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Plasma kisspeptin levels in male cases with hypogonadism

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Abstract. The hypothalamic hormone kisspeptin (metastin) regulates human reproduction by modulating gonadotropin-releasing hormone (GnRH) secretion. Kisspeptin is detected in peripheral blood, although GnRH is not. In this study, we measured plasma kisspeptin levels in four male cases with hypogonadism and seven normal male controls using enzyme immunoassay (EIA) to elucidate the clinical implications of kisspeptin levels in male hypogonadism. The results showed a variety of plasma kisspeptin levels: 6.0 fmol/ml in a male with isolated hypogonadotropic hypogonadism (IHH), 43.2 fmol/ml in a male with Kallmann’s syndrome, 40.7 fmol/ml in a male with azoospermia, 323.2 fmol/ml in a male with hypergonadotropic hypogonadism, and 12.3 ± 2.5 fmol/ml (mean ± SD) in seven normal controls. Except for the case with IHH, the plasma kisspeptin levels were elevated in the three cases with Kallmann’s syndrome, azoospermia, and hypergonadotropic hypogonadism. The reason why the three cases had high values was their lesions were downstream of the kisspeptin neuron in the hypothalamic-pituitary-gonadal axis, suggesting that elevated kisspeptin levels were implicated in hypothalamic kisspeptin secretion under decreased negative feedback of gonadal steroids. The result that the plasma kisspeptin levels were decreased by gonadotropin therapy in the case with Kallmann’s syndrome supported this hypothesis. In conclusion, to measure plasma kisspeptin levels could be useful for better understanding of male hypogonadism.

Key words: Kisspeptin, Metastin, Gonadotropin, Hypogonadism, Azoospermia

IN HUMAN reproduction, gonadotropin-releasing hormone (GnRH) plays an important role as a central regulator in the hypothalamus. GnRH regulates pituitary gonadotropin secretion, modulates gonadal steroid feedback, and brings about fertility [1]. Since GnRH is secreted from the hypothalamus and exerts physiological function mostly in the pituitary [2], GnRH is not detected in human peripheral blood [3].

In 2003, kisspeptin (metastin), which was the processed product of the KiSS-1 gene and an endogenous ligand of the G protein-coupled receptor GPR54 (also known as KISS1R), was discovered to be a critical gatekeeper of GnRH secretion in mice and humans [4-8]. Kisspeptin is closely related to the GnRH neuron which is colocalized with GPR54 transcripts in mouse hypothalamus [9]. As with GnRH, kisspeptin is detected in human peripheral blood using enzyme immunoassay (EIA) [3, 10-12]. In pregnant subjects and some cases of pancreatic cancer, the kisspeptin levels are elevated because it is expressed not only in the hypothalamus but also in the peripheral organs such as the placenta, pancreas, testis, liver, and small intestine [3, 7, 8]. On the other hand, the clinical implications of peripheral kisspeptin in hypogonadism have yet to be fully elucidated. There are some reports that the plasma kisspeptin are not elevated in cases with isolated hypogonadotropic hypogonadism (IHH) [3, 12]. In this study, we measured plasma kisspeptin levels in four male cases with hypogonadism in order to better understand the clinical implications of peripheral kisspeptin in hypogonadism.

Materials and Methods

This study was registered with the ethical committee of Fujieda Municipal General Hospital and UMIN Clinical Trials Registry with the registration numbers...
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Fujibyou 823 and 000012579, respectively. Each case and normal control received information on the clinical purposes of this study and gave their informed consent.

After collecting blood, the plasma fraction was rapidly separated by a centrifuge at 4°C and frozen at -20°C until use. Plasma kisspeptin concentrations were measured by previously established EIA [3]. Briefly, the EIA for plasma kisspeptin was specific and sensitive. Its detection limit was 0.52 fmol/ml and its coefficient of variance did not exceed 10%. EIA showed complete cross-reactivity with synthetic kisspeptin-13, -14, and -54, and no cross-reactivity with neuropeptide FF, neuropeptide AF, prolactin-releasing peptide or RFamide-related peptide-3 [3]. In human plasma, the molecular ratio of kisspeptin-54 and kisspeptin-13 and -14 was 1:3 using high performance liquid chromatography [4]. Size of testes was measured with an orchidometer (Accurate Surgical & Scientific Instruments Corp.). Hormone stimulation tests were performed by single intravenous administration with 100 μg of gonadorelin acetate (Mitsubishi Tanabe Pharma Corp.) as GnRH [13].

Case 1 was in his twenties. He consulted a hospital due to pubertal delay at age twenty-one. He received a hormone stimulation test, as described above, which slightly increased his luteinizing hormone (LH) from 0.1 to a peak of 1.3 mIU/ml and his follicle stimulating hormone (FSH) from 0.2 to 1.2 mIU/ml. He received 3000 IU of human chorionic gonadotropin (hCG) injection for five days which increased his testosterone from 0.28 to 1.44 ng/ml which was within normal range from 1.31 to 8.71 ng/ml [14]. He had normosmia without anatomic lesion in brain magnetic resonance imaging (MRI) (not shown). Therefore, he was diagnosed as IHH. His plasma kisspeptin levels are as shown in Table 1.

Case 2 was in his twenties. He consulted a hospital due to pubertal delay. At age eighteen, his testosterone of 0.09 ng/ml was low. He received hormone stimulation test which slightly increased his LH from 0.1 to a peak of 0.5 mIU/ml and his FSH from 0.2 to 1.9 mIU/ml. He had anosmia with hypoplasia of the olfactory bulb in MRI (not shown). He was diagnosed as Kallmann’s syndrome. Then he started to receive hormone therapy with 3000 IU of hCG and 150 IU of human menopausal gonadotropin (hMG) injection twice a week [1]. Sperm was observed in his seminal fluid one year later. After that, his plasma kisspeptin and sex hormone levels were measured after a withdrawal of the hormone therapy for a month and after resuming the therapy with 5000 IU of hCG and 150 IU of hMG as shown in Table 1.

Case 3 was in his thirties with normal secondary sexual development. He was diagnosed as azoospermia due to defect of spermatogenesis in his twenties. His testosterone was normal although his LH and FSH were elevated as shown in Table 1.

Case 4 was in his thirties. He had a height of 183 cm and lacked secondary sexual development. His LH and FSH were elevated although his testosterone was low as shown in Table 1. He was diagnosed as hypergonadotropic hypogonadism [1].

We collected blood samples from seven males with normal secondary sexual development as normal controls. The mean age of normal controls from 14 to 46 was 27.9 years old. Values of normal controls were

<table>
<thead>
<tr>
<th>Case No. treatment</th>
<th>BMI (kg/m²)</th>
<th>testis (ml)</th>
<th>kisspeptin (fmol/ml)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hCG baseline</td>
<td>22.0</td>
<td>6/6</td>
<td>6.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>3.9</td>
<td>0.1</td>
<td>0.3</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GnRH baseline</td>
<td>2.8</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak</td>
<td>6.3</td>
<td>1.3</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>baseline</td>
<td>19.3</td>
<td>6/6</td>
<td>43.2</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>hCG and hMG</td>
<td>22.0</td>
<td>0.1</td>
<td>2.1</td>
<td>3.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hCG and hMG</td>
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<td>0.1</td>
<td>3.2</td>
<td>7.38</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>baseline</td>
<td>25.0</td>
<td>12/12</td>
<td>40.7</td>
<td>15.7</td>
<td>41.0</td>
</tr>
<tr>
<td>4</td>
<td>controls</td>
<td>22.7 ± 4.9</td>
<td>22 ± 3</td>
<td>12.3 ± 2.5</td>
<td>5.1 ± 1.6</td>
<td>2.9 ± 0.9</td>
</tr>
</tbody>
</table>

*; injection twice a week, #; injection three times a week, values of seven controls were mean ± SD.
described as mean ± SD in Table 1.

**Results and Discussion**

Table 1 shows the body mass index (BMI), sizes of testes, plasma kisspeptin, and sexual hormones levels in four cases with hypogonadism and normal controls. The plasma kisspeptin levels varied widely among the four cases. The kisspeptin level of normal controls was 12.3 ± 2.5 fmol/ml (mean ± SD) which was consistent with a previous report [3].

In case 1 with IHH, his plasma kisspeptin of 6.0 fmol/ml at baseline was not elevated as compared with those of normal controls, which was consistent with a previous report [3]. His kisspeptin levels after administration of hCG for five days or GnRH were also low. IHH was due to deficiency of gonadotropins at the level of the hypothalamus [1]. In case 1, the lesion in the hypothalamus, the cause of which remains to be determined, might be involved in the relatively low plasma kisspeptin levels in the four cases with hypogonadism and normal controls since the kisspeptin neuron in arcuate nucleus co-expressing neurokinin B and/or dynorphin plays an important role as a GnRH pulse generator in mammals [5].

In case 2 with Kallmann’s syndrome, his plasma kisspeptin of 43.2 fmol/ml was high after a withdrawal of gonadotropin therapy. After we resumed the therapy, his testosterone levels increased to 3.72 and 7.38 ng/ml and his plasma kisspeptin levels decreased to 22.0 and 22.3 fmol/ml, respectively. Kallmann’s syndrome was caused by migrating defects of the GnRH neuron which was downstream of the kisspeptin neuron in the hypothalamus [9]. In case 2, his plasma kisspeptin levels were suggested to be influenced by the negative feedback of testosterone.

In case 3 with azoospermia, his plasma kisspeptin of 40.7 fmol/ml was elevated. Although his testosterone level was normal, his high levels of FSH and LH suggested insufficient gonadal steroid production associated with defect of spermatogenesis in his testis.

In case 4 with hypergonadotropic hypogonadism, his kisspeptin level of 323.2 fmol/ml was markedly elevated. His low testosterone level and small testes suggested testicular dysfunction of gonadal steroid production.

To summarize the results, the lesions of cases 2, 3 and 4 were downstream of the kisspeptin neuron, suggesting that their elevated kisspeptin levels were implicated in hypothalamic kisspeptin secretion under decreased negative feedback of gonadal steroids.

In conclusion, the plasma kisspeptin levels in male hypogonadism varied widely. To measure plasma kisspeptin levels could be useful for better understanding of male hypogonadism.

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**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

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