Research Article

Relationship of Serum Leptin Level with Chronological Age and Body Mass Index in Adolescent Girls

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Abstract |

Objective: The study aimed to evaluate the e ect of chronological age and Body Mass Index (BMI) on concentration of leptin in serum.

Methods: It is a cross-sectional comparative study. One hundred and fifty two girls were selected from di erent schools in Lahore. Their demographic data was recorded. 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 height and weight were measured to determine their BMI. Blood samples were collected to determine the serum leptin level by Enzyme Linked Immunosorbent Assay (ELISA) method. Pearson correlation test were applied to observe the correlation between serum leptin and BMI.

Results: The mean BMI is 18.20±3.70 kg/m2 and mean serum leptin value is 6.653±7.11 ng/ml over all groups. A positive correlation was observed between serum leptin and chronological age, ($r = 0.225$, and $p < 0.005$). A strong positive correlation was observed between BMI and serum leptin concentration ($r = 0.604$, $P<0.000$). The mean BMI in underweight, normal weight and overweight subjects were 14.35±1.63 kg/m2, 17.85±2.45 kg/m2, 24.08±2.98 kg/m2, and mean serum leptin concentrations 3.28±3.90 ng/ml, 5.32±5.46 ng/ml, 16.42±8.39 ng/ml respectively ($r = 0.511$, and $p < 0.000$) .

Conclusion: Serum leptin level is positively correlated with chronological age and BMI. This study indicates that serum leptin increases with advancement in chronological age and higher BMI results in higher serum leptin level.

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Introduction

Leptin is a hormone, protein in nature with important e ect in modulating body weight, and metabolism in rodents, decreasing weight and increasing energy consumption[(1)], but little is known about its mechanism of action in human being. It is expressed predominantly by adipose tissue. Over the last two centuries especially in last few decades, rapid development in economics, leads to better nutrition, resulting in more weight gain in early childhood and adolescence leading to childhood obesity, which is now a worldwide problem. The body fat is usually clinically measured as Body Mass Index (BMI).[(2,3)] Serum leptin level represents as a marker of adipose tissue and in turn of increased BMI.[(4)] Relationship of serum leptin with age was also established in many

studies revealing that from five years upto 10 years of age serum leptin increases in both sexes along with BMI but after word in male it decreases but in female it continue to increase till 13 to 15 years. The rise of serum leptin with age is related to BMI.[5,6]

Concentration of serum leptin is more in obese subject than normal weight subjects, and low in underweight which rises after increase in weight.[7,8] In addition, serum leptin level is influenced by di erent hormones and food intake. Fasting leads to reduction in leptin level by 30% and over eating increases leptin level by 50%. This increase is greater if food is rich in fat.[9] Fat in subcutaneous tissue contains leptingenes. These genes are expressed in prepubertal and pubertal girls. A positive relationship between serum leptin level and subcutaneous tissue has been demonstrated.[1,9,10]

Many studies are carried out about leptin and its relationship with puberty, pregnancy, adulthood obesity but this is the first study carried in adolescent girls, to observe the relationship of serum leptin with chronological age and BMI in young girls in Pakistan. We will compare our results with literature taking literature as control group.

Materials and Method

It is Cross-sectional Comparative study. One hundred and fifty eight school going adolescent female students, ages between 8 to 17.99 years, were recruited in the study from di erent schools of Lahore. Six children were excluded from the study having incomplete data information so a total 152 were the final participants. Approval of this study was taken from Ethical Committee of Post Graduate Medical Institute and Advanced Study Review Board of University of Health Sciences Lahore and was completed within one year from September, 2011 to June, 2012.Simple random sampling (using balloting method) was applied.

Sample size was calculated with the following formula:

$$N = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2}{\frac{1}{4}\left[\log_e\left(\frac{1+r}{1-r}\right)\right]^2} + 3$$

Where, Z 1 – β is the desired power of study = 90%, Z 1-α/2 is the desired level of significance = 5%, r is the correlation coe cient = 0.26[11]. The calculated sample size turned out 152subjects.

The children voluntarily participated in this study. Children with any known metabolic syndromes, any chronic disease and congenital anomalies were excluded. Informed and voluntary consent was taken from parents and girls. Confidentiality of the data was ensured. Laboratory work was performed in the Postgraduate Medical Institute, Lahore and University of Health Sciences, Lahore.

Height, weight and age of girls were taken and recorded on a proforma. Date of birth was asked and confirmed from school register. The decimal age of each child was calculated from decimal chart[12]. Their body weight (up to 0.1 kg) was recorded by a portable standard weighing scale (Kinlee, YRBB-120) in kilograms in normal school clothes without shoes. Their height was measured in meters (up to 0.1cm) without shoes with mandible parallel to floor with portable manual height board, (Seca gmbhf co. kg Model:210, 1721009, CE 0123. Made in Germany).[8,13] To assess the e ect of age on parameters measured, the children were grouped according to their chronological age. From 8 to17.99 years, girls were divided into 10 groups almost approximately 15 students in each age group. BMI was calculated by dividing weight in kg by square of height in meters. BMI less than 5th percentile considered as underweight (BMI group 1), between 5th to 85th percentile as normal weight (BMI group 2) and more than 85th percentile considered as overweight Body Mass Index BMI group.[3,14,16]

Participants were asked to fast over night. 5-ml of blood was obtained from each girl by venepuncture with aseptic measures. Universal precautions were observed, including spirit swabbing of skin, use of sterile needles and syringes, proper disposal of used syringes in sharp bin, disinfection of bio-hazardous material (used swab and syringes), and cleaning of table surface with disinfectants[17]. To minimize the influence of diurnal variation in serum leptin concentration, all samples were collected between 10 am to 12 noon 18 and after clotting at 4°C; the serum was separated by centrifugation at 2000xg for 10 min. The samples were stored in serum cups at -70°C.Serum leptin was measured by Enzyme Linked Immunosorbent Assay (ELISA) method.[8,18] Serum leptin was estimated by using commercially available

ELISA Kit (AviBion Research ELISA, Orgenium Laboratories Finland). Procedure was performed as described in manual of the kit.

Data was entered into Statistical Package for the Social Sciences (SPSS) (Statistical package for social sciences) version 16 and analyzed. Mean ± Standard Deviation (SD) was given for quantitative variables i.e. serum leptin concentration, chronological age, and BMI. Pearson's correlation test was applied to observe the relationship of serum leptin concentration with chronological age and body mass index. A value of $p < 0.05$ was considered as statistically significant.

Results

The data of all the groups, pertaining to mean chronological age, mean BMI and mean serum leptin level is given in Table 1.This table revealed a significant increase in BMI (P < 0.000) with age, but non significant di erence in serum leptin concentration (P < 0.436) in di erent age groups.

A positive correlation between serum leptin and chronological age was observed, (r = 0.225, and p <0.005) (Figure1).Serum leptin concentrations varied among the girls over almost 30-fold range, changing with chronological age of the subjects.

Table 1: *Chronological Age, BMI, and Serum Leptin Levels of the Subjects.*

Group	N	Age Range(Mean Age)(Years)	BMI[a](Kg/m^2)Mean ±S.D	Serum leptin[b](ng/ml)Mean ±S.D
Group 1	15	8-8.99(8.40)	15.62±1.50	4.39±5.21
Group 2	16	9-9.99(9.37)	15.81±2.45	4.91±4.91
Group 3	17	10-10.99(10.57)	16.66±2.96	4.90±4.81
Group 4	15	11-11.99(11.40)	17.01±3.45	5.08±5.40
Group 5	15	12-12.99(12.60)	17.11±2.92	5.75±5.37
Group 6	15	13-13.99(13.57)	18.44±2.50	8.11±7.36
Group 7	15	14-14.99(14.44)	18.91±2.78	8.14±8.37
Group 8	16	15-15.99(15.40)	19.63±4.08	8.85±9.56
Group 9	13	16-16.99(16.54)	22.82±4.34	8.80±9.81
Group 10	15	17-17.99(17.45)	20.84±3.28	8.07±8.41
Mean	152	12.89	18.20±3.70	6.65±7.11

a, P< 0.000 b, P< 0.436

Pearson's correlation test was applied to observe the relationship between serum leptin and BMI (figure 2). A moderately positive correlation between serum leptin and BMI was observed (r = 0.604, and p <0.000).

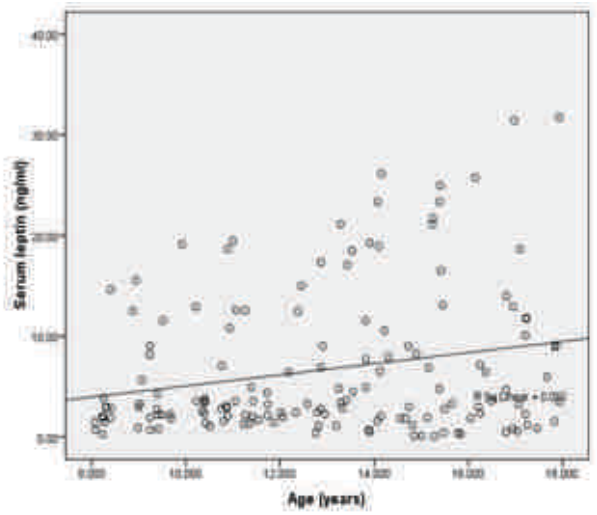

Figure 1: *Relationship of Serum Leptin with Chrono-Logical Age ®= 0.225, p<0,005)*

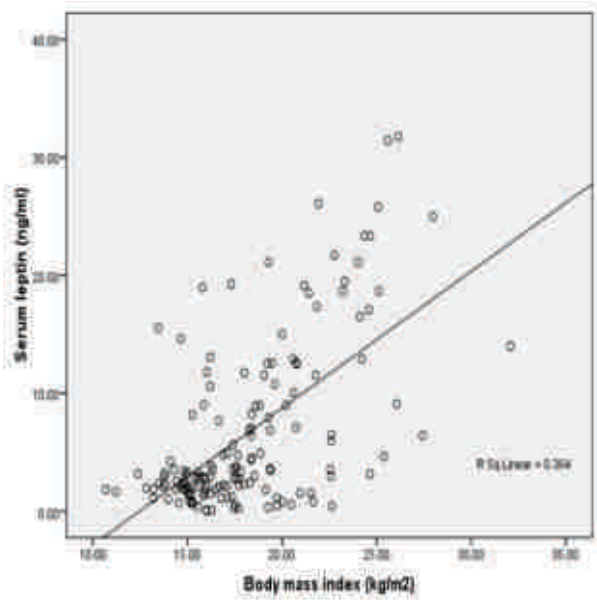

Figure 2: *Relationship of Serum Leptin with BMI. (r = 0.604, p<0.000)*

Table 2: *BMI Groups and Relationship with Serum Leptin.*

BMI Groups According to Percentile	Number of Subjects N=152	Body Mass Index Kg/m^2 (Mean ±SD)	Serum Leptin ng/ml (Mean ±SD)
Group 1: under weight <5th percentile	26 (17.10%)	14.35±1.63	3.28±3.90
Group 2: Normal weight 5th to 85th percentile	103 (67.80%)	17.85±2.45	5.32±5.46
Group 3: Overweight >85th percentile	23 (15.10%)	24.08±2.98	16.42±8.39
Total and mean ±SD of BMI and Serum Leptin	152 (100%)	18.19±3.70	6.65±7.11

Pearson's correlation test was applied to observe the relationship between serum leptin concentration and BMI groups. A moderately significant positive correlation between serum leptin concentration and BMI groups was observed showing that as BMI groups advanced, serum leptin concentration significantly increased, ($r = 0.511$, and $p < 0.000$).

Discussion

The relationship of serum leptin concentration with chronological age and BMI appears to be controversial and intriguing. This study has determined a relationship between serum leptin concentration, chronological age and BMI in adolescent girls.

The leptin rose progressively with increase in age from 8 to 17.99 years from 4.40±5.21 ng/ml to 8.07±8.41 ng/ml with mean of 6.65±7.11 ng/ml. A positive correlation between serum leptin and chronological age was observed in this study showing that leptin level increases with increase in age, ($r = 0.225$, and $p < 0.005$). When leptin was correlated with age, wide distribution was observed in our study population. These results revealed that concentration of serum leptin varies by the di erence in age and BMI. This gain of weight and BMI is due to higher fat mass in body.[19] Our study also showed wide variation of serum leptin level in di erent individuals of same age group as evident from scatterplot in figure 1 ($P = 0.005$). Di erent studies highlighted these finding showing a wide variation of serum leptin level of same age group.[20,21] Variation of serum leptin level in di erent subjects of same age group may be due to di erence in BMI, body fat, level of adiposity, nutrition, nourishment, variability in feeding habits, exercise, regular physical training, ethnicity, and variability in body composition, environmental di erences, di erent life style and mutation in gene.[11, 22, 23] Our population is thin and lean, and has relatively low BMI as compared to other adolescent population so the leptin is also low.[15,19,24,25,26]

Our results of serum leptin level in relationship with age are comparable to German children of same age group 27, but concentration of serum leptin was greater in Americans and in Chinese.[18,21] The serum leptin level, and increase in its level with age in Pakistani girls showing a positive relationship although weak ($r = 0.225$, and $p < 0.005$), is quite comparable with other studies carried in Spain, Brazil, Japan, and China.[5, 28,29,30] The study conducted in Brazil revealed that leptin concentration in girls showed a positive linear correlation with chronological age ($r = 0.35$, $P = 0.0012$).[28] An other study also reported almost same relationship of serum leptin level with age as in our study ($r = 0.26$, $P < 0.001$).[11] The study carried out in Japan also showed age related increase in serum leptin level with a weakly positive relationship ($r = 0.32$, $p < 0.001$)[29] Almost similarrise of serum leptin level were detected in a study carried in Colombia 22. Another study carried in Tibet China determines the serum leptin level in children of age range 12 to 16 years demonstrating the same trend, but level of serum leptin was higher in Tibtian girls compared to our study population (5.74±2.14ng/dl at 12 years to 13.33±2.56 ng/dl at 17 years).[18] Positive correlation of leptin with age in children was also observed in another study carried in India ($r = 0.56$).[8] In our study, there was substantial rise in leptin concentration between 12 and 13 years of age group probably related to weight gain during adolescence in majority of girls at this age group. In our study there was mild decrease observed after 15.99 years of age as serum leptin falls from 8.85±9.56 to 8.80±9.81 ng/ml and 8.07±8.41 ng/ml respectively in next two groups. Similar findings were also recorded by Wang et al. and Poveda et al.[22,29] This decrease in serum leptin level seen at this age may be due to either stabilization or reduction of weight of subjects leading to slight fall in leptin level.[23,29,30] It could be deduced that serum leptin level, despite considerable variation, increases with the increase in chronological age of adolescent girls and di erence in leptin level may be due to racial di erence.[19]

Our study showed strong correlation of serum leptin concentration with BMI showing that serum leptin level increases with increase in BMI ($p < 0.000$, $r = 0.604$).These results were consistent with various studies which revealed that serum leptin concentrations correlate well with BMI ($r = 0.60$)[8] ($r = 0.71$)[20] ($r = 0.331$, $p < 0.01$)[18] ($r = 0.65$, $P < 0.0001$).[28]

A longitudinal study conducted in Michigan conducted to examine the relationship between leptin as an index of energy balance, weight, adiposity, and fitness. This study revealed that one percent increase in body fat is related to a seven percent increase of leptin ($P < 0.0001$) whereas 1 unit increase in BMI

increases leptin concentration by 15%.[22] BMI is measure of total body fat although it is a good indicator of body fat in adults may be less accurate indicator of fat in adolescents.[20] During sexual maturity in girls, increase in body fat has been approximated 120% and this would lead approximately two fold rise in leptin concentration.[11,20] Most probably, the amount of serum leptin present in blood during sexual maturation in adolescent girls may be manifestation of gain in body fat.

Accurate amount of fat cannot be determined by BMI but strength of correlation between BMI and fat is much more significant than other variable that is age. The mechanism by which increase in body fat is translated into an increase in serum leptin appears to involve expression of ob gene. An other study concluded that significant amount of ob mRNA is found from adipocyte in obese subjects than from normal weight subjects.[10] When adipocyte become hypertrophied, production of leptin by individual cells increase to approximately twice the initial value. It mean that changes in body fat is translated into changes in serum leptin level at the level of induction of ob gene, it revealed that small change in body fat produces significant change in serum leptin level revealing that body fat is the major modulator of leptin levels.[11]

A study carried in Brazil tried to construct reference ranges for leptin concentration according to BMI ranges (14-18, 19-23, >24) showing a positive correlation between two.[28] In our study, the serum leptin concentration increased with BMI rise, it is almost double in girls with BMI between 5th and 85th percentile than girls with BMI less then 5th percentile and overweight girls (BMI< 85th percentile) had the value three times than normal weight. Significant positive correlation between serum leptin concentration and BMI groups was observed showing that as BMI groups advanced, serum leptin concentration significantly increased,(r = 0.511, and p <0.000) (Table 2).

Despite a good correlation of serum leptin and BMI, there is wide variation in serum leptin level among the girls of same BMI; this may be related to di erence in body composition and fat distribution. It may also be due to di erent sensitivity of individuals to leptin. This leptin resistance is induced due to high fat diet.[8] This wide variation may be due to high concentration of free fatty acids, triglycerides, cholesterol and high blood sugar levels in these subjects. These findings are also supported by other studies[8,22,27,29] indicating that factors other than BMI also have e ect on it. Variation of serum leptin is also associated with values of insulin which is low in fasting and high after meal.[31] In addition to this leptin is also influenced by the concentration of growth hormone.[2]

Serum leptin level reflect adiposity status well when condition are in steady state but during dynamic conditions this relation is not very consistent, energy balance is dependent upon nutrition which may change leptin concentration.[32] In our study the children's are in developmental stage their fat mass and body composition is in dynamic state that's why there is wide variation in serum leptin level.

Conclusion

Serum leptin concentration has weak positive relation with chronological age. There is wide variation in leptin concentration in individual of same age group. As the BMI increased with age the serum leptin concentrations also increased progressively, showing significant positive correlation bet-ween serum leptin and BMI. There is marked di erence in serum leptin concentration when the subjects were catego-rized as underweight, normal weight, and overweight, according to their BMI.

Limitations of this study: One should interpret these results under the light of our study limitations. One of the major limitations of this study is that it is cross-sectional in nature but the strength of our study is that we used large sample size and methodology is purely population based. A long study may be more helpful in understanding these relationships.

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References

1. Kulik-Rechberger B, Tarkowski R, Mago n DA, Osemlak P, Rechberger T, Jakimiuk AJ. Leptin gene expression in subcutaneous adipose tissue in girls before and during puberty. Eur J Obs Gynaecol Reprod Biol [Internet]. 2008; 136(2):210–4. Available from: pmid: 17137703 DOI: 10.1016/ j. ejogrb. 2006.10.028
2. Kasa-Vubu JZ, Ye W, Borer KT, Rosenthal A, Meckmongkol T. Twenty-four hour growth hormone and leptin secretion in active postpubertal adolescent girls: Impact of fitness, fatness, and age at menarche [Internet]. Vol. 91, J. Clin. Endocrinol. Metab. 2006. p. 3935–40. Available from: doi: 10. 1210/jc. 2005-2841
3. Zhang K, Pollack S, Ghods A, Dicken C, Isaac B, Adel G, et al. Onset of ovulation after menarche in girls: A longitudinal study [Internet]. Vol. 93, J. Clin. Endocrinol. Metab. 2008. p. 1186–94. Available from: doi: 10.1210/jc.2007-1846. Epub 2008 Feb 5.
4. Mounir GM, El-Sayed N a, Mahdy NH, Khamis SE. Nutritional factors a ecting the menarcheal state of adolescent school girls in Alexandria. J Egypt Public Heal Assoc. 2007;82:239–60.
5. Fujii K, Demura S. Relationship between change in BMI with age and delayed menarche in female athletes.J Physiol Anthropol Appl Human Sci [Internet]. 2003;22(2):97–104. Available from: http:// www.ncbi.nlm.nih.gov/pubmed/12672973
6. Marwaha RK, Tandon N, Ganie MA, Kanwar R, Shivaprasad C, Sabharwal A, et al. Nationwide reference data for height, weight and body mass index of Indian schoolchildren. Natl Med J India [Internet]. 2011;24(5):269–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22680077
7. Garcia-Mayor R V, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum Leptin Levels in Normal Children : Relationship to Age , Gender , Body Mass Index , Pituitary-Gonadal. J Clin Endocrinol Metab [Internet]. 1997;82(9):2849–55. Available from: http://jcem.endojournals.org/content/82/9/2849.full.pdf+html
8. Dubey S, Kabra M, Bajpai A, Pandey RM, Hasan M, Gautam RK, et al. Serum leptin levels in obese Indian children relation to clinical and biochemical parameters. Indian Pediatr [Internet]. 2007; 44(4): 257–62. Available from: http://www.ncbi.nlm.nih.gov/ pubmed/17468520
9. Venner AA, Lyon ME, Doyle-Baker PK. Leptin: A potential biomarker for childhood obesity? Clin Biochem. 2006;39(11):1047–56.
10. Considine RV, Sinha MK, Heiman ML et al. Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans. N Eng J Med [Internet]. 1996;334(5):292–295. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00019616-199607000-00020
11. Mclntosh, N., Helms, P.J. and Smyth R. Physical growth and development, In, Forfar and Arneil's Textbook of Pediatrics, 6e_ 9780443071928_ Medicine & Health Science Books @ Sixth edition. Churchill Livestone, Elsevier limited. Spain Amazon 458-567. 2003. p. 458–567.
12. Ersoy B, Balkan C, Gunay T, Egemen A. The factors a ecting the relation between the menar-cheal age of mother and daughter. Child Care Heal Dev. 2005; 31(3):303–8.
13. Harris MA, Harris MA, Sc M, Prior JC, Koehoorn M, Ph D. Age at Menarche in the Canadian Population: Secular Trends and Relationship to Adulthood BMI. J Adolesc Heal [Internet]. Elsevier Ltd; 2008; 43: 548–554. Available from: http://dx.doi.org/10.1016/j.jadohealth.2008.07.017
14. Mushtaq MU, Gull S, Mushtaq K, Abdullah HM, Khurshid U, Shahid U, et al. Height, weight and BMI percentiles and nutritional status relative to the international growth references among Pakistani school-aged children. BMC Pediatr [Internet]. BioMed Central Ltd; 2012;12(1):31. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3337223&tool=pmcentrez&rendertype=abstract
15. de Onis M, Garza C, Onyango AW, Rolland-Cachera MF. WHO growth standards for infants and young children. Arch Pediatr [Internet]. 2009;16(1):47–53. Available from: pmid: 19036567 DOI: 10.1016/j.arcped.2008.10.010
16. Chow VTK, Phoon MC. Measurement of serum leptin concentrations in university undergraduates by competitive ELISA reveals correlations with body mass index and sex. Adv Physiol Educ. 2003; 27(1–4):70–7.
17. Xi H, Zhang L, Guo Z ZL. Serum leptin concentration and its e ect on puberty in Naqu Tibetan adolescents. J Physiol Anthr. 2011;30(3):111–7.
18. Kuzawa CW, Quinn EA, Adair LS. Leptin in a lean population of Filipino adolescents. Am J Phys Anthr. 2007;132(4):642–9.
19. Carlsson B, Ankarberg C, Rosberg S, Norjavaara E, Albertsson-Wikland K, Carlsson LMS. Serum leptin concentrations in relation to pubertal development. Arch Dis Child [Internet]. 1997; 77(5):396–400. Available from: http://adc.bmj.com/content/77/5/396.abstract
20. Horlick MB, Rosenbaum M, Nicolson M, Levine LS,

Fedun B, Wang J, et al. E ect of puberty on the relationship between circulating leptin and body composition. J Clin Endocrinol Metab. 2000; 85(7): 2509–18.

21. Poveda E., Callas NE., Baracaldo CM., Castillo C., Hernández P. Leptin levels in school age children associated with anthropometric measurements and lipid profiles. Biomedica [Internet]. 2007; 27(4): 505–14. Available from: http://www.scopus. com/inward/record.url?eid=2-s2.0-43649108896 & partnerID=40&md5=9bb1cd926c9ea70fbcab412f3986c46b
22. Bandini LG, Must A, Naumova EN, Anderson SE, Caprio S, Spadano-Gasbarro JL, et al. Change in leptin, body composition and other hormones around menarche - A visual representation. Acta Paediatr. 2008;97(10):1454–9.
23. Kelly AM, Shaw NJ, Thomas AMC, Pynsent PB, Baker DJ. Growth of Pakistani children in relation to the 1990 growth standards. Arch Dis Child. 1997;77:401–5.
24. Onyango AW, Onis M De, Caroli M, Shah U, Sguassero Y, Redondo N, et al. Field-Testing the WHO Child Growth Standards in Four Countries 1. J Nutr. 2007;137(3):149–52.
25. Aziz S, Noor W ul A, Majeed R, Amanullah Khan M, Qayum I, Ahmed I, et al. Growth centile charts (Anthropometric measurement) of Pakistani pediatric population. Vol. 62, J Pak Med Associ. 2012. p. 367–77.
26. Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab. 1997; 82(9): 2904–10.
27. Brandão CMA, Lombardi MT, Nishida SK, Hauache OM, Vieira JGH. Serum leptin concen-tration during puberty in healthy non-obese adole-scents. Braz J Med Biol Res. 2003; 36(10): 1293–6.
28. Wang T, Morioka I, Gowa Y, Igarashi Y, Miyai N, Yamamoto H, et al. Serum leptin levels in healthy adolescents: E ects of gender and growth. Env Heal Prev Med. 2004;9(2):41–6.
29. Zhu HJ1, Pan H, Zhang DX, Wu QY, Zhang K, Li M, Gong FY, Wu XY, Deng JY SY. [E ect of body weight on the onset of puberty of female children and adolescents]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2010;32(1):25–28.
30. Thomas T, Burguera B, Melton LJ, Atkinson EJ, O'Fallon WM, Riggs BL, et al. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. Bone. 2001; 29(2): 114–20.
31. Di Stefano G, Bini V, Papi F, Celi F, Contessa G, Berioli MG, et al. Leptin serum concentrations predict the responsiveness of obese children and adolescents to weight excess reduction program. Int J Obes Relat Metab Disord [Internet]. 2000; 24(12): 1586–91. Available from: http://www.ncbi.nlm. nih.gov/pubmed/11126210