¹⁰; in Turkey it is 12.74 years⁴³; in Indonesia is 12.96 years⁴⁴; in Germany it is 12.8 years⁽⁴⁵⁾. Previous reported average age at menarche in Pakistani girls is 13.6 years^{42,46}. So there is strong evidences of early onset of menarche not only in Pakistan but literature proved that it is worldwide problem⁴⁷.

In our study pre-menarche serum leptin level was significantly less as compared to post-menarche serum leptin (4.63 ± 4.74 vs 8.62 ± 8.41 ng/ml, $P<0.000$). Similar findings were observed by Wang et al, 2004⁽³³⁾. Carlson et al also reported that before menarche leptin concentration were 5.4 ± 0.5 $\mu\text{g/L}$ and post menarche it was 13.7 ± 1.4 $\mu\text{g/L}$ ($p<0.0001$)⁴¹.

As it is suggested that there may be a signal to initiate and maintain pubertal development or there may be presence of some permissive agent in the blood circulation preceding the beginning of biochemical events of sexual maturation, leptin exactly plays this role^{35,18}. These important findings support the theory that leptin acts as messenger through which body tell the hypothalamus that energy reserves have reached the desired levels to initiate complicated events leading to puberty couple of years afterward. All above findings are supported by the fact that females who are deficient in leptin are prepubertal in spite of adult age, when treated with leptin they had spontaneous menarche and other sign of puberty⁴⁸.

CONCLUSION

The results of present study indicate that puberty and menarche are experiencing a decreasing trend. Serum leptin concentration is positively with correlated with pubertal stages and menarche. Leptin concentration in normal range acts as a

permissive agent for pubertal development. In adolescent girls, rise of serum leptin with puberty shows that leptin is an informative biomarker of puberty. The values obtained from the present study can provide baseline data for analysis of timing of puberty in Pakistan as well as for international comparisons. It may also be concluded that physiological level of serum leptin may be necessary for the onset and maintenance of puberty.

Future directions: The query whether early pubertal development is the reason of higher serum leptin level or the raised serum concentration is responsible for early pubertal development, can be answered by the longitudinal studies which proposed that a rapid rise of serum leptin in body determines the early onset of pubertal development⁽⁴⁹⁾. Still more research is needed to explore many more details about leptin and reproduction, it is progressively evident that serum leptin plays an important role in the secretion of FSH and LH, and establishes a linkage among the body fat reserves and onset of sexual maturation in human as well as rodents.

Acknowledgement: We appreciate the contribution by the department of medical education Postgraduate Medical Institute Lahore for financial help and University of Health Science Lahore for conducting laboratory workup for this research.

We also acknowledge all the participants and their parents who participated in the study and provided required information for this study.

Conflict of interest disclosure: None

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