

Dynamic Changes in Serum Inhibin B Levels in 6- to 24-Month-Old Children Receiving Cryptorchidism Surgery

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Da Ma
Zhi-Guang Yao
Yan-Ping Guo
Rui-Fa Wu

Department of Pediatric General Surgery,
Dongguan Eighth People's Hospital
(Dongguan Children's Hospital),
Dongguan, 523000, People's Republic of
China

Objective: Cryptorchidism is the most common congenital disability and is defined as the absence of one or both testicles in the scrotum during fetal development. In patients with cryptorchidism, lowered serum inhibin B levels suggest testicular dysfunction. The practical application of serum inhibin B levels in childhood remains controversial. The purpose of this study was to observe the post-operational changes of serum inhibin B levels in patients with cryptorchidism and provide evidence that serum inhibin B is a potential marker for the efficacy of testicular surgery.

Methods: A total of 83 male children with cryptorchidism were enrolled in this study. All the children had blood samples taken by venipuncture on the day of surgery. The serum inhibin B levels in the blood samples were measured using an ELISA kit. Paired *t*-tests were used to assess differences between the groups.

Results: The results show that, compared with the preoperative values, there was a statistically significant increase in serum inhibin B values one month after the operation in each group ($P < 0.05$). Compared with the one-month post-operation results, there were no significant changes six months after operation in each group ($P > 0.05$). However, serum inhibin B values decreased significantly in all groups at 12 months post-operation ($P < 0.05$), suggesting that serum inhibin B values are significantly correlated with age. Correlation analysis showed that serum inhibin B was negatively correlated with age from 6 to 36 months, and the correlation coefficient was -0.488 .

Conclusion: Serum inhibin B can be used as a short-term evaluation index of cryptorchidism surgery rather than as a long-term indicator.

Keywords: serum inhibin B, children, cryptorchidism surgery

Introduction

Cryptorchidism is the most common congenital disability in males, occurring in 1.8–4.1% of full-term newborns.¹ The condition mainly affects mesenchymal cells, Sertoli cells, and spermatogenic cells in various stages of differentiation. It can cause testicular atrophy, malignant transformation, infertility, and sexual dysfunction in adulthood, thereby seriously affecting male reproductive health.²

Inhibins are heterodimeric protein hormones secreted by the Sertoli cells in the testis.³ They consist of dimers of two homologous subunits: inhibin A and inhibin B. In the testis before puberty, inhibin B is reported to be a circulating biomarker for monitoring the state of seminiferous epithelium because it is synthesized by Sertoli cells and/or germ cells.⁴ High inhibin B levels persist for longer periods than elevated

Correspondence: Da Ma
Department of Pediatric General Surgery,
Dongguan Eighth People's Hospital
(Dongguan Children's Hospital),
Dongguan, People's Republic of China
Tel/Fax +86 769-86186253
Email mada66_doc@163.com

follicle-stimulating hormones, luteinizing hormones, and testosterone levels.⁵ Many studies have emphasized that inhibin B may be a marker of testicular function. In patients with cryptorchidism, serum inhibin B levels are positively correlated with testicular function.^{6–8} Unfortunately, the practical application of inhibin B in childhood remains controversial.⁹ The purpose of this study was to observe the changes in serum inhibin B levels in patients with cryptorchidism before and after surgery and to provide an experimental basis for the effect of testicular surgery.

Patients and Methods

Patients

From October 2015 to February 2019, 83 children with cryptorchidism who were treated at the Dongguan Children's Hospital were enrolled in this study. All cases of pediatric cryptorchidism were diagnosed by physical examination and ultrasound examination in accordance with the diagnostic criteria. The children were aged between 6 and 24 months, with 32 aged 6–12 months, 27 aged 13–18 months, and 24 aged 19–24 months. There were 60 cases of unilateral cryptorchidism and 23 cases of bilateral cryptorchidism. A total of 49 of the children received traditional surgery, while 34 received laparoscopic surgery. The results are shown in Table 1.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Dongguan Children's Hospital. All examinations and procedures were performed with the informed consent of the child's relatives.

Methods

One day before the samples were taken, vigorous activity and medications that could affect hormone levels were forbidden. The blood sample was quickly collected on an empty stomach between 7:00am and 9:00am from the cubital vein (10.0 mL). Before usage, the serum was stored

at -20°C until use. The concentrations of inhibin B were measured using the enzyme-linked immunosorbent assay (ELISA kit, Immunotech, Beckman Coulter Ltd, Webster, USA) with an assay sensitivity of 3.0 pg/mL. Intra- and inter-assay coefficients of variation were 5.1% and 7.3%, respectively.

Statistical Analysis

For statistical analysis, two-tailed paired *t*-tests were used to assess differences between the groups, and correlation analysis was performed between age and serum inhibin B levels. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the Student's *t*-test function in bioconductor R package (version 3.5.1, <http://www.r-project.org>) as 2-tailed *t*-tests assuming unequal variances.

Results

Comparison of Preoperative and Postoperative Serum Inhibin B Levels in Different Age Groups

The results showed that each group's serum inhibin B values fluctuated greatly prior to surgery, with the highest value (258 pg/mL) in the 6–12-month group and the lowest value (167 pg/mL) in the 19–24-month group. Compared with the preoperative values, the serum inhibin B values of each group increased significantly one month after operation ($P < 0.01$ or $P < 0.05$). However, there was no statistically significant difference in the serum inhibin B values measured after 6 months in each group compared with the preoperative values. Although there was an increase in the serum inhibin B levels in the 6–12-month group, there was no statistically significant difference ($P = 0.065$) (Table 2).

Correlation Analysis Between Age and Serum Inhibin B Levels

Preliminary analysis of the results showed that serum inhibin B values were significantly correlated with age. According to the age of the children at the time of detection, serum inhibin B values were taken as abscissa and numerical values as longitudinal coordinates. Each child had four values measured for statistical analysis (preoperative, 1 month, 6 months, and 12 months after operation). The results showed that serum inhibin B levels were negatively correlated with age from 6 to 36 months; the correlation coefficient was -0.488 , with $P = 2.504 \times 10^{-11}$ (Figure 1).

Table 1 General Patient Information

Patient Age (Months)	6–12	13–18	19–24
Cryptorchidism type			
Unilateral cryptorchidism	22	20	18
Bilateral cryptorchidism	10	7	6
Surgical methods			
Laparoscopic surgery	15	16	18
Traditional surgery	17	11	6

Table 2 Comparison of Preoperative and Postoperative Inhibin B Levels in Different Age Groups

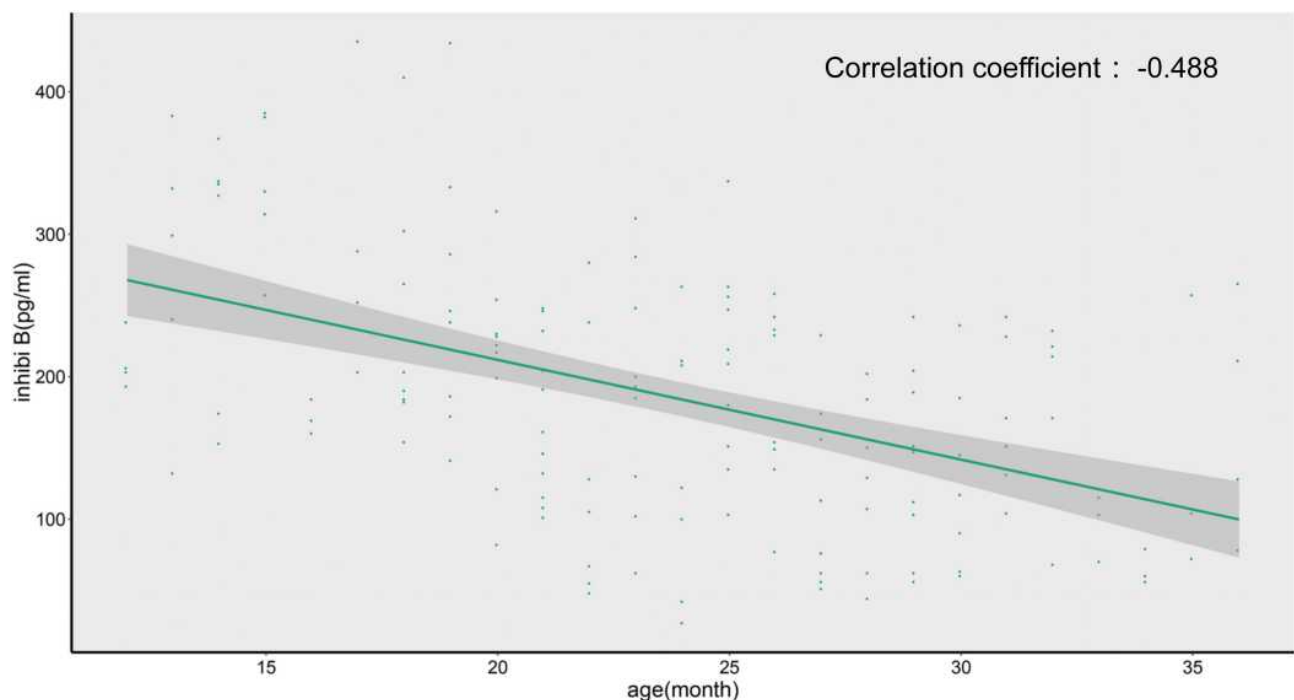
Age Group (Months)	Number of Patients	Preoperative Value (pg/mL)	1-Month Postoperative Value (pg/mL)	6-Month Postoperative Value (pg/mL)	12-Month Postoperative Value (pg/mL)
6–12	32	258±107	276±111**	269±85	169±75
13–18	27	203±109	209±106**	209±85	140±79
29–24	24	167±74	181±76*	168±56	147±71
6–24	83	215±105	226±107**	220±87	153±75

Notes: Compared with preoperative value, ** $p < 0.01$, * $p < 0.05$.

Discussion

Cryptorchidism is the most common sexual development abnormality in boys during childhood and puberty, and its incidence can increase in premature infants, especially if they also have a low birth weight.¹⁰ Cryptorchidism can be an isolated abnormality or associated with other anatomical changes of the male genitalia.¹¹ The exact pathogenesis of cryptorchidism is still not completely clear, and different causes lead to different degrees of testicular function and fertility damage. The descent of the testis to the scrotum is the result of a multifactorial process involving anatomical structure and hormonal function.¹¹ It is generally considered that the testicles initially form in the abdominal cavity and then descend through a two-stage process. In the first stage, from the 8th week to the 15th

week of the fetus, the testis is fixed on the internal inguinal ring by the gubernaculum.¹² The Leydig cytokine named insulin-like 3 (INSL3) and its receptor relaxin family peptide receptor 2 (RXFP2) are the main regulators. In addition, androgen regulated regression of the testis cranial suspensory ligament also plays a driving role.¹³ In the second stage, the testicles migrate from the internal inguinal ring to the scrotum, mainly due to androgens and intra-abdominal pressure. Therefore, prematurity is associated with a higher incidence of cryptorchidism, and spontaneous decline may still occur in the first few months after birth. Anti-Müllerian hormone (AMH) is also used to evaluate the condition of cryptorchidism patients. In boys with bilateral unpalpable testes, for example, AMH, can be detected in serum indicating the presence of testicular

**Figure 1** Correlation analysis between age and serum inhibin B levels.

tissue. Some treatment guidelines and clinical studies also recommend serum AMH detection for the differential diagnosis of cryptorchidism and inguinal hernia.¹⁴

Serum inhibin B is a heterodimer glycoprotein hormone that belongs to the growth factor superfamily and consists of an α subunit and a β subunit.⁴ It is mainly secreted by immature support cells in early childhood and is synthesized and secreted by supporting cells, spermatogenic cells, and mesenchymal cells in adulthood.¹⁵ Serum inhibin B in adolescent boys increases immediately after the increase of serum FSH, indicating that FSH can stimulate Sertoli cells.¹⁶ Prepubertal Sertoli cells can also produce serum inhibin B under the stimulation of human chorionic gonadotropin (HCG), but it cannot be produced in more mature Sertoli cells.¹⁷ A previous study found no significant difference in serum inhibin B levels between a cryptorchidism group and a control group at 6 months of age, indicating that the testicular support cell function of 6-month-old children with cryptorchidism had not been affected.¹⁸ However, serum inhibin B levels in children with cryptorchidism were significantly lower than those in the control group at 12 months of age, indicating that cryptorchidism had significantly damaged testicular support cells at 12 months of age. This supports the idea that early surgery is beneficial for the testicular development of these patients.^{19–21}

The present study investigated the serum inhibin B levels in children with cryptorchidism before corrective surgery and at 1 month, 6 months, and 12 months after the surgery. There was a statistically significant increase in serum inhibin B levels 1 month after cryptorchidism surgery, and the follow-up results at 6 months and 12 months showed decreases in serum inhibin B levels compared to the values 1 month after surgery. This suggests that serum inhibin B levels can be used to evaluate surgical outcomes 1 month after corrective surgery but that it is unsuitable as an evaluation index 6 months or 12 months after surgery. However, a study by Irkilata showed that the level of serum inhibin B increased significantly 6 months after surgery and that it can therefore be used as a follow-up parameter after orchiopexy as a marker, which differs from the conclusion of the present study: whereas Irkilata concluded that serum inhibin B can be used as a marker after 6 months, the present study concludes that it can only be used as a marker for 1 month (the data do not support that it can be used as a marker for 6 months or longer). This difference in conclusions could be due to the age and condition of the selected patients.

Sex hormones vary greatly between prepubertal boys and between individuals. Similarly, in children with

cryptorchidism, previous studies have reported that the serum inhibin B response after short-term HCG treatment varies at different ages, with the results showing that only prepubertal boys have an increased serum inhibin B response (older boys have no response or even a downward trend).¹⁷ In the present study, the correlation between the age of the patient and serum inhibin B values was analyzed in 83 children. The results showed that in children aged between 6 and 36 months, serum inhibin B levels were negatively correlated with age; the correlation coefficient was -0.488 . This suggests that there is a negative correlation between serum inhibin B and age and that even the correlation intensity may be weak. Serum inhibin B levels dynamically fluctuate from infancy to adolescence, which is enough to cover up the effect of surgery on serum inhibin B levels. Based on the results of the present study, it can be suggested that the impact of surgery on serum inhibin B levels can be masked by age-related changes in the levels. In this study, 60 cases of unilateral cryptorchidism and 23 cases of bilateral cryptorchidism were enrolled, of which 49 underwent traditional surgery and 34 underwent laparoscopic surgery. Stratified statistical analysis found no difference in clinical serum inhibin B values due to differences in surgical methods, nor were any differences identified in serum inhibin B values between unilateral and bilateral cryptorchidism, which may be due to the small sample size.

There are also limitations in this study, other hormone levels are not included in the statistics and analysis, such as AMH and testosterone, and the data of ultrasonic evaluation of patients' condition are not included. It is necessary for more detailed, systematic and comprehensive research work in the future.

Conclusion

In conclusion, there is a strong correlation between inhibin B and age in patients with cryptorchidism. Serum inhibin B can be used as a short-term evaluation index of the efficacy of cryptorchidism surgery, and the data of this study does not support that inhibin B can be used as a marker for long-term evaluation.

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Disclosure

The authors declare that they have no competing interests.

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