

A Prospective Observational Study to Evaluate the Change in Inhibin-B as a Marker of Sertoli Cell Function in Children Subjected to Surgical Correction for Undescended Testes

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Abstract

Background and Objectives: Undescended testes (UDT) or cryptorchidism is a common congenital disability characterised by the absence of at least one testicle from the scrotum. The primary aim of surgical correction is to preserve fertility potential and prevent complications including trauma, malignancy, hernia and torsion. Often, children, especially in developing countries, present late with UDT. The effect of surgical correction in the recovery of Sertoli cell function in children aged more than 2 years is not apparent. The present study was conducted to study the change in inhibin-B level as a marker of Sertoli cell function in surgically corrected UDT in a heterogeneous population. **Materials and Methods:** A prospective observational study conducted over a 3-year period at a tertiary care paediatric surgery centre recruited 76 children with UDT undergoing surgical correction. Inhibin-B as a marker for Sertoli cell function was studied preoperatively and postoperatively. Continuous variables were summarised by calculating mean, standard deviation, median and interquartile range (IQR). Quantile versus quantile plotting was done to assess the distribution of the data. Data were analysed in two groups, with participants aged <2 years (Group A) and more than 2 years (Group B). Wilcoxon signed-rank test was used to compare the pre-operative and post-operative value. **Results:** In Group A ($n = 39$), the median (IQR) of pre-operative inhibin-B was 181 pg/ml (148–254) and post-operative inhibin-B was 230 pg/ml (176–296). In Group B, the median (IQR) of pre-operative inhibin was 70 pg/ml (44–104) and post-operative inhibin was 102 pg/ml (46–176). There was a significant increase in post-operative inhibin when compared to the pre-operative inhibin ($P = 0.015$ and 0.012 , respectively, in Group A and B). Luteinizing hormone (LH) showed a significant decrease ($P = 0.002$) in Group A following surgery but bordering on significance in Group B ($P = 0.43$). On the other hand, follicle-stimulating hormone showed a significant decrease ($P < 0.01$) in Group B following surgery but not in Group A ($P = 0.87$). **Conclusion:** The mean post-operative inhibin-B levels were increased significantly as compared to the pre-operative levels indicating either a successful orchiopexy/adequate germ cell number or both. The benefit of orchiopexy may extend even to children presenting late for evaluation.

Keywords: Inhibin-B, orchiopexy, undescended testes

INTRODUCTION

Undescended testes (UDT) or cryptorchidism is a common congenital disability characterised by the absence of at least one testicle from the scrotum. The incidence varies from approximately 3% in full-term to 30% in premature male infants. Nearly 80% of cryptorchid testes descend by the 3rd month of life, making the actual incident approximately 1.2%.^[1]

The primary treatment modality of UDT is surgery, as hormonal therapy is not effective.^[2] The surgical correction is

recommended as soon as possible after 4 months of age and should be completed before 2 years of age.^[3,4]

The primary aim of surgical correction is to preserve fertility potential and prevent complications including trauma, malignancy, hernia and torsion. Men with a history of UDT

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have increased incidence of sub-fertility, manifest as lower sperm counts, sperm of more inferior quality and lower fertility rates.^[5,6]

Testicular biopsy and gonocytes and spermatogonia per tubular cross section or S/T ratio indicate the fertility potential in UDT.^[7,8] However, testicular biopsy is an invasive procedure with its share of complications. There are possible risks of the formation of anti-sperm antibodies against the normal testis. Inhibin-B is a hormone produced by the Sertoli cells and indicates Sertoli cell function and partly reflects the histological state of the seminiferous tubules.^[9] Basal and stimulated inhibin-B levels and inhibin-B/follicle-stimulating hormone (FSH) ratio can be helpful to evaluate Sertoli cell function in children <4 years of age following surgical correction of UDT.^[7,10]

There is a lack of studies from the Indian subcontinent on the fertility potential post-surgical correction of UDT. Often children, especially in developing countries, present late with UDT. The effect of surgical correction in the recovery of Sertoli cell function in children aged more than 2 years is not apparent. The present study was conducted to study the change in inhibin-B level as a marker of Sertoli cell function in surgically corrected UDT in a heterogeneous population.

MATERIALS AND METHODS

This prospective observational study was conducted at a tertiary care paediatric surgical centre from January 2018 to December 2020 (36 months). The study participants included all male patients younger than 10 years of age referred to our centre for unilateral or bilateral UDT. The UDT were confirmed by two separate paediatric surgeons on two different occasions. The following participants were excluded from the study:

1. Patients reporting for reoperation after a failed earlier surgery for UDT
2. Patients with disorders of sexual differentiation with UDT
3. Syndromic children or children with any chromosomal anomaly
4. Children on hormonal therapy
5. Patients with anorchia discovered during surgery
6. Patients with retractile testis
7. Patients refusing to be a part of this study.

Detailed history, including age at presentation, was taken. Pre-operative samples for luteinizing hormone (LH), FSH and inhibin-B were drawn. The participants were reviewed after an interval of at least 6 months post surgery as, by that time, recovery of Sertoli cell function was anticipated. Nomograms proposed by Kelsey *et al.* were used as a reference for S inhibin-B levels.^[11]

Five millilitre of venous blood was drawn during sample collection by venipuncture between 8 am and 11 am. The samples were labelled and stored at -80°C till the time of analysis. The sample was analysed as a batch using commercially available enzyme-linked immunosorbent assay kits from M/S Beckman

Coulter, UK. The lowest detectable range for inhibin was 5 pg/ml and for LH/FSH was 0.01 IU/L. The inter-assay and intra-assay coefficient of variation was <7%.

The data were analysed using SPSS ver. 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Continuous variables were summarised by calculating mean, standard deviation, median and IQR. Quantile versus quantile plotting was done to assess the distribution of the data. As inhibin-B, LH and FSH values were not distributed normally, Wilcoxon signed-rank test was used to compare the pre-operative and post-operative value. The data were analysed in two groups –Group A consisted of participants <2 years of age and Group B consisted of more than 2 years of age. This was done to decrease the confounding caused by the mini-puberty of infancy.

Formal sample size calculation was not done, as data for pre-operative and post-operative inhibin-B in the study population were not available. On average, 40 patients per year are referred to evaluate UDT at our paediatric surgery centre. All patients presenting during the study period were invited to participate in the study.

Ethical clearance was obtained from the institutional ethical committee, and the protocol was validated by the institutional scientific committee.

RESULTS

During the 3-year study period, a total of 110 children were evaluated at the Department of Paediatric surgery with the presentation of UDT. Eighty-eight children satisfied the inclusion criteria and were invited to participate in the study. Informed parental consent was obtained at the time of enrolment. Twelve children were lost to follow up. A total of 76 participants were analysed in the study, 39 participants in Group A and 37 participants in Group B [Figure 1].

The average age of the study participants was 36.54 months (median age: 24 months; range 6–98 months). The average time for review for repeat evaluation was 7.5 months (median time: 7 months; range: 6–18 months) [Table 1]. Twelve (15.8%) of the study participants had bilateral UDT, 28 (36.8%) had left-sided UDT and 36 (47.4%) had right-sided UDT.

Table 1: Patient statistics

	Group A	Group B	Total (Group A and B)
Number of participants	39	37	76
Mean age (months)	16.6	57.6	36.4
SD	5.82	19.07	24.8
Median age (months)	18	50	24
Minimum age (months)	6	30	6
Maximum age (months)	24	98	98

SD: Standard deviation

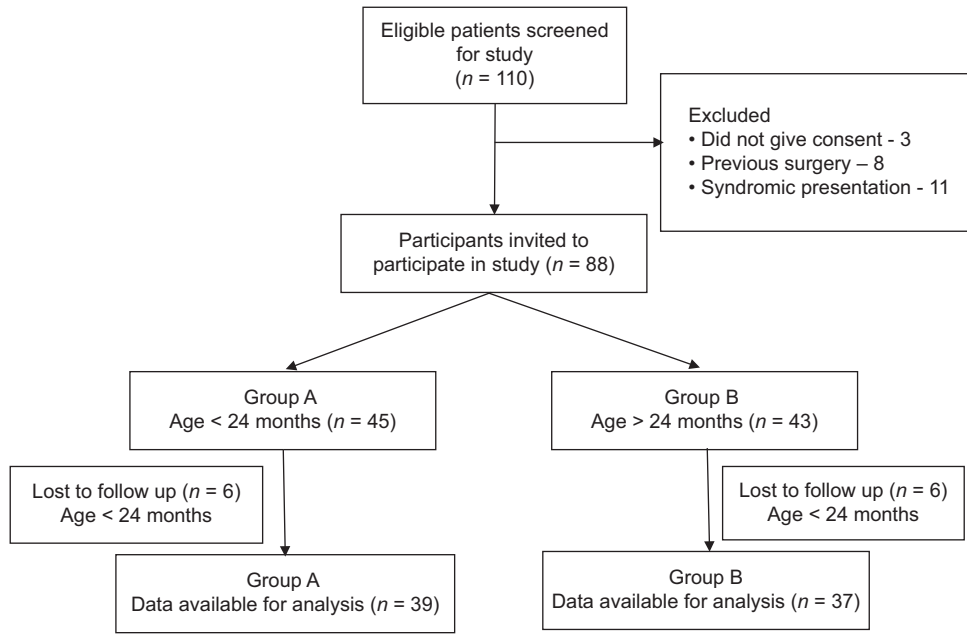


Figure 1: Flow diagram of the study

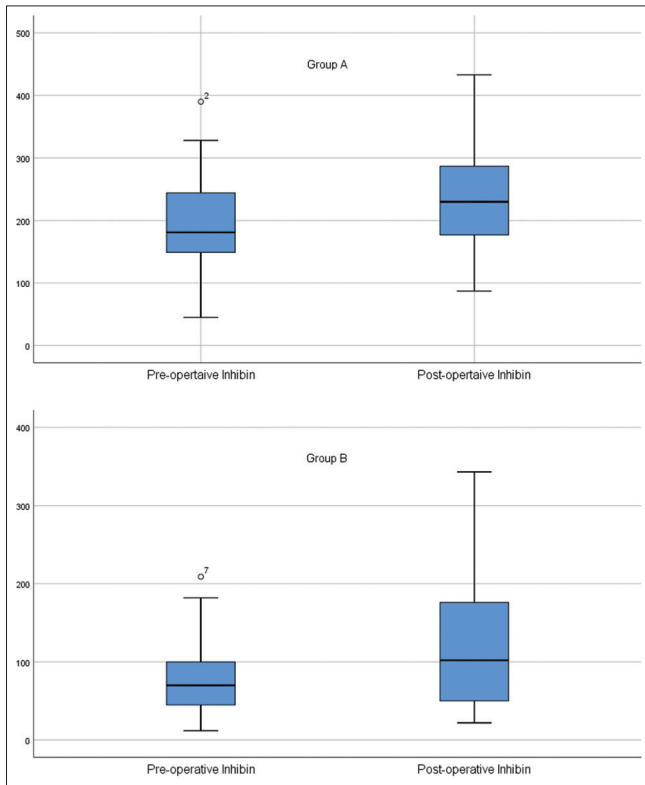


Figure 2: Pre-operative and post-operative inhibin-B levels

All patients had a successful post-operative outcome measured after 6 months with correctly positioned testes in respective hemi-scrotum and normal morphology. Post-operative complications in the form of wound infection were seen in 3 cases (4%) and hematoma in the scrotum was seen in two patients (2.6%). All complications could be managed successfully.

Table 2: Pre-operative and post-operative values of inhibin-B, luteinizing hormone and follicle-stimulating hormone

Parameter	Mean (SD)	P	Median (IQR)
Group A (n=39)			
Pre-operative inhibin (pg/ml)	195.82 (74.49)	0.015	181 (148-254)
Post-operative inhibin (pg/ml)	233 (84.14)		230 (176-296)
Pre-operative LH (IU/L)	0.43 (0.65)	0.002	0.07 (0.01-0.68)
Post-operative LH (IU/L)	0.37 (0.58)		0.09 (0.01-0.50)
Pre-operative FSH (IU/L)	1.03 (0.77)	0.87	0.98 (0.45-1.11)
Post-operative FSH (IU/L)	1.15 (1.01)		0.87 (0.34-1.96)
Group B (n=37)			
Pre-operative inhibin (pg/ml)	79.22 (46.83)	0.012	70 (44-104.5)
Post-operative inhibin (pg/ml)	118 (79.13)		102 (46-176)
Pre-operative LH (IU/L)	0.56 (0.88)	0.43	0.23 (0.03-0.66)
Post-operative LH (IU/L)	0.54 (0.91)		0.20 (0.05-0.50)
Pre-operative FSH (IU/L)	0.37 (0.40)	<0.01	0.12 (0.05-0.75)
Post-operative FSH (IU/L)	0.28 (0.59)		0.06 (0.01-0.23)

LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, SD: Standard deviation, IQR: Interquartile range

In Group A, the median (IQR) of pre-operative inhibin was 181 pg/ml (148–254) and post-operative inhibin was 230 pg/ml (176–296). In Group B, the median (IQR) of pre-operative inhibin was 70 pg/ml (44–104) and post-operative inhibin was 102 pg/ml (46–176) [Table 2]. Thus, there was a significant increase in post-operative inhibin when compared to the pre-operative inhibin in both groups [Figure 2].

In Group A, one (2.56%) participant had pre-operative inhibin <2.5th centile, which normalised postoperatively, and three participants (7.69%) had pre-operative inhibin less 10th centile, which normalised in two participants postoperatively. Out of the three participants who had inhibin-B value <10th centile

preoperatively, all improved with a value $>10^{\text{th}}$ centile. Another participant whose value was as expected in the pre-operative period dropped to $<10^{\text{th}}$ centile but remained above 2.5^{th} centile post surgery. In Group B, one (2.7%) of the participants had pre-operative inhibin $<2.5^{\text{th}}$ centile and six (16.21%) had pre-operative inhibin less 10^{th} centile. In the post-operative period, none of the participants had a value <2.5 centile and three participants had a value $<10^{\text{th}}$ centile. Out of six participants who had inhibin-B value $<10^{\text{th}}$ centile preoperatively, there were four patients who improved with a value $>10^{\text{th}}$ centile, and two continued to have similar value. One patient deteriorated with a value $<$ the 10^{th} centile.

LH showed a significant decrease ($P = 0.002$) in Group A following surgery, but bordering on significance in Group B ($P = 0.43$). On the other hand, FSH showed a significant decrease ($P < 0.01$) in Group B following surgery but not in Group A ($P = 0.87$) [Table 2].

DISCUSSION

We studied 76 children presenting with UDT and attempted to see the change in inhibin-B levels after surgical procedure. There was a significant increase in inhibin-B levels in both groups. We could also demonstrate that there was an improvement in percentile score of inhibin-B levels for respective ages. A study by Irkilata *et al.* showed a significant increase in inhibin-B levels in both early and late orchiopexy groups related to the testicular biopsy score. However, in their analysis, the effect of mini-puberty of infancy and the normative value for inhibin-B for respective age was not considered.^[12]

Another interesting finding was an increase in the inhibin-B levels and inhibin-B percentile scores in children older than 2 years of age at the time of surgery. The ideal time for surgical treatment of UDT is between 6 months and 2 years of age and not later.^[3,4] However, we could demonstrate that there may be some beneficial effect of surgery even if the child presents beyond 2 years with respect to Sertoli cell function.

The LH levels in normal male infants peak between the 2nd and 10th week of life and reach a prepubertal range by 4–6 months. Similarly, the FSH value also reaches a prepubertal range by 4 months of age.^[13,14] A value of LH and FSH above the prepubertal range indicates Sertoli and Leydig cell dysfunction in the developing gonads. A correction of UDT and recovery of Sertoli and Leydig cell function, therefore, should cause resolution of raised LH and FSH value. We could demonstrate a significant decrease in LH in both groups. However, FSH showed a significant reduction only in Group B but not in Group A. This finding may reiterate that early surgery may have a beneficial effect on the future potential of the gonads.

Biopsy of the testicular tissue has been used to predict fertility potential in individuals with UDT.^[9,15,16] However, in recent time, testicular biopsy has been associated with the formation

of anti-sperm antibodies.^[17] For this ethical concern, we did not subject our patients to testicular biopsy.

Chinya *et al.* demonstrated in 33 participants with palpable UDT that mean FSH and inhibin-B levels were higher in the bilateral as compared to the unilateral group. However, no such trends emerged in our study. In subgroup analysis, there was no difference in unilateral versus bilateral or palpable versus impalpable group.

Although the study has a limitation of a small sample size, the results do give a pattern of Inhibin-B in the two study groups. The average follow-up of 6 months may not be adequate to study the long-term fertility potential. The limitations of this study were also the lack of testicular biopsy and assessment of the testicular volume to complement the hormonal values of the patients. Further data and long-term fertility potential can be ascertained by longer follow-up of this cohort or similar children.

CONCLUSION

Surgical repair of UDT will increase the inhibin-B levels postoperatively, indicating either a successful orchiopexy/adequate germ cell number or both. The benefit of orchiopexy may extend even to children presenting late for evaluation.

To the best of our knowledge, this is the largest study to be carried out in the subcontinent on this subject. Further collaborative studies involving multiple high volume paediatric surgical centres and longer follow-up periods would help us obtain invaluable data and further our knowledge on this topic.

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Conflicts of interest

There are no conflicts of interest.

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