Could miRNAs be used as markers for distinguishing undescended testicles from retractile testicles

No NCT number assigned yet

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1. Study protochol

1.1.Sample size

This study is designed as a prospective controlled trial to compare miRNA changes across UDT, RT, and normal control groups. The sample size was determined via G*Power 3.1.9.6. A one-way ANOVA power analysis for miR-34c, which compared three groups, utilized an effect size (f=1.2955357) from a previous study [10]. To achieve 99% power and a 1% error rate, a total sample size of 24 was needed. To increase confidence, the study ultimately enrolled 30 patients, who were divided into three groups of 10.

1.2.Patient population

Initially, 10 boys with undescended testicles (UDTs), 10 with retractile testicles (RTs), and 10 healthy volunteers (controls) from our urology clinic (dates between March 2022 and March 2023) were enrolled. However, one RT patient and one control patient were excluded because of parental refusal for blood sampling. Only palpable (unilateral or bilateral) UDT cases were included, excluding nonpalpable types. To ensure accurate RT diagnosis, initial physician examinations were performed in three positions (supine, semisupine, standing), followed by a 1-month parental examination (twice daily). Only RT patients whose testicles spent >50% of their time in the scrotum were included. The exclusion criteria also included prior inguinal/scrotal surgery, defective datasets, or unsuitable serum samples.

1.3.Collection of patient data and control samples

Detailed patient histories, physical examinations, and routine biochemical tests were performed. UDT blood samples were collected presurgery to prevent misinterpretation. Blood from all the groups was collected in 5 mL biochemistry tubes and centrifuged at 3000 rpm for 10 minutes. The resulting

particle-free serum was transferred to 1.5 mL tubes and stored at -80°C until analysis.

1.4.Validation of the expression levels of miR-210, miR-449a and miR-34c via real-time PCR

Serum samples from the urology clinic were subjected to RNA isolation via the Quick-cfRNA™ Serum & Plasma Kit (Zymo Research). The extracted miRNA was reverse transcribed into cDNA via the miRNA All-In-One cDNA Synthesis Kit (ABMGood). cDNA quality and quantity were assessed spectrophotometrically with a BioSpec-nano instrument (Shimadzu) before real-time PCR. miRNA expression analysis was performed via a Rotor Gene Q (Qiagen) instrument. The levels of miR-210, miR-449a, and miR-34c were quantified with ready-to-use primers and BlasTaq™ 2X qPCR MasterMix (ABMGood).

1.5.Statistical analysis

Data normality was assessed via the Shapiro–Wilk test, and variance equality was assessed via Levene's test. Descriptive statistics are presented as the means ± SDs for parametric data and medians (IQRs) and means (ranks) for nonparametric data. For group comparisons, one-way ANOVA was used for parametric data, and the Kruskal–Wallis test was used for nonparametric data, followed by the Dunn–Bonferroni correction for post hoc multiple comparisons. Spearman's rank correlation coefficient was used to analyse correlations. Statistical significance was set at $p < 0.05$.

miRNA expression was quantified via qRT–PCR via Ct (crossing point) values and normalized to RNU6B_13. Relative miRNA expression levels were compared among all groups via the 2-ΔCt method and calculated as 2^{Ct}

$^{(\text{target gene}) - Ct (\text{reference gene})}$. All the statistical analyses were performed via IBM SPSS v28 (IBM Inc., Chicago, IL, USA).