ABSTRACT

Puberty is the critical period of human development, which results in sexual maturation and the ability to reproduce. At puberty, there is an increase in the concentration of gonadotropin releasing hormone (GnRH), which in turn causes an increase in the concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex steroids (Estradiol (E_2), progesterone). Furthermore, many factors like genetic characteristics, nutrition, socioeconomic status, stress and exercise are involved in determination of the timing of puberty. Any alteration in these factors results in disruption in normal span of pubertal development and leads to early or delayed onset of puberty. The specific aim of the present study was to determine the secular trends of normal pubertal onset and its relationship with demographic factors; etiological classification of pubertal disorders; and establish the genetic basis of pubertal disorders in females. The survey analysis for the onset of normal puberty was conducted in 1296 subjects. A total of 94 subjects with early and 105 with delayed puberty were recruited during study period. The genetic analysis was performed in 17 subjects with central precocious puberty (CPP), 52 with hypogonadotropic hypogonadism (HH) and 69 controls. The exon and exon-intron boundaries of *KISS1* and *KISS1R* genes were evaluated for CPP. Whereas, the genetic screening of exon and exon-intron boundaries of *KISS1*, *KISS1R*, *GNRH1*, *GNRHR*, *PROK2* and *PROKR2* genes were selected for HH. Genotyping was performed through direct Sanger sequencing. The downward secular trend of age at menarche (AAM) in Pakistani females residing in urban areas of Punjab was in accordance with the global pattern in developing countries. A highly significant decrease (P=0.0001) in the mean AAM was observed in the girls born during 2000s as compared to those born during 1990s and 1980s. The mean AAM of girls was significantly different between Central and North Punjab (P=0.01), upper lower and upper middle socioeconomic group (P=0.03), and Jutt and Mughal ethnicity (P=0.02). The etiological characterization of early puberty demonstrated that peripheral precocious puberty (PPP) (45.74 %) was the leading cause of early puberty followed by idiopathic CPP (18.09 %) in local population. The most common cause of pubertal delay in female residing in Punjab was HH (49.52%). The genetic analysis revealed that genotypes AG and GG of rs10407968 in exon 1 (P=0.0013) and AT of rs350132 in exon 5 (P=0.0002) of *KISS1R* gene were strongly associated with HH. The haplotype A-T of rs10407968-

rs350132 was protective against HH (P=0.033) and G-A was associated with HH (P=0.041). The SNP rs12998 in exon 2 and three SNPs (rs4889, rs71745629, rs35431622) in exon 3 of *KISS1* gene were identified in HH patients. However, none of them had a significant association (P>0.05) with HH. The haplotype G-C-A-A of rs12998-rs4889-rs71745629-rs35431622 appeared to be protective against HH (P=0.038). Genetic results of *GNRHR* indicated that rs4986942 in exon 1 had no association (P=0.40) with HH. The genotypes AG and AA of CM015157 in exon 3 (P=0.04) and novel mutations c.385G>A, p. Ala129Thr in exon 1 were associated with HH. Another synonymous novel mutation c.665T>C, p.221Hist= in exon 2 of *GNRHR* was also observed. The SNP rs1865 in exon 1 and a novel mutation (c.284_286del3) in exon 3 of *GNRH1* were associated with HH (P<0.05).

In conclusion, the present study demonstrates that downward trend of age at menarche in Pakistani females in accordance with the global pattern in developing countries. The main disorders of pubertal development are peripheral precocious puberty and hypogonadotropic hypogonadism (HH). The genetic polymorphism in *KISS1R* and *KISS1* was not associated with CPP in Pakistani Girls. However, the genetic polymorphism in *KISS1R*, *KISS1*, *GNRHR* and *GNRH1* genes were associated with HH in Pakistani females. Further studies in the local population are required to find the association of CPP and HH with other genes involved in development and migration of GnRH neurons and GnRH secretion or action.