

## Original Article

## DETERMINATION OF AGE OF PUBERTY USING LEPTIN LEVEL IN MALE POPULATION

Ahmad Raza Khan, Memona Yasmin, Khalid Mahmood, Muhammad Abaidullah and Naveera Ahmed

**Objective:** Innovating puberty analysis via utilization of leptin as a biomarker to revolutionize existent archetype prototype paragon

**Methods:** It was a cross sectional study based on pubescence markers utilized as landmark criterion. Healthy volunteer males of disparate maturescens were selected. Their physical maturity was substantiated via gold standard tanner staging and correlative algorithm was established with serum leptin levels. Sample size calculative of 150 was collected.

**Results:** Leptin levels dependent on age groups were stratified. These leptin level ranged from a minimum pre-pubertal level of 0.174 ng/ml to maximum 7.704 ng/ml at puberty initiation, which exhibited a peak uprise of the leptin mean value of 3.57 ng/ml  $\pm$  Std. Dev. 1.608 ng/ml, during the puberty startup averaging up to 12.5 years, with a progressive decline from mid-puberty to post-puberty age represented by the fall in leptin level from 2.539 ng/ml to 0.174 ng/ml respectively, with a mean value of 1.021 ng/ml  $\pm$  0.611 ng/ml

**Conclusions:** Leptin incentivized study was in pursuance of establishing a correlation between leptin in regards with juvenility along with evaluating range of leptin levels at which the maturity is initiated. The prospective study was deployed via utilizing pinnacle paradigm of tanner staging supplemented by leptin analysis. Data obtained was analyzed statistically for computation of puberty attainment age. Epitome of research conclusively analyzed a correlation between serum leptin and puberty initiation and an incontrovertible definitive leptin level range to assess the maturescence staging from initiation to complete adulthood.

**Keywords:** puberty, leptin, tanner, age of majority, forensic

### Introduction

Maturescence into adulthood is a multisystem web of complex yet organized programmed pattern dependent on various physiological processes that once initiated at a particular time results in a cascade of events, generating sexual maturity.<sup>1</sup> The decisive time for sexual dimorphism is dependent on various prerequisites. The main initiation signal for the body to go into such transformation is thermogenesis. When the balance tilts in the favor of increased heat production in comparison to at which level heat is dissipated, is when the body knows it is time to develop sexual organs to its full functional level.<sup>2</sup> The second triggering feature of adipose tissue other than heat is hormone generated known as leptin.<sup>3</sup> Positive balance of the heat production signals the body to produce enough leptin to play triggering role in puberty initiation. Leptin is governed by multifactorial array of BMI, dexamethasone, insulin, NPY and T3. Cascading out the map of attainment of puberty has so far been traced up to trigger/permissive role of leptin. In pre-pubertal phase, leptin remains in the bound state to its receptors throughout the

body. However, metabolic rates decline, signal the detachment of the leptin from its receptors in brain and subsequently from soluble receptors throughout the body. Hence, just at puberty initiation, surge of free form of leptin triggers the puberty process.

Finally coming to the legal aspect of the sexual maturity i.e. time of all sorts of experimentation under the multiple hormonal siege. A behavioral pattern trend studied by Peper and Dahl elaborates on the urges and surges of hormones having variable outcome mostly not positively driven.<sup>4</sup> This leads to juvenile criminological behavior resultantly producing sexual deviants and aggressive attitude personalities. This results in explicit extrovert behavior such as dacoity, rape, aggression and hostility towards society, leading to violence and disturbance of communal peace. A study conducted by Fakhurnissa Talpur et al. narrates multiple influences on the youngsters leading them astray dependent upon the social factors, domination of peer impact and domestic crisis.<sup>5</sup> Hence the study outcome and its benefits would include suggested proposal of reduction in legal age of majority to implicate the threat of punishment as an effective

tool to curb the violence in society, rather than to apply the actual penalty after the unrest being created which itself is a source of disturbance. Statistical details exhibit the number of juvenile delinquents in Punjab jails in a research project by Khalid Mahmood and Mohammad Asghar Cheema. It analyses the causative factors resultantly producing unharnessed adolescent offenders.<sup>6</sup>

## Methods

Sample size calculated according to the statistical formula was 160. This sample size was divided into 4 groups of different age ranges as shown in table 1. The male subjects were categorized in 4 groups as follows:

**Table-1:** Age stratification according to puberty range.

Groups	Age	Blood Sample
Group 1 (pre-pubertal)	9-11 years	3-5cc
Group 2 (initiation of puberty)	11.5-13 years	3-5cc
Group 3 (mid-puberty)	13.1-1 years	3-5cc
Group 4 (post-puberty)	15.1-18 years	3-5cc

The total number of 160 subjects included pre-pubescent, developing teenage, along with fully mature boys with an age range of 9-18 years.

Proper, valid and informed consent was taken from the parents/ guardians of subjects of age groups 1, 2 and 3 for the tanner staging and withdrawal of the venous blood. Informed consent of major subjects was obtained from themselves after elaborate information. All the major and consenting participants, willingly and actively cooperated in sample collection activity. None of the individuals had any chronic or debilitating disease.

Blood samples were collected from group 1, 2 and 3 from three schools of district Kasur and group 4 blood samples were collected from different individuals from Lahore. 5cc blood was withdrawn from vein in cubital fossa, of age ranging from 9-18 year old boys, in the disposable syringes, by the expert medical officer. The procedure was thoroughly elucidated regarding sample collection and likely risks accompanying it along with purpose of entire activity. Blood was preserved in the EDTA vacutainer tubes, stored with ice packs, maintaining the cold chain till transport to the laboratory.

Comprehensive global precautionary measures were taken, including alcohol swab use on skin, aseptic disposable syringes, with prompt dispensation of the used material in the disposal

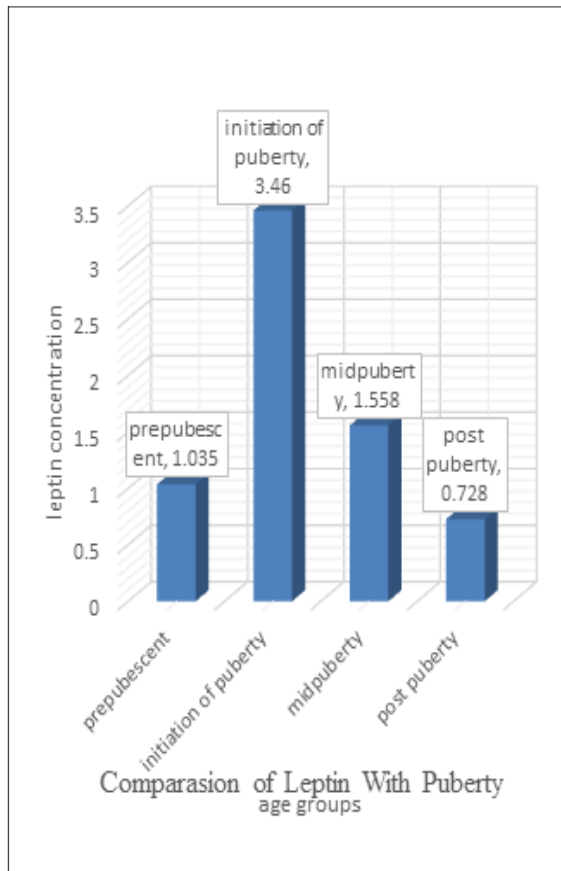
containers. Upon arrival in the laboratory in the department of Forensic Sciences, UHS, Lahore, the blood samples were centrifuged at 1000 rpm and stored at -20°C. Later ELISA was performed for serum leptin analysis.

A detailed biodata was collected on a proforma which stated the name, age and tanner staging of male subjects.

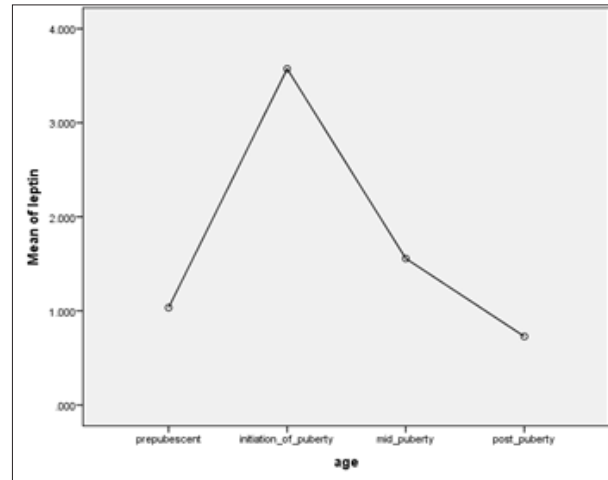
## Results

Three schools were visited and 120 samples were collected from District Kasur and 14 samples were collected randomly from District Lahore. Random samples were taken from age 9-18 years and later the data was arranged as per tanner stages. 46 candidates were recorded in age group of 9-11 years, 51 in age group of 11.1-13 years and data of 14 was recorded in age group of 13.1-15 years and finally 23 candidates were in age group of 15.1-18 years. Later on according to tanner stages 70 sorted out as tanner 1, 18 as tanner 2, 12 each as tanner 3 & 4 and 22 as tanner 5. These groups were stratified as pre-pubescent, initiation of puberty, mid-puberty and post-puberty, which included 70, 30, 12 and 22 candidates respectively. In our cross sectional research project, boys of age ranging from 9 years up to 18 years in varying tanner stages were evaluated as per their physical and sexual developmental levels by criterion of pubic, axillary and face hair as secondary sexual markers. Penile length and testicular size were measured as genitalia characteristics. Pileouspuberal growth was one of the two pathways chosen for tanner staging, the other being the testicular volume measure. Some candidates exhibited spurt of scanty pubic hair initially without TV more than 3 ml, while others presented with a simultaneous virilization, i.e. nascent growth of pubic hair and increase in TV ranging from 4 ml to 8 ml initially ranging its way up from 12 ml to a maximum of 20 ml. Pre-pubescent age group according to tanner staging ranged from 9 years minimum up to maximum age of 14 years, with mean age of  $10.764 \pm \text{Std. Dev. of } 1.3125$ . Puberty initiation fell within the range of 9-16 years of age according to tanner assessment with mean age of  $12.533 \pm \text{Std. Dev. } 1.5916$ . Mid-puberty group was within range of 12-18 years, mean of which was  $15.5 \pm \text{Std. Dev. } 1.9188$ . The last group of post-puberty fell within the range of minimum 12.5-18 years maximum, with mean of  $16.523 \pm \text{Std. Dev. } 1.8158$ . Leptin levels dependent on age groups were stratified. These leptin level ranged from a minimum pre-pubertal level of 0.174 ng/ml to maximum 7.704 ng/ml at puberty initiation, which exhibited a peak

upraise of the leptin mean value of  $3.57 \text{ ng/ml} \pm \text{Std. Dev. } 1.608 \text{ ng/ml}$ , during the puberty startup, with a progressive decline from mid-puberty to post-puberty age represented by the fall in leptin level from  $2.539 \text{ ng/ml}$  to  $0.174 \text{ ng/ml}$  respectively, with a mean value of  $1.021 \text{ ng/ml} \pm 0.611 \text{ ng/ml}$ . Comparative table in the statistical ANOVA displayed  $p = 0.000$  for comparing the means of prepubescent vs puberty initiation depicting the presence of significant difference between leptin levels which were  $1.035 \text{ ng/ml}$  pre-pubertal as compared to  $3.573 \text{ ng/ml}$  at the puberty initiation. Pre-pubertal vs post-pubertal levels exhibited a  $p = 0.451$ , with the resultant evaluation that no meaningful difference, exists between the mean values of these two groups which were  $1.035 \text{ ng/ml}$  pre-pubescence vs  $0.728 \text{ ng/ml}$  in the post-puberty phase. Similarly the pre-pubertal levels vs mid-puberty also had a  $p = 0.000$  showing a significant mean difference as  $1.035 \text{ ng/ml}$  pre-puberty vs  $1.558 \text{ ng/ml}$  in the mid-puberty age. Finally the mid-puberty vs post-puberty had a  $p = 0.036$  which also showed a valid difference among means of the groups respectively as  $1.558 \text{ ng/ml}$  vs  $0.728 \text{ ng/ml}$ .



**Fig-1:** Laptin concentration.



**Fig-2:** Means plot leptin association with puberty.

### Discussion

Human body is a pool of amazing combination of multiple genetic codes and epigenetic factors influencing the outcome. The transitional phase of metamorphosing into an adult capable of procreation is a cascade with has yet not be completely deciphered. However so far the code has been traced up to leptin, an ob gene product, playing a mandatory role in puberty attainment.<sup>7</sup> Leptin although present throughout life playing different role in human biochemistry is only enhanced in enough effective exertive level to take the body into pubescence upon the various markers including thermal signal, insulin, triiodothyronine, glucocorticoids and neuropeptide Y.<sup>8</sup> The cumulative effect of all these signals, detaches the plasma-protein-mopped up leptin along with increased leptin production by the body fat resultantly initiating the puberty cascade.

Sexual desirability is a significant milestone of puberty emergence. This sexuality can be objectified by four landmarks, namely, sexual desire, vivification, attitude and activity. This tends to comprehend the advancement of sex sempiternity of human evolution into a procreative being, spanning from pubescence to adulthood. It is an interconnected web of desire, awareness, interpretation and respondent functionality<sup>9</sup>. Pattern of crime in adolescence is called juvenile delinquency. Puberty brings along various physical and hormone driven alterations, which if uncontrolled ends up as delinquent behavior.

The covert alternating overt juvenile delinquent outcome among the young puberty attaining individuals is influenced by none other than peers, socio-economic factors as well as the electronic dilemma of internet, smart phones,

and easy availability of the cable television. Every society has its norms and legal legislature to keep the community in harmony of decree and justice. The statute law prevailing in Pakistan, categorizes age for the punitive actions against the criminal rebels. A child under 7 years is exempt of any criminal and legal obligation, as stated in the sec. 82 of P.P.C. The reason being a lack of intent, in the act, if any, committed by the child. However an individual between 7-12 years can be penalized for his deplorable deeds if the child realizes the lamentable consequences of his act, as per sec. 83 of P.P.C. Next age in the annexure is of prime concern, declared as juvenility most prone to delinquencies of almost every kind, time of stepping up the maturation process, experimenting every thought. This is the age which if not exposed to proper paternalism, may become contingent with permanent atrocious, nefarious criminality. Finally 18+ years are treated as adults for all capital punitive actions due for the committed crimes<sup>10</sup>. This is the legal practice in most of the countries with the exception of Saudi Arabia, Iran, Sudan and Yemen, where the legal age for capital punishment is 15 years as Shariat law.<sup>11</sup> Despite of the legislature which states that a juvenile is to be sent to correctional facility, around 800 minors are on death row in adult jails of Pakistan. If the heinous crime obligates the court to go beyond clemency and award prime punitive sentence to a minor, may be its time to revise the legal age for criminal responsibility<sup>12</sup>. Deterrence is to be conceptualized in a reformed way, to discourage an act via introducing a fear of consequence. If effective threat is imposed in society, cognizance of risk would be a factor enough for dissuasion.<sup>13</sup> Presently, offenders even if apprehended, rarely go through the process of conviction. The reason behind is a legal technicality being so very conveniently manipulated by the solicitors, to benefit their clients, that any age below 18 years, is exempt of punitive action. The lawyers very successfully orchestrate the legal loophole maneuvers, to maximally exploit the situation<sup>14</sup>. The inception of reasonable uncertainty is seeded during the criminal proceedings which are eventually engineered strategically for the acquittal of the client. Recidivism is the repercussion of the ironic statutory rulings of the honorable court. These impudent juveniles when unleashed upon the society become a hideous audacity, as they have the understanding that the law gives them, benefit of doubt of being underage. Subsequently these

unabashed delinquents become a malevolent element. This malignant element, ultimately is then uprooted, by extrajudicial killings, a most disapproved yet indispensable method which, still leaves behind the metastases<sup>15</sup>. Henceforth promulgation of the Sharia law is de rigueur, as it already states the age for capital crime is 15 years. Accountability needs to be liable, in the present scenario where “accidental hump” avows a secular decline in the age of pubescence from 18 years to 13 years. A pattern has emerged from mid-18th to 1900s, eliciting a 3 month decrease per decade in pubescence. 134 volunteer available ethnic individuals were taken for the research conducted by the author and his team. 70 candidates ranging from ages 9-14 years were sexually dormant, labelled as pre-pubescent. The next batch of 30 candidates exhibited puberty initiation signs, in the age range of 9-16 years.<sup>12</sup> Mid-puberty individuals were of age range of 12-18 years. Finally the post puberty group of 22 candidates showed a variation of age from 13-18 years.

The disequilibrium of the fluctuating data exhibited a non-linear regression correlation. It showed that a pre-pubescent child had an age range from no minimum to a maximum of 14 years. Our present research quantifies the statistics that boys as young as 9 years are experiencing the initiation of puberty, as substantiated by tanner stage 2, backed up by leptin level.

Ages 10-12 years fell in the range of tanner 2 and 3, with age 13 years exhibiting full attained puberty with tanner 5 staging and corresponding serum leptin levels. However variability of the data does not preclude possibility of early initiation in the population of Pakistan. ANOVA results also discern the distinctive peak of leptin at puberty initiation. Hence this inflating generation gap amidst adolescence and adulthood enhances the time span for escalation of risk taking behavior. This shall consequently cause an upsurge of the erratic attitude and illicit predilection<sup>15</sup>. It is imperative to state here that most of the puberty attainment procedures, follow the trend of tanner staging backed up by radiological examination. It exposes an individual to entire body radiological irradiation, for age estimation, in cases of violence including sexual assault. This obsolete health hazardous technique can very conveniently be replaced by leptin analysis especially where puberty attainment is under scrutiny. A 3-5cc venous blood can efficiently support the physical tanner examination for the puberty staging. Our study narrates the physical examination done as per tanner guidelines. This is further supported by the

serum leptin analysis showing marked correlation with Puberty initiation. This would be of much help in settling legal disputes of sexual assaults by categorizing the individual as to prepubescent or otherwise, capable of accrediting sexual act.

Hence the budding generation making a sexual debut at age of 12 years is definitely prone to vandalism, sexual experimentation besides sexual exploitation by other juveniles, and other risk taking behavior. Urbanization and industrialization has caused secular decline in the attainment of puberty<sup>16</sup>. This has consequently resulted in increase in “maturity gap”. The system needs to be revitalized by revolutionary amends in social and legal architecture. Finally it is better to be safe rather sorry for a situation that could have been handled more competently. It is about time for a long due reformatory effort to improvise the legal framework. The harmonious prevalence is dependent only on the effective and just society.

## Conclusion

In the conclusive summation, it is merely, not an

understatement to narrate that although prevalent finale outcome of puberty is by and large, apparently survived by the youth, with not much perilous results. This approach shall be cliché, addressing only the triviality of the profound scenario. The many facet juvenile delinquent behavior includes vandalism, aggression, violence and sexual offences including deviances. Introduction of leptin analysis in Forensic Medicine, is an ancillary accessory for detection of puberty initiation. Puberty process is spread over years. The biochemical analysis of serum leptin, can help assess the pubescence initiation mid-puberty phase, clearly demarcating these stages from pre-pubertal and post-puberty phases<sup>17</sup>. Therefore the medicolegal cases, being presented in the department of Forensic Medicine, in cognizance of sexual assault, can be processed by tanner staging supplemented by serum leptin analysis requiring only 3-5cc of blood.

*Department of Forensic Medicine  
SIMS/ Services Hospital, Lahore  
[www.esculapio.pk](http://www.esculapio.pk)*

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