Serum AMH concentration as a marker evaluating gonadal function in boys operated on for unilateral cryptorchidism between 1st and 4th year of life

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Abstract The aim of this study was to measure the serum AMH (anti-Mullerian hormone) concentrations in a group of boys with or without cryptorchidism, evaluation of karyotypes, testicular position, morphology, and major length of the undescended testes. Fifty boys who were 1–4 years old (median = 2.4 years) with unilateral cryptorchidism were evaluated. All of them underwent karyotyping to exclude chromosomal abnormalities. Fifty healthy boys within the same age range (median = 2.1 years) admitted for planned inguinal hernia repair in 2010, served as controls. Blood samples were collected, while obtaining blood for standard laboratory tests routinely performed before the surgeries. Medians of AMH in boys with cryptorchidism were lower than in boys with inguinal hernia and differed significantly between two groups. Undescended testes were generally found in superficial inguinal pouch (n = 46), in two cases were noted to be in the external ring of the inguinal canal, and in another two instances, in the abdominal cavity. The major lengths of the undescended testes were smaller in comparison to the testes positioned normally (mean of 1 cm vs. a mean of 1.5 cm, respectively). In nine of the cases, the testes had turgor deficit, a drop shape, with epididymides that were small, dysplastic, and separated from the testis. The authors found that AMH was lower in boys with unilateral cryptorchidism (also found to have smaller testis) when compared with the control group.

Keywords Anti-Mullerian hormone · Cryptorchidism · Gonadal function · Orchidopexy · Testis descent

Introduction

The descend of testis from a temporary intra-abdominal site in fetal life to the permanent scrotal location after birth is crucial to spermatogenesis in mature testis. The etiology of undescended testis is still surrounded by much controversy. It is largely believed that disrupted endocrine regulation and several gene defects may play a role in the process [1]. Nevertheless, the first phase of typical testicular descent takes place between the 10th and 15th week of human gestation [2]. This occurrence is independent of androgen levels, as the process has been found to transpire in both animals and humans with complete androgen insensitivity and may also be controlled by anti-Mullerian hormone (AMH) and insulin-like hormone 3 (INSL3) [3–5]. INSL3 is secreted by the Leydig cells shortly after the onset of testicular development, and controls the thickening of the gubernaculum anchoring the testis to the inguinal region [6]. Disruption of the InsL3 gene in mice results in bilateral intra-abdominal testes [7, 8]. In humans, on the other hand, it was found that only 1.9% of the cases of cryptorchidism were caused by INSL3 gene mutations, and that the mutations of the INSL3 receptor on the whole, were uncommon [9, 10]. This is in accordance with the fact that intra-abdominal descended testes comprise only 5% of operations for undescended testes [11]. AMH, also named Mullerian inhibiting substance, is a tissue-specific TGF-beta superfamily growth factor. AMH is secreted by immature Sertoli cells during the 8th week of gestation, and is responsible for the regression of Mullerian ducts in the male fetus as part of the sexual differentiation process.
[12]. No extra gonadal AMH source has been found thus far. On the other hand, mutations in the gene for the ligand or the AMH receptor cause the persistent Mullerian duct syndrome in males, as well as the maldescent of the testis [13]. Conversely, other studies have shown that AMH receptor-deficient mice undergo normal testis descent [14].

With the onset of puberty and spermatogenesis in healthy men, the secretion of AMH declines corresponding to the maturation of the Sertoli cells [15, 16]. The second, or inguinoscrotal phase of testicular descent occurs between 26th and 40th weeks of gestation [2]. During this phase, the testis migrates through the inguinal canal and across the pubic region to the scrotum. The testis and epididymis then remain within the diverticulum of the peritoneum, that elongates within the elongating gubernaculum [17]. Furthermore, the gubernaculum growing out of the abdominal wall might be under the control of Hox genes—a group of genes that determines the basic structure and orientation of an organism. Disruption of some Hox genes in mice has been shown to lead to cryptorchidism, but the relevance of this observation is debatable in human studies of cryptorchidism [18]. There is much clinical evidence on the other hand, that shows reduced androgen action to be associated with undescended testes [19–21].

In this study, we measured the serum AMH concentrations in a group of boys with or without cryptorchidism. We evaluated karyotypes of these boys, as well as the position, morphology, and major length of the undescended testes.

Methods

Study population

Fifty boys aged 1–4 years (median = 2.4 years) with unilateral cryptorchidism, and without previous human chorionic gonadotropin treatment, were evaluated. All of them underwent orchidopexy in 2010. An abnormal karyotype, as well as the presence of any endocrine disorders, constituted grounds for exclusion from the study. Prior to their orchidopexy, all of the subjects had their karyotypes (to exclude chromosomal abnormalities) and AMH serum levels evaluated. During the actual orchidopexy, the position and morphology of the testes were evaluated, and their major length was measured.

Control group

Fifty healthy boys aged 1–4 years (median = 2.1 years) admitted to the Pediatric Surgery Department for planned inguinal hernia repairs in 2010, served as controls. All boys in the control group had their testes in the scrotum. Their karyotypes and AMH serum concentrations were also determined prior to their procedures.

Blood samples were drawn from antecubital vein. Blood was collected in tubes containing EDTA. The serum was separated from cells by centrifugation. Serum samples were made anonymous and stored at −20°C, until the hormonal analyses were performed. AMH was measured by a sensitive immunoassay (Immunotech, Beckman Coulter Ltd., Marseilles, France (A11893)). Assay sensitivity indicated by the manufacturer was 1 pM or 0.14 ng/ml and intra and interassay coefficients of variation (CV) were less than 12.3 and 14.2%, respectively. This immunoassay was a two-immunological-step sandwich type assay.

Data was collected from patients admitted to Pediatric Surgery Department for either a planned orchidopexy or hernia repair, and blood samples were drawn while obtaining blood for standard laboratory tests performed prior to the scheduled surgeries. All parents of the patients gave informed consent for both clinical and biochemical follow-up.

The study was approved by the local Ethics Committee as an audit of a clinically agreed-upon protocol of investigation and treatment.

The data is hereby shown as mean ± SD. Two-tail, Mann–Whitney U test was used consistently throughout the study. Correlations were analyzed using a Pearson test. The P values less than 0.05 were considered significant.

Results

There was no statistically significant difference in the age distribution of the two groups. All boys had karyotypes 46XY. Medians of AMH in boys with cryptorchidism were lower than in boys with inguinal hernia, and differed significantly—74.8 and 95.6 ng/ml, respectively. The two-tailed P value was 0.0042, and considered to be very significant. The 95% confidence interval for cryptorchidism was 67.9–86.5, while the 95% confidence interval for the inguinal hernia was 89.0 to 103.4. The standard error of mean (SD) was 18.66 in cryptorchidism, and 19.66 in inguinal hernia.

The undescended testes were generally found in the superficial inguinal pouch (n = 46), but in two of the instances were located in the external ring of inguinal canal, while two more subjects had theirs in the abdominal cavity. The major lengths of the undescended testes differed from 0.8 to 2 cm, and in most cases these glands were found to be smaller in comparison to the testes positioned normally (mean 1 cm and mean 1.5 cm, respectively, ±0.3SD). They also correlated with lower AMH concentration. In nine of the cases of cryptorchidism, the testes
had turgor deficit, a drop shape, and the epididymides were small, dysplastic, and separated from the testis.

Discussion

In healthy boys, there is a steep increase in circulating AMH concentrations during the first months of life. It is followed by a decline to a stable level until the time of puberty arrives, between Tanner stages II and III, where a renewed decline to another stable level through adolescence and adulthood is found [16]. Bearing in mind the aforementioned fact, we studied the population of boys with and without cryptorchidism, who were between 1 and 4 years old (Tanner stage I).

Serum AMH is known to be valuable in the evaluation of bilateral cryptorchidism and furthermore in assessing gonadal function [22, 23]. A measurable value in a boy with bilateral cryptorchidism is predictive of undescended testes, while an undetectable value is highly suggestive of anorchia [24]. Only Sertoli cells remain active during childhood, so the evaluation of gonadal function in the prepubertal male relies on the assessment of Sertoli cell products [15, 25–27].

Unilateral cryptorchidism carries an increased risk of infertility in adulthood. Up to 30% of men operated on in childhood for unilateral cryptorchidism are likely to be subfertile in later life [28–31]. Men who undergo an operation for bilateral cryptorchidism are more affected—up to 54% are infertile according to their semen and hormonal analysis [29, 32]. The position of the testes at the time of orchiopexy is also important. In fact, a lack of fertility has been reported in men who underwent bilateral abdominal orchiopexy in childhood [33]. Even though we evaluated boys with unilateral cryptorchidism, whose testes were positioned in most cases in inguinal pouch, the median serum AMH—a Sertoli cell marker evaluating gonadal function—was lower than in boys with both testes in the scrotum. According to Lukas-Croisier et al. [34] low serum AMH correlates with small testes. In our study, mean diameters of undescended testes were smaller in comparison to the normally developing ones (1 × 0.5 and 1.5 × 0.8 cm, respectively).

Testicular size and sperm density are positively correlated to germ-cell status in the cryptorchid testes in childhood [28, 35]. Lower serum AMH concentrations in otherwise healthy boys with cryptorchidism, who were compared with their age-matched counterparts with palpable testes, have also been reported in two previous studies [36, 37]. In contrast, Aksglaede et al. [38] did not find the difference in AMH concentrations between patients with Klinefelter Syndrome, with or without a history of cryptorchidism. The exception to this was noted in untreated patients, 10–14 years old, in whom the expected puberty decline in AMH tended to occur later than in the non-cryptorchid patients of the same age. Some authors argue that the negative correlation between testosterone and AMH suggests that androgens are responsible for AMH down-regulation [23]. The coexistence of high levels of androgens and AMH during fetal life and the first months after birth is thereby explained by the androgen insensitivity of Sertoli cells during this period, due to the lack of androgen receptor expression [39–42].

Conclusions

We found that serum AMH concentration—a Sertoli cell marker evaluating gonadal function—is lower in boys with cryptorchidism operated upon between the 1st and 4th year of life. This reflection of a Sertoli cell defect may subsequently be primary or secondary to testicular descent failure.

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Conflict of interest Authors do not declare any conflict of interest.

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