CLINICAL, HISTOLOGICAL AND CYTOGENIC OBSERVATIONS IN PURE GONADAL DYSGENESIS

By

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ABSTRACT

Clinical observations, gonadal histology and cytogenic studies are described in eight cases of pure gonadal dysgenesis. The general appearance of the patients was feminine. They had never menstruated spontaneously. Most cases showed eunuchoidal body proportions and absence of breast development. The external genitalia were infantile. All patients had the streak gonads which consisted of undifferentiated embryonic stroma without any germinal elements. The gonadotrophin level was normal or high. In four cases, apparently normal male karyotype and in three cases apparently normal female karyotype was found. In one case chromosomal mosaic XO/XX was encountered. No clinical or histological differences between patients with male and female karyotype were found, except for phallic enlargement which was present in three out of four cases with male chromosome complement.

«Pure gonadal dysgenesis» is a syndrome in phenotypic females with gonadal dysgenesis but without the somatic malformations seen in Turner’s syndrome. Cases of pure gonadal dysgenesis are not always classified as a separate clinico-pathological entity, which they should be, and are often presented in publications as cases of Turner’s syndrome.

Although not many cases have so far been reported it is possible to give the clinical characteristics of pure gonadal dysgenesis from the reports which have already been published, though the clinical diagnosis was not confirmed by morphological examination of the gonad in all of them – Pelà (1935),...
Albright et al. (1942), Varney et al. (1942), Dux et al. (1952), Søyer (1955), Greenblatt et al. (1956), Hauser et al. (1956), Kerkhof & Stolte (1956), Hoffenberg & Jackson (1957 a, 1957 b), Elliot et al. (1959), Sele & Trolle (1960), Teter (1960). The general appearance of the patients is always rather feminine. The patients are of average height or tall. Most cases have eunuchoidal proportions, but in some cases the body build is quite feminine. Axillary and pubic hair in some cases is poorly developed. The external genitalia are fairly normal and the uterus is smaller than normal. Usually no gonads can be palpated. On laparotomy, »streak« gonads are found, which microscopically consist of immature ovarian like stroma. The gonadotrophin levels are markedly elevated, although in rare cases they are within normal limits.

The cytogenic studies in »pure gonadal dysgenesis« in most cases revealed normal male or female karyotype. The apparently male chromosome pattern 46/XY was found by Harnden & Stewart (1959) and Stewart (1960), de Grouchy et al. (1960) and Netter et al. (1960), Barr & Carr (1961), case 24 of de la Chapelle (1962), Hartog (1963), Frasier et al. (1964). Court Brown et al. (1964), Armstrong (1964), Boczkowski, Philip & Teter (1964), Graham et al. (1964), Brogger & Strand (1965), Moszkowski et al. (1965), Kinch et al. (1965). The female karyotype 46/XX was found in cases 18 and 32 of Jacobs et al. (1961), cases 7 and 8 of Haddad (1962), cases 1 and 2 of Hauser (1963), Lozzi et al. (1964), Kincl et al. (1965). An abnormal chromosomal complement XO/XY was found by Judge et al. (1962). Jones et al. (1963) encountered an XO/XX chromosome mosaic in cases 2 and 12, an XO/XXD mosaic in case 7, and an XO/XY/XYY pattern in case 5. Vague et al. (1964) found an XX/XXX mosaic and an XO/XX mosaicism was found by Warren et al. (1964) and Sohval et al. (1964).

The frequency of the occurrence of pure gonadal dysgenesis seems to be greater than the number of published cases indicates. Since, however, pure gonadal dysgenesis does not co-exist with somatic malformations as seen in Turner’s syndrome, it is often not diagnosed.

The main purpose of this study which presents 8 cases of pure gonadal dysgenesis, is to define more fully the clinico-pathological syndrome called »pure gonadal dysgenesis«.

MATERIAL AND METHODS

265 patients with primary amenorrhea were examined in the Department of Endocrinology during 1951–1963. After seven cases with a syndrome of testicular feminization and two cases with ambiguous external genitalia had been excluded, all the remaining 256 cases were found to have female external genitalia and uterus. These cases were divided into the following two main groups. Subjects with short stature and those with average height or tall; the height of 155 cm (5 ft.) was regarded as being the border height – Court Brown & MacGregor (1962). Most cases with short
stature exhibit other characteristic somatic malformations and were classified as cases of Turner’s syndrome.

Cases with average height or tall were divided into two groups. The first one consisted of cases in which there was a local defect of the uterine mucosa which was incapable of responding to endogenous and exogenous stimulation; most of these cases were diagnosed as having tuberculosis of the genital tract. To the second group belonged cases in which primary amenorrhea was connected with absence or abnormal hormonal excretion. Following lapararhea and morphological analysis of the gonads in 30 such cases, they were classified into five groups: pure gonadal dysgenesis (streak gonads consist of undifferentiated stroma without any germinal elements) – twelve cases; mixed gonadal dysgenesis (undifferentiated streak gonad on one side and testis on the other, both situated abnormally) – two cases; syndrome of rudimentary testes (extremely small testicles with highly pronounced underdevelopment, situated abnormally) – two cases; unclassified – »pure« or »mixed« gonadal dysgenesis (fibrous streak on the one side and gonadal tumour on the other) – four cases; ovarian dysplasia (hypoplastic ovaries containing only a few primordial follicles) – ten cases.

The subjects of this report consist of eight patients with pure gonadal dysgenesis who were hospitalized again for the assessment of their chromosomal pattern and clinical conditions. The other four cases were not examined cytogenetically.

The cytogenic analysis was performed according to the method of Moorhead et al. (1960), i. e. on white blood cells in the peripheral blood, and in one case, the chromosomal pattern was also studied in the skin by the method of Philip (1963) – personal communication.

One case (no. 1) described in this paper, was published earlier (Boczkowski, Philip & Teter 1964).

RESULTS

The clinical characteristic of these patients are summarized in Table 1. The patients ranged in age between 18–36, when first seen. They were hospitalized because of primary amenorrhea and poor secondary female sex characteristics. None of the patients had menstruated and all except no. 6 had hot flushes. Substitution therapy with oestrogen and progesterone given in a cyclic fashion produced uterine bleeding which, however, never re-occurred spontaneously. Every patient who was questioned regarding her sexual life admitted to a complete lack or a marked lowering of her libido.

Biotypological examination

In biotypological examination seven cases showed typical features of pure gonadal dysgenesis. The height of these seven patients was 159 to 173 cm (5 ft. 2 in. to 5 ft. 7 in.). All of them had eunuchoidal body proportions, with a span exceeding their height and absent or prepubertal breasts (Figs. 1 and 2).

In case no. 6 the height was 155 cm (5 ft.). Despite dysgenetic gonads (Fig. 4) her body build was feminine and she had well formed breasts. This phenomenon could not be explained.
Table 1.
Clinical features of patients with pure gonadal dysgenesis.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Height Span (cm)</th>
<th>Breasts</th>
<th>Axillary and pubic hair</th>
<th>Clitoris</th>
<th>Gonadotrophin mouse units</th>
<th>17-ketosteroids mg/24 h</th>
<th>Percentage of Barr bodies</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. H. A.</td>
<td>36</td>
<td>173 180</td>
<td>Prepubertal</td>
<td>Scanty</td>
<td>Hyper-trophied</td>
<td>&gt; 200</td>
<td>12.4</td>
<td>0</td>
<td>46/XY*</td>
</tr>
<tr>
<td>2. W. M.</td>
<td>22</td>
<td>169 174</td>
<td>Absent</td>
<td>Absent</td>
<td>Hyper-trophied</td>
<td>&gt; 400</td>
<td>18.6</td>
<td>3</td>
<td>46/XY</td>
</tr>
<tr>
<td>3. B. R.</td>
<td>31</td>
<td>164 175 159 166</td>
<td>Prepubertal</td>
<td>Almost normal</td>
<td>Hyper-trophied</td>
<td>&gt; 100 &lt; 200</td>
<td>7.2</td>
<td>0</td>
<td>46/XY</td>
</tr>
<tr>
<td>4. S. A.</td>
<td>18</td>
<td>160 171</td>
<td>Absent</td>
<td>Scanty</td>
<td>Normal</td>
<td>&gt; 100</td>
<td>4.3</td>
<td>1</td>
<td>46/XY</td>
</tr>
<tr>
<td>5. S. J.</td>
<td>36</td>
<td>155 153</td>
<td>Absent</td>
<td>Scanty</td>
<td>Hypo-plastic</td>
<td>&gt; 200</td>
<td>13.4</td>
<td>25</td>
<td>46/XX</td>
</tr>
<tr>
<td>6. O. K.</td>
<td>30</td>
<td>164 174</td>
<td>Normal</td>
<td>Almost normal</td>
<td>Normal</td>
<td>&gt; 200</td>
<td>7.8</td>
<td>14</td>
<td>46/XX</td>
</tr>
<tr>
<td>7. M. A.</td>
<td>26</td>
<td>164 179</td>
<td>Absent</td>
<td>Absent</td>
<td>Normal</td>
<td>&gt; 14 &lt; 90</td>
<td>9.2**</td>
<td>12</td>
<td>46/XX</td>
</tr>
<tr>
<td>8. J. P.</td>
<td>35</td>
<td></td>
<td>Prepubertal</td>
<td>Scanty</td>
<td>Normal</td>
<td>&gt; 100</td>
<td>9.2</td>
<td>21</td>
<td>45/XO / 46/XX</td>
</tr>
</tbody>
</table>

* In this case an XY constitution was found both in the blood and skin.
** In this case oestrogen excretion in the urine was determined by the method of Brown (1955).
Oestrone – 4.5 g/24 h; 17β-Oestradiol – 3.3 g/24 h; Oestriol – 3.8 mg/24 h.
All data were obtained before substitution therapy.
Fig. 1. Case No. 2 Karyotype 46/XY. a) Note eunuchoidal body proportions, and absence of axillary and pubic hair. b) After hormonal therapy (oestrogens with progesterone given in a cyclic fashion). Note characteristic hyperchromatin around nipples.

The axillary and pubic hair in all cases was sparse or reduced, and absent in two cases.

Gynaecological examination

The external genitalia without exception were infantile. Three cases showed hypertrophy of the clitoris and scrotal appearance of labia majora (Fig. 2 b). In all of them the karyotype was 46/XY. In six cases, the labia minora were almost absent and were formed only in the upper third (Fig. 3). In all cases a narrow vagina with nonelastic walls was noted. The uteri were rudimentary or infantile and no gonads were palpable.

Hormonal assays

The gonadotrophin titers in all cases were either very high or within normal limits (Klinefelter-Dekanski method). Urinary 17-ketosteroid outputs (measured by the method of Dreker with the Zimmermann reaction) were within the upper limits of normal or were elevated (Table 1).
Fig. 2. Case No. 3. Karyotype 46/XY. a) Anterior view after hormonal therapy. b) Hypertrophied clitoris and rudimentary labia minora, which are thin and developed only in the upper third.

Cytological examination

Vaginal smears did not show any cyclic changes and were characterized by the presence of mainly intermediate and superficial cyanophilic cells, in some places folded and in others crowded in clusters. After oestrogen therapy, a moderate or even a marked oestrogenic effect was observed.

In two cases (no. 2 and no. 3) with 46/XY karyotype the androgenic type of clear intermediate cells was found.

Radiological examination

Six patients (nos. 1, 2, 4, 5, 7 and 8) had X-rays of the skeleton and urinary tracts. All cases showed osteoporosis and characteristic features of eunuchoidism, e.g. delayed epiphyseal fusion and elongation of the phalanges. Shortening of the fourth metatarsal and metacarpal bones was observed in cases no. 1, 2, and 5. In cases no. 2, 5, and 8 an abnormal angular shape of the proximal carpal bones was found. These abnormalities of the hand were not as pronounced as in the cases of Turner's syndrome described by Kosowicz (1959),
Fig. 3.
Case No. 6. Karyotype 46/XX. Note rudimentary labia minora which are thin and developed only in the upper part.

Fig. 4.
Case No. 6. Karyotype 46/XX. Microscopic picture of cortical part. Haematoxylin and eosin. Magn. 100 X.
van der Werff ten Bosch (1959). Finally in cases no. 2, 7 and 8, flattening of the knee joint epiphyses was observed. In all cases pyelography was normal.

**Laparotomy**

At laparotomy the uteri were found to be infantile, and the Fallopian tubes were long and thin. In the place normally occupied by ovaries rudimentary gonadal streaks were found.

**Histological examination**

Histological examination was performed on both gonads, except in case no. 3, in which biopsy was only performed on the right gonad. Morphologically the gonads appeared as fibrous streaks without any germinal elements and consisted mainly of a cortical part. The only difference, noticed in cases no. 1, 6 and 7 was that the cortical part was well developed and consisted of connective tissue with ovarian-like stroma (Fig. 5), while the others showed atrophy and hyalinization (Fig. 6). Finally in cases no. 1, 7 and 8 the medullary part, which in no instance was large, contained interstitial Leydig-like or theca-like cells (Table 2).

**Cytogenetical examination**

Sex chromatin pattern was negative in four patients. Sex chromatin could not be found in the oral mucosa cells in two patients, and in two cases only one to three per cent of the cells had a sex chromatin. In all these four

![Fig. 5. Case No. 7. Karyotype 46/XX. Section of the gonad: a) Well developed cortical part composed of connective tissue fibres. Note, wavy ovarian-like stroma. The so-called germinal epithelium is present in this section (top). b) Mesonephric tubules with a highly convoluted lumen are visible. Haematoxylin and eosin. Magn. 135 X.](image-url)
Fig. 6. Case No. 2. Karyotype 46/XY. Section of the gonad: a) Showing rudimentary ovarian-like cortical part. Haematoxylin and eosin. Magn. 100 X. b) Remnants of Wolfian ducts. Haematoxylin and eosin. Magn. 80 X.

Table 2.
Gonadal morphology of patients with pure gonadal dysgenesis.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cortical part</th>
<th>Interstitial cells in medullary part</th>
<th>Mesonephric remnants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well developed</td>
<td>Small clusters of Leydig-like cells</td>
<td>Many convoluted tubules</td>
</tr>
<tr>
<td>2</td>
<td>Atrophied, partially hyalinized</td>
<td>Absent</td>
<td>Small tubules</td>
</tr>
<tr>
<td>3*</td>
<td>Fibrous, partially hyalinized</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Fibrous</td>
<td>Absent</td>
<td>Small tubules</td>
</tr>
<tr>
<td>5</td>
<td>Atrophied, fibrous</td>
<td>Absent</td>
<td>Small tubules</td>
</tr>
<tr>
<td>6</td>
<td>Well developed</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Well developed</td>
<td>Small clusters of Leydig-like cells</td>
<td>Small tubules</td>
</tr>
<tr>
<td>8</td>
<td>Fibrous, partially hyalinized</td>
<td>Small nests of Leydig-like cells</td>
<td>Rather small tubules</td>
</tr>
</tbody>
</table>

* In this one case only one gonad was examined.
Table 3.
Results of chromosome counts, peripheral blood.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>No. of chromosomes</th>
<th>No. of cells counted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;44</td>
<td>44</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

* Chromosome counts from the skin.

patients a normal male karyotype 46/XY was found. In four patients the sex chromatin pattern was positive. Sex chromatin was found in 12 to 25 per cent of the cells. In three of these four patients normal female karyotype 46/XX was found, and in case no. 8 chromosomal mosaic XO/XX was encountered. In case no. 7 the low percentage of sex chromatin in the oral mucosa cells suggested the presence of chromosomal abnormalities in tissues other than the blood (Table 3).

In other cases no evidence of mosaicism or other chromosomal abnormalities were obtained. In case no. 1 and 3, two cells with 45 chromosomes were analyzed and showed no constant pattern. The missing chromosomes belonged to the group 19-20, 13-15 and 17-18, 6-12-X respectively. It was concluded that these were artifacts, which probably arose during the making of the preparations, possibly by heating. Two cells with 47 chromosomes in case no. 1 were also analyzed and showed no constant pattern. The extra chromosome in one cell belonged to group 13-15 and in the other, to group 6-12-X.

**DISCUSSION**

The aetiology of pure gonadal dysgenesis was suggested by Ezes (1949). He pointed out an analogy with the experiments of Jost (1947) on rabbits and Raynaud & Frilley (1947) on mice to explain the syndrome of pure gonadal dysgenesis. Gonadectomy in the early foetal state leads to a subject with a female phenotype which is independent of the sex chromosome constitution.

In considering sex determination and differentiation in cases of pure gonadal
dysgenesis, when examining the first link in this chain—chromosomes, it must be noted that in most cases chromosomal aberrations are not a cause of this syndrome. The finding of a normal karyotype in cases of pure gonadal dysgenesis differentiates the aetiology of the gonadal dysgenesis in this group of patients from the aetiology of dysgenetic gonads in Turner’s syndrome, in which in almost all cases, chromosomal abnormalities are present (de la Chapelle 1962; Lindsten 1963).

Since chromosomal aberrations are not a main cause of gonadal dysgenesis in pure gonadal dysgenesis, the question as to what factor or factors cause gonadal dysgenesis remains open. The underdevelopment of the gonads during foetal life may be the result of either a destruction of the genital ridge, or of failure of the primitive germ cells to reach the genital ridge (Everett 1945; Dantchakoff 1950; Heller & Jones 1964). In our cases it was not possible to ascertain the existence of a pathological factor which could conceivably have brought about these abnormalities.

We found no clinical or histological differences between cases with male and female karyotype, except for phallic enlargement which was present in three out of four cases with male chromosomal complement. In our previous report (Boczkowski, Philip & Teter 1964) case no. 1 was delineated as pure gonadal dysgenesis with phallic enlargement. To this group no. 2 and 3 may be added. Only four similar cases of pure gonadal dysgenesis with phallic enlargement have been reported. These are the chromatin negative case of Swyer (1955), the chromatin positive case of Greenblatt et al. (1956), the chromatin positive case, quoted by Grumbach et al. (1957) and case no. 2 of Hauser (1963) with karyotype 46/XX. Thus it can be concluded that it is not possible to link phallic enlargement with the presence of chromosome Y.

We wish to emphasize that the condition for the classification of pure gonadal dysgenesis should be a complete absence of follicles. Two of our cases, which are not included in this paper, and which on clinical grounds, were initially diagnosed as pure gonadal dysgenesis were re-classified following a histological examination of the gonads. In sections of the gonad an atretic follicle was found in one case. A few atretic follicles surrounded by nests of thecalutein cells were found in the second case. It is important that in both these cases spontaneous uterine bleeding occurred for only a short period. This finding is in accordance with our supposition—that several spontaneous episodes of uterine bleeding excludes gonadal dysgenesis and indicates the presence of hypoplastic ovaries with follicles. A review of cases of pure gonadal dysgenesis indicates that only in the cases of Greenblatt et al. (1956), Greenblatt (1958, p. 353) and Hoffenberg et al. (1957) was spontaneous uterine bleeding reported. The first case had only one episode of uterine bleeding and here the histological examination of the gonads revealed typical gonadal dysgenesis, while the remaining two cases showed primordial follicles.
In almost all of the reported cases of gonadal dysgenesis the external genitalia were described as normal. In all our cases, the external genitalia were infantile and in six cases there was an almost complete absence of labia minora which were formed only in their upper third. This characteristic development of only the upper part of the labia minora was also frequently found in other different cases of gonadal dysgenesis. Hence we consider this symptom as characteristic for abnormal sex determination and differentiation.

In all cases of pure gonadal dysgenesis reported here, only slight oestrogen deficiency or even a slight oestrogenic effect was found in the vaginal smears. We have never noticed such a hormonal pattern in Turner's syndrome, in which generalized atrophy of the vaginal epithelium is characteristic. The smears are usually scanty. Parabasal and a few small intermediate cells are present. The response of the vaginal epithelium to exogenous oestrogen stimulation in pure gonadal dysgenesis was good. A moderate or even a marked oestrogenic effect was obtained. It is impossible to obtain similar results in Turner's syndrome. After intensive oestrogen therapy we found only very little or a slight oestrogenic effect. This significant difference between pure gonadal dysgenesis and Turner's syndrome is probably one more sign of the different genetic background of these two entities.

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509

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