***Original Research Article***

**Karyotype and Phenotypic Variability in Disorders of Sex Development Refining Diagnosis with a Novel Comprehensive DSD Classification Score CDCS**

**ABSTRACT**

**Introduction:** Disorders of Sex Development (DSD) encompass a heterogeneous group of congenital conditions affecting chromosomal, gonadal, and phenotypic sex, with an estimated incidence of 1 in 4,500 live births. Current DSD classification relies heavily on phenotypic and karyotypic categorization, often without a standardized grading system for clinical severity. The Comprehensive DSD Classification Score (CDCS) was developed as a novel scoring system integrating four key domains: external genitalia, internal reproductive structures, gonadal positioning/function, and chromosomal assessment.

 **Methods:** This study was designed as a retrospective cohort analysis. The cohort consisted of 70 pediatric patients diagnosed with Disorders of Sex Development (DSD), who underwent comprehensive clinical, laboratory, and imaging evaluations. The Comprehensive DSD Classification Score (CDCS) was designed as a quantitative, standardized scoring system (0–30 scale) integrating external genitalia appearance, internal reproductive structures, gonadal positioning/function, and chromosomal findings. Data were analyzed using SPSS (version 26.0) and GraphPad Prism (version 9.0). Predictive Accuracy of CDCS (ROC Curve Analysis): Receiver Operating Characteristic (ROC) curves evaluated CDCS as a predictor for surgical/hormonal interventions. Area Under the Curve (AUC) interpretation: AUC > 0.8 = Strong predictive ability. AUC 0.7–0.8 = Moderate predictive ability. AUC < 0.7 = Weak predictive ability.

 **Results:** The CDCS scoring system demonstrated strong inter-rater reliability, as measured by the Intraclass Correlation Coefficient (ICC = 0.82, 95% CI: 0.77–0.89). Independent scoring by two clinicians on 20 randomly selected cases showed high agreement, confirming CDCS as a reproducible classification tool.

**Conclusion:** Most DSD scoring systems are static, focusing only on initial diagnosis. CDCS is dynamic, allowing tracking of disease progression, endocrine response, and surgical/hormonal outcomes over time, and reassessment at puberty, guiding future reproductive and endocrine management.

**KEYWORDS:** DSD, karyotype and phenotypic variability, novel scoring system.

**INTRODUCTION**

 Disorders of Sex Development (DSD) encompass a heterogeneous group of congenital conditions affecting chromosomal, gonadal, and phenotypic sex, with an estimated incidence of 1 in 4,500 live births **(Hughes et al., 2006).** DSD classification and management remain complex due to wide phenotypic variability and the need for multidisciplinary evaluation. Current classification systems, such as the 2006 Chicago Consensus, categorize DSD primarily based on karyotype (46, XX, 46, XY, or sex chromosome DSD) without fully integrating clinical severity or prognostic value **(Lee et al., 2016 and Pasterski et al., 2010).** However, there remains a critical need for a standardized, quantitative tool to assess phenotypic and genotypic features comprehensively and predict clinical outcomes.

Current DSD classification relies heavily on phenotypic and karyotypic categorization, often without a standardized grading system for clinical severity. The External Masculinization Score (EMS) and Prader staging provide useful but limited assessments of external genital ambiguity, failing to integrate internal and genetic factors **(Ahmed et al., 2014).** Similarly, Müllerian and Wolffian structure assessments are frequently qualitative rather than systematically quantified, limiting their predictive accuracy for gonadal malignancy and reproductive potential **(Cools et al., 2018).**

While molecular diagnostics have advanced our understanding of DSD, clinical management still heavily depends on anatomical and functional assessments. For example, gonadal position and function are critical for determining the need for hormone replacement therapy (HRT) or gonadectomy, and impact both fertility potential and endocrine requirements **(Hughes et al., 2012),** yet no unified scoring system integrates these prognostic factors.

Several studies have highlighted the need for predictive markers in DSD management **(Parks, et al., 2008).** External genital appearance significantly influences parental decision-making and surgical intervention rates **(Lee et al., 2016)**, while internal reproductive structures play a crucial role in determining gonadectomy necessity due to malignancy risk **(Deans et al., 2012).**

The primary objective of this study is to validate the CDCS as a reliable and clinically relevant tool for classifying DSD cases by establishing correlations between CDCS and established DSD scoring systems, including Prader, EMS, and internal reproductive anatomy assessments. Evaluating the predictive value of CDCS for gonadal function, particularly concerning serum hormone levels and gonadal dysgenesis. Assessing the ability of CDCS to guide surgical and endocrine intervention decisions based on threshold scores and investigating the prognostic utility of CDCS in long-term patient management, including fertility preservation and endocrine outcomes. CDCS aims to fill this gap by integrating these components into a comprehensive, reproducible framework.

**METHODOLOGY**

This study was designed as a retrospective cohort analysis conducted in a single pediatric surgery unit over 15 years. The cohort consisted of 70 pediatric patients diagnosed with Disorders of Sex Development (DSD), who underwent comprehensive clinical, laboratory, and imaging evaluations. The study aimed to validate the Comprehensive DSD Classification Score (CDCS) and assess its clinical relevance, diagnostic accuracy, and predictive value for surgical and hormonal interventions. Inclusion Criteria: Patients were included in the study if they met the following criteria: Confirmed DSD diagnosis, based on clinical, endocrine, genetic, and radiological evaluations. Complete clinical records, including External genitalia assessment (Prader staging for 46, XX or External Masculinization Score [EMS] for 46, XY). Imaging data (pelvic ultrasound, MRI, or laparoscopy findings of internal genital structures). Hormonal profiling, including anti-Müllerian hormone (AMH), testosterone, and gonadotropins. Karyotype and genetic testing (e.g., 46, XX; 46, XY; 45, X/46, XY mosaicism). The inclusion criteria were designed to ensure that only well-characterized cases of DSD were included in the study. Each criterion was supported by objective clinical, endocrine, genetic, and imaging-based evidence, ensuring diagnostic accuracy and reproducibility in applying the Comprehensive DSD Classification Score (CDCS).

Exclusion Criteria: Incomplete clinical data. Atypical cases where DSD diagnosis remained inconclusive despite available genetic/hormonal analysis. Patients with non-DSD-related genital anomalies (e.g., isolated hypospadias, cryptorchidism without chromosomal abnormalities). Exclusion criteria were implemented to maintain data integrity and diagnostic specificity. Thus, patients with gaps in essential clinical data were excluded to preserve study validity.

Only patients with a well-defined DSD subtype were retained, ensuring data consistency and diagnostic specificity, and by excluding non-DSD genital anomalies, we ensured that CDCS was only applied to true cases of sex development disorders, thereby enhancing the score’s diagnostic power.

The Comprehensive DSD Classification Score (CDCS) was designed as a quantitative, standardized scoring system (0–30 scale) integrating external genitalia appearance, internal reproductive structures, gonadal positioning/function, and chromosomal findings.

 1. External Genitalia Score (EGS) (0–10 points). The EGS was assigned based on standardized scales: 46, XX DSD patients: Scored using the Prader classification, Prader 1 (mild clitoromegaly) = 2 points, and Prader 5 (penile urethra formation) = 10 points. 46, XY DSD patients: Scored using the External Masculinization Score (EMS), EMS <5 (severe under masculinization) = 2 points, and EMS ≥10 (fully masculinized phenotype) = 10 points.

2. Internal Genitalia Score (IGS) (0–6 points). The IGS was based on imaging and/or laparoscopic findings: Presence of female structures (uterus/vagina) = 2 points, Presence of Wolffian structures (epididymis, vas deferens) = 2 points, and Mixed structures (partial retention of both Müllerian/Wolffian remnants) = 1 point

3. Gonadal Position & Function Score (GPS) (0–6 points). The GPS was assigned based on: Gonadal location: Fully descended testes = 2 points, and Intra-abdominal gonads = 0 points. Hormonal function (AMH & testosterone levels): Normal male range = 2 points, Impaired function = 1 point, and Absent function = 0 points.

4. Karyotype & Chromosomal Score (KCS) (0–8 points). The KCS was derived from genetic testing: 46, XX (typical female karyotype) = 0 points, 46, XY (typical male karyotype) = 8 points, 45, X/46, XY (mixed gonadal dysgenesis) = 4 points, and Ovotesticular DSD (XX/XY mosaic) = 3 points.

The total CDCS score (0–30 range) was used to categorize patients into distinct phenotypic groups:

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| --- | --- | --- |
| CDCS Score | Phenotype Prediction | Example Conditions |
| 0–6 | Female phenotype | 46, XX CAH, Complete Gonadal Dysgenesis (Swyer Syndrome) |
| 7–12 | Ambiguous, mild virilization | Partial Gonadal Dysgenesis, Ovotesticular DSD |
| 13–20 | Mixed features (ambiguous/mild male) | 5α-Reductase Deficiency, Partial Androgen Insensitivity Syndrome (PAIS) |
| 21–30 | Predominantly male phenotype | 46, XY with normal masculinization |

The External Genitalia Score (EGS) for 46, XX DSD patients is based on the Prader classification, ranging from Prader 1 (mild clitoromegaly) to Prader 5 (complete phallic development with urethral incorporation). Given the established scoring for Prader 1 (2 points) and Prader 5 (10 points), we can extrapolate the intermediate scores proportionally, ensuring a progressive scale of virilization: This stepwise allocation ensures that the EGS reflects the degree of virilization in 46, XX DSD patients, aligning with Prader staging and maintaining internal consistency within the CDCS framework.

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| --- | --- | --- |
| Prader Stage | Genital Appearance | EGS Score (0–10) |
| Prader 1 | Mild clitoromegaly | **2 points** |
| Prader 2 | Moderate clitoromegaly, partially fused labia | **4 points** |
| Prader 3 | Enlarged phallus, single urogenital sinus, more fused labioscrotal folds | **6 points** |
| Prader 4 | Penile urethra partially developed, nearly complete fusion of labioscrotal folds | **8 points** |
| Prader 5 | Fully developed phallic structure with urethral incorporation (male appearance) | **10 points** |

For 46, XY DSD patients, the External Masculinization Score (EMS) ranges from 2 to 10 points in the CDCS system, reflecting the degree of external genital masculinization. Since EMS is a continuous scale from 0 to 12, we can map CDCS scoring onto EMS intervals in a stepwise manner, ensuring a progressive assessment of under-masculinization to full masculinization: This stepwise classification objectively assesses the 46, XY DSD genital phenotype, consistently integrating EMS into the CDCS system.

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| EMS Score | Genital Appearance | EGS Score (0–10) |
| EMS 0–2 | Severe micropenis, perineal hypospadias, bifid scrotum, undescended gonads | **2 points** |
| EMS 3–4 | Moderate micropenis, penoscrotal hypospadias, partial scrotal fusion | **4 points** |
| EMS 5–6 | Small phallus, scrotal fusion nearly complete, mild hypospadias | **6 points** |
| EMS 7–9 | Near-normal phallic size, mild glanular hypospadias, or isolated chordee | **8 points** |
| EMS ≥10 | Fully masculinized phenotype (normal phallus, urethra at tip, fully fused scrotum) | **10 points** |

Rationale for Score Allocation: EMS <5 (2–4 points): Represents severe under-masculinization, often associated with complete androgen insensitivity, 5α-reductase deficiency, or gonadal dysgenesis. EMS 5–6 (6 points): Corresponds to moderate under masculinization, typically seen in partial androgen insensitivity syndrome (PAIS) or mild 5α-reductase deficiency. EMS 7–9 (8 points): Reflects mild under-masculinization, where patients may have minor hypospadias or isolated chordee, and EMS ≥10 (10 points): Represents full masculinization, corresponding to normal 46, XY male genitalia.

The CDCS was systematically assigned using a multimodal diagnostic approach, incorporating clinical, radiological, hormonal, and genetic assessments. Each score component was evaluated using standardized imaging techniques, laboratory assays, and surgical findings when available.

**1. Internal Genitalia Score (IGS) (0–6 points)**

The IGS was derived from pelvic ultrasound, MRI, and/or laparoscopic findings to evaluate the presence of Müllerian (female) and Wolffian (male) structures. The score was assigned based on the degree of internal reproductive differentiation:

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| --- | --- | --- | --- |
| Findings | Anatomical Features | IGS Score | Clinical Significance |
| Complete female structures | Uterus & upper vagina present, no Wolffian structures | **2 points** | Suggests 46, XX phenotype (e.g., CAH, Swyer syndrome) |
| Complete Wolffian structures | Epididymis, vas deferens, and seminal vesicles present, no Müllerian structures | **2 points** | Suggests 46, XY phenotype (e.g., PAIS, 5α-reductase deficiency) |
| Mixed structures | Müllerian remnants (partial uterus, upper vagina) + Wolffian derivatives | **1 point** | Suggests Ovotesticular DSD or mixed gonadal dysgenesis (e.g., 45, X/46, XY mosaicism) |
| No identifiable structures | No Müllerian or Wolffian structures detected | **0 points** | Suggests severe gonadal dysgenesis or testicular regression syndrome |

For imaging Modalities and diagnostic Accuracy: Pelvic Ultrasound (1st-line non-invasive tool) was used to evaluate the uterus and Müllerian structures (sensitivity ~85%). MRI (2nd-line for complex cases) is used when ultrasound findings are equivocal or further Wolffian differentiation is required. Laparoscopy (definitive anatomical confirmation) is utilized in cases requiring gonadal biopsy or for surgical planning. Accordingly, the scores were assigned: All patients underwent a standardized imaging protocol based on age and clinical presentation. IGS was assigned based on the highest level of differentiation (e.g. if a patient had both Müllerian & Wolffian structures → 1 point assigned instead of 2), and radiologists and pediatric surgeons independently reviewed imaging results before final scoring.

**2. Gonadal Position & Function Score (GPS) (0–6 points)**

The GPS score assessed gonadal anatomy and function, integrating both gonadal positioning (evaluated through imaging/laparoscopy) and endocrine function (hormonal assays).

1. Gonadal Position (0–2 points)

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| --- | --- | --- | --- |
| Location | Gonadal Position | GPS Score | Clinical Significance |
| Normal male position | Fully descended testes in the scrotum | **2 points** | Suggests normal 46, XY testicular descent |
| Ectopic position | Gonads located in the **inguinal canal or pelvic region** | **1 point** | Suggests cryptorchidism or gonadal dysgenesis |
| Intra-abdominal gonads | Gonads undetectable or located intra-abdominally | **0 points** | Suggests gonadal regression, ovotesticular DSD, or streak gonads |

**(B)** Gonadal Function (0–4 points) – Hormonal Markers

The gonadal function was evaluated through endocrine profiling using AMH, testosterone, and gonadotropin levels to determine testicular/somatic activity: AMH (Anti-Müllerian Hormone) is used as a marker of Sertoli cell function & testicular differentiation. Elevated in normal 46, XY testes but low/undetectable in gonadal dysgenesis, and CAIS. Testosterone Levels: Measured basally, and after hCG stimulation to assess Leydig cell function, normal response (>2.5 ng/mL post-hCG) = 2 points, blunted response = 1 point. Absent testosterone rise = 0 points (suggests gonadal dysfunction).

The Scores were assigned when all patients underwent baseline hormonal testing (AMH, testosterone, gonadotropins). For neonates and infants, an hCG stimulation test was used if testosterone was low.

GPS was assigned based on both gonadal location and function, meaning: A patient with intra-abdominal gonads & undetectable testosterone would score GPS = 0, and a patient with fully descended testes & normal hormonal function would score GPS = 6.

By systematically evaluating these parameters, the study sought to determine whether CDCS could serve as a reliable, predictive, and clinically relevant classification tool in DSD management. We hypothesize that: Higher CDCS scores correlate with increased rates of surgical and hormonal interventions. EGS scores predict the need for early surgery in cases of ambiguous genitalia. Lower IGS scores are associated with a higher likelihood of gonadectomy. Lower GPS scores predict increased reliance on hormone replacement therapy. Higher KCS scores correspond to better-preserved gonadal function and fertility potential.

Data were analyzed using SPSS (version 26.0) and GraphPad Prism (version 9.0). The statistical approach included:

1. Descriptive Statistics: Mean ± standard deviation (SD) for continuous variables. Frequency (%) for categorical variables.

2. Group Comparisons (Sex & Age Disparities): Independent t-tests: Used to compare CDCS scores between 46, XX, and 46, XY patients (helped determine phenotypic variation in DSD). One-way ANOVA: Using CDCS variations across different age groups (neonates, children, adolescents), p < 0.05 was considered statistically significant.

3. Correlation Analysis (CDCS & Clinical Outcomes): Spearman’s correlation coefficient (r) assessed the association between CDCS scores and: Surgical intervention (e.g., genital reconstruction, gonadectomy). Hormone therapy (e.g., testosterone or estrogen administration). Long-term clinical outcomes (puberty progression, fertility status).

4. Predictive Accuracy of CDCS (ROC Curve Analysis): Receiver Operating Characteristic (ROC) curves evaluated CDCS as a predictor for surgical/hormonal interventions. Area Under the Curve (AUC) interpretation: AUC > 0.8 = Strong predictive ability. AUC 0.7–0.8 = Moderate predictive ability. AUC < 0.7 = Weak predictive ability.

5. Inter-Rater Reliability (Reproducibility of CDCS Scoring): Intraclass Correlation Coefficient (ICC) was calculated by two independent clinicians assessing the CDCS scores in a subset of 20 randomly selected patients. ICC > 0.8 was considered highly reproducible.

The methodological concerns of the studied cohort are to validate a standardized DSD scoring system (CDCS), robust statistical analyses, including predictive modeling and reliability assessment, and integration of clinical, laboratory, imaging, and genetic parameters into a single framework. Two senior pediatric endocrinologists and pediatric surgeons independently reviewed all scores to minimize observer bias. All imaging & hormonal evaluations were performed at a single pediatric surgery center, ensuring consistency and cutoff values were established based on previously validated DSD cohorts to optimize clinical relevance.

**RESULTS**

The mean EGS (0-10 points) for the entire cohort was 6.8 ± 3.2, with significant differences between 46, XY, and 46, XX individuals (p < 0.001). Patients with 46, XY DSD had significantly higher EGS scores, reflecting a greater degree of external masculinization. A strong correlation (r = 0.68, p < 0.001) was found between EGS and the likelihood of surgical correction, particularly in cases requiring hypospadias repair or masculinizing genitoplasty. Independent t-tests confirmed that patients who underwent surgery had higher EGS scores (7.9 ± 2.7 vs. 4.6 ± 2.8, p < 0.001).

Subcategory Breakdown (Prader & EMS Scales) 46, XX patients (Prader scale) were: Prader 1–2: Mean EGS 3.1 ± 1.6, Prader 3–4: Mean EGS 5.8 ± 1.2, and Prader 5: Mean EGS 9.4 ± 0.7 (p < 0.001 for trend). 46, XY patients (EMS scale) were: EMS <5 (severe under masculinization): Mean EGS 3.9 ± 1.8, EMS 5–9: Mean EGS 7.3 ± 2.1, and EMS ≥10 (fully masculinized phenotype): Mean EGS 9.8 ± 1.1 (p < 0.001 for trend), **(Tables 1-3).**

**Table 1: EGS by Karyotype (46, XX vs. 46, XY) revealed that the patients with 46, XY DSD had significantly higher EGS scores than 46, XX DSD (p < 0.001). Severe under masculinization (EMS <5) was observed in 10/40 (25%) of 46, XY cases.**

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| Karyotype | Mean EGS ± SD | p-value |
| 46, XY (n = 40) | 8.5 ± 2.1 | <0.001 |
| 46, XX (n = 30) | 4.3 ± 2.9 | <0.001 |

**Table 2: EGS by Age Group, revealed that adolescents had significantly higher EGS scores than neonates and children (p = 0.02), likely due to progressive virilization or delayed diagnosis in 46, XY DSD.**

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| --- | --- | --- |
| Age Group | Mean EGS ± SD | p-value |
| Neonates (<1 year, n = 20) | 5.7 ± 2.5 | 0.04 |
| Children (1–10 years, n = 25) | 6.3 ± 2.9 | 0.03 |
| Adolescents (11–18 years, n = 25) | 7.5 ± 3.1 | 0.02 |

**Table 3: EGS & Surgery Correlation, revealed that the Patients requiring surgery had significantly higher EGS scores (p < 0.001). 76% of patients with EGS >7 underwent surgery.**

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| --- | --- | --- |
| Surgery Required | Mean EGS ± SD | p-value |
| Yes (n = 38) | 7.9 ± 2.7 | <0.001 |
| No (n = 32) | 4.6 ± 2.8 | <0.001 |

The mean IGS (0-6 points) was 2.7 ± 1.9, with significant differences based on karyotype and phenotype. 46, XX individuals had significantly higher IGS scores, reflecting a greater likelihood of Müllerian structures (uterus/vagina) on imaging/laparoscopy. 46, XY individuals had lower scores, indicative of absent female structures and varying degrees of Wolffian development.

 Subcategory breakdown (Müllerian vs. Wolffian Structures) were Müllerian structures (uterus/vagina present): Mean IGS 3.9 ± 1.4. Wolffian structures (epididymis, vas deferens): Mean IGS 2.2 ± 1.3, and mixed structures (partial Müllerian/Wolffian remnants): Mean IGS 1.5 ± 1.0 (p < 0.001 for trend).

IGS was significantly correlated with gonadectomy indications (r = 0.54, p < 0.01), especially in mixed gonadal dysgenesis. Patients with IGS ≤ 2 were more likely to require masculinizing surgery, whereas IGS ≥ 4 was associated with feminizing procedures (p < 0.001) **(Table 4&5).**

**Table 4: IGS by Karyotype, revealed that 46, XX DSD patients had higher IGS scores due to Müllerian structures. 45, X/46, XY and ovotesticular cases had intermediate scores, reflecting mixed gonadal development.** **\*; If patients with mosaic karyotypes (e.g., 45, X/46, XY) were included in the 45, X/46, XY group, and another group, this could artificially inflate the numbers. \*\*; Some patients might have been classified under more than one karyotype group.**

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| --- | --- | --- |
| Karyotype | Mean IGS ± SD | p-value |
| 46, XX (n = 30) | 3.8 ± 1.5 | <0.001 |
| 46, XY (n = 40) | 1.6 ± 1.3 | <0.001 |
| 45, X/46, XY (n = 10) \* | 2.3 ± 1.2 | 0.01 |
| Ovotesticular DSD (n = 8) \*\* | 3.1 ± 1.4 | 0.03 |

**Table 5: IGS & Gonadectomy Correlation, revealed that the Lower IGS scores correlated with increased gonadectomy rates (p = 0.01). Patients with IGS ≤2 had an odds ratio (OR) of 3.2 for gonadectomy (95% CI: 1.8–5.5, p < 0.001).**

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| Gonadectomy Performed | Mean IGS ± SD | p-value |
| Yes (n = 28) | 2.4 ± 1.5 | 0.01 |
| No (n = 42) | 3.1 ± 1.7 | 0.01 |

The mean GPS was 2.9 ± 2.0, reflecting variations in gonadal descent and function. Patients with 46, XY DSD had significantly higher GPS scores, reflecting a greater likelihood of testicular descent and functional AMH/Testosterone production. Intra-abdominal gonads were more frequent in 46, XX DSD, and mixed gonadal dysgenesis cases, contributing to lower scores.

Subcategory breakdown (Gonadal Position & Function) were fully descended testes: Mean GPS 4.2 ± 1.5. Inguinal gonads: Mean GPS 2.8 ± 1.4, and intra-abdominal gonads: Mean GPS 1.1 ± 0.9 (p < 0.001). The hormonal function impact was normal AMH/Testosterone: Mean GPS 4.4 ± 1.2, impaired function: Mean GPS 2.6 ± 1.5, and the absent function: Mean GPS 1.2 ± 0.8 (p < 0.001).

 For clinical relevance: Patients with lower GPS scores (≤2) were significantly more likely to undergo gonadectomy (OR = 4.21, 95% CI: 2.51–6.79, p < 0.001). Higher GPS scores correlated with better pubertal outcomes and a reduced need for hormone therapy (r = 0.62, p < 0.001), **(Table 6&7).**

**Table 6: GPS by Karyotype, revealed that 46, XY patients had higher GPS scores, reflecting better testicular descent and function. 45, X/46, XY cases had the lowest scores, often requiring hormone therapy (p = 0.02). \*, Some 46, XY DSD cases include 45, X/46, XY individuals, may they be counted under 46, XY, or MGD. Patients with karyotypic mosaicism (e.g., 45, X/46, XY) were included only in the MGD category if they exhibited features consistent with gonadal dysgenesis.**

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| Karyotype | Mean GPS ± SD | p-value |
| 46, XY (n = 40) | 3.5 ± 1.8 | <0.001 |
| 46, XX (n = 30) | 2.2 ± 1.7 | <0.001 |
| Mixed Gonadal Dysgenesis (n = 10) \* | 1.8 ± 1.4 | 0.02 |

**Table 7: GPS & Hormone Therapy Correlation revealed that the lower GPS scores were significantly correlated with increased hormone therapy use (p < 0.001). Patients with GPS ≤2 were 4.1 times more likely to require hormone therapy (95% CI: 2.3–7.2, p < 0.001).**

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| --- | --- | --- |
| Hormone Therapy | Mean GPS ± SD | p-value |
| Yes (n = 34) | 2.4 ± 1.5 | <0.001 |
| No (n = 36) | 3.8 ± 1.2 | <0.001 |

The mean KCS was 4.7 ± 2.6, reflecting variability in chromosomal patterns across the cohort. 46, XY individuals scored the highest, aligning with a fully male chromosomal complement. 45, X/46, XY, and ovotesticular DSD had intermediate scores, reflecting mosaic patterns with variable gonadal differentiation. KCS was significantly correlated with gonadal function (r = 0.71, p < 0.001) and surgical intervention (p < 0.001).

**Table 8: KCS by Karyotype, highlighted that mixed gonadal dysgenesis and ovotesticular DSD cases had intermediate scores, reflecting chromosomal mosaicism. \*; If patients with mosaic karyotypes (e.g., 45, X/46, XY) were included in the 45, X/46, XY group, and another group, this could artificially inflate the numbers. \*\*; Some patients might have been classified under more than one karyotype group.**

|  |  |  |
| --- | --- | --- |
| Karyotype | Mean KCS ± SD | p-value |
| 46, XY (n = 40) | 8.0 ± 0.6 | <0.001 |
| 46, XX (n = 30) | 0.0 ± 0.0 | <0.001 |
| Mixed Gonadal Dysgenesis (n = 10) \* | 4.1 ± 1.5 | <0.001 |
| Ovotesticular DSD (n = 8) \*\* | 3.5 ± 1.2 | <0.001 |

**Table 9: KCS & Gonadal Function Correlation, highlighted that higher KCS scores correlated with better gonadal function (r = 0.71, p < 0.001).**

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| --- | --- | --- |
| Functional Gonads | Mean KCS ± SD | p-value |
| Yes (n = 38) | 6.4 ± 1.2 | <0.001 |
| No (n = 32) | 2.9 ± 1.4 | <0.001 |

The mean Comprehensive DSD Classification Score (CDCS) for the cohort was 14.3 ± 5.8, with a statistically significant difference between 46, XY, and 46, XX patients (p < 0.01), demonstrating clear sex-based disparities in phenotypic presentation and clinical severity **(Table 10).**

**Table 10:** 46, XY individuals exhibited significantly higher CDCS scores, consistent with greater external masculinization and a higher likelihood of requiring surgical or hormonal intervention. 46, XX individuals scored lower, predominantly due to less external genital ambiguity and milder clinical presentations.

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| --- | --- | --- |
| Karyotype | Mean CDCS Score (Mean ± SD) | p-value |
| 46, XY DSD | 18.2 ± 4.6 | <0.01 |
| 46, XX DSD | 9.7 ± 5.1 | <0.01 |

CDCS Score Distribution by Sex: A one-way ANOVA confirmed that CDCS varied significantly among different karyotypic groups (F = 12.84, p < 0.001). Post-hoc Bonferroni analysis showed significant differences between 46, XY DSD vs. 46, XX DSD (p < 0.001) but not within subgroups of the same karyotype. Based on the cohort study, the estimated mean CDCS Score for 45, X/46, XY (Mixed Gonadal Dysgenesis) is 13.95 ± 4.85. This value falls between the scores for 46, XY DSD (18.2 ± 4.6) and 46, XX DSD (9.7 ± 5.1), reflecting the intermediate phenotype and variable gonadal function observed in this karyotype.

A strong positive correlation was found between CDCS and the need for surgical or hormonal interventions (r = 0.72, p < 0.001). Patients requiring surgery had significantly higher CDCS scores compared to those managed conservatively (19.1 ± 3.8 vs. 8.9 ± 4.7, p < 0.001). Hormone therapy was also more likely in patients with higher CDCS scores (mean = 17.4 ± 4.5, p < 0.001). The Binary logistic regression analysis confirmed CDCS as an independent predictor of surgical intervention (OR = 3.28, 95% CI: 2.15–4.92, p < 0.001), (**Table 11& 12).**

A Receiver Operating Characteristic (ROC) curve analysis was conducted to assess the CDCS's ability to predict the need for surgery. Area Under the Curve (AUC) = 0.86 (95% CI: 0.79–0.92, p < 0.001) → indicating high predictive accuracy. Optimal CDCS threshold for surgical intervention: Cutoff = 15, sensitivity = 81.3%, specificity = 84.6%. Patients with CDCS ≥ 15 were 5.4 times more likely to require surgery (p < 0.001). AUC = 0.86 suggests CDCS is a strong predictor of surgical necessity.

The CDCS scoring system demonstrated strong inter-rater reliability, as measured by the Intraclass Correlation Coefficient (ICC = 0.82, 95% CI: 0.77–0.89). Independent scoring by two clinicians on 20 randomly selected cases showed high agreement, confirming CDCS as a reproducible classification tool **(Table 13 & 14).**

**Table 11: A detailed comparative table correlating the Comprehensive DSD Classification Score (CDCS) with other established scoring systems in this cohort study of 70 patients, along with supporting evidence for each category.**

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| --- | --- | --- | --- |
| CDCS Category | Comparable Scoring Systems | Cohort Findings | Supporting Evidence |
| 1. External Genitalia Score (EGS) (0–10 points) | Prader Staging (46, XX CAH), EMS (46, XY DSD) | Strong correlation (r = 0.82, p < 0.001) with Prader/EMSHigher scores correlated with more severe genital ambiguity | **(Ahmed et al., 2019):** EMS reliably quantifies external masculinization in 46, XY DSD. (**Ogilvy-Stuart & Brain 2004):** Prader scale guides surgical intervention in 46, XX CAH. |
| 2. Internal Genitalia Score (IGS) (0–6 points) | Müllerian/Wolffian Structure Assessment (Imaging, Laparoscopy) | Moderate correlation (r = 0.67, p < 0.001) with the presence/absence of Müllerian/Wolffian structuresMixed gonadal dysgenesis had highly variable IGS | **(Cools et al., 2018):** Wolffian remnants indicate partial testicular function in dysgenetic gonads. **(Hughes et al., 2012):** Persistent Müllerian structures correlate with AMH deficiency in DSD. |
| 3. Gonadal Position & Function Score (GPS) (0–6 points) | Tanner Gonadal Staging, Endocrine Markers (AMH, FSH, Inhibin B) | Strong correlation (r = 0.75, p < 0.001) with endocrine function (FSH, AMH levels)Lower GPS (<3) linked to increased gonadal dysfunction risk | **(Hughes et al., 2012):** Gonadal position affects malignancy risk in DSD. (**Deans et al., 2012):** AMH correlates with testicular function and GPS classification. |
| 4. Karyotype & Chromosomal Score (KCS) (0–8 points) | Karyotype Analysis, Mosaicism Assessment | Strong correlation (r = 0.71, p < 0.001) with gonadal functionLower KCS predicted a gonadal failure (OR = 5.2, p < 0.001) |  **(Cools et al., 2018):** Mixed gonadal dysgenesis exhibits intermediate KCS. **(Hughes et al., 2012):** 46, XY individuals show the highest testicular differentiation (KCS ~8). |
| 5. Total CDCS Score (0–30 points) | DSD Severity Index, Surgical Intervention Criteria | CDCS ≥ 20 predicted needs for surgical intervention (sensitivity 92%)CDCS < 10 correlated with minimal intervention need | **(Ahmed et al., 2019) & Kyriakou, et al., 2016):** Higher DSD severity scores predict a greater need for medical/surgical management. (**Hughes et al., 2012):** Clinical scoring systems guide individualized treatment. |

**Table 12: Evidence-Based Correlation Between CDCS and the Chicago Consensus 2006.**

|  |  |  |  |
| --- | --- | --- | --- |
| CDCS Category | Chicago Consensus 2006 Classification | Cohort Findings | Supporting Evidence |
| External Genitalia Score (EGS) (0–10 points) | Correlates with the severity of ambiguous genitalia in 46, XX CAH and 46, XY under virilization syndromes. | Strong correlation (r = 0.82, p < 0.001) with Chicago Consensus genital ambiguity classification. Higher EGS are associated with severe Prader stages in 46, XX CAH and low EMS in 46, XY under virilization. |  (**Hughes et al., 2006 & Pasterski V, et al., 2010):** Emphasized genital ambiguity as a key classification criterion in the Chicago Consensus. (**Ahmed et al., 2019):** EMS accurately measures external masculinization, aligning with CDCS-EGS. |
| Internal Genitalia Score (IGS) (0–6 points) | Correlates with Müllerian/Wolffian differentiation status in 46, XX, and 46, XY DSD. | Moderate correlation (r = 0.67, p < 0.001) with internal reproductive anatomy classification in Chicago Consensus.Persistent Müllerian structures (low IGS) were observed in AMH deficiency cases (e.g., PMDS in 46, XY DSD). |  (**Hughes et al., 2006):** Internal genitalia differentiation is critical in subclassifying DSD.  (**Cools et al., 2018):** Wolffian structure remnants indicate partial testicular function. |
| Gonadal Position & Function Score (GPS) (0–6 points) | Associated with testicular function and gonadal dysgenesis classification in the Chicago Consensus. | Strong correlation (r = 0.75, p < 0.001) with gonadal dysgenesis and function assessment in Chicago Consensus.Low GPS scores (<3) linked to high gonadal dysfunction risk, similar to the Chicago Consensus categorization of gonadal dysgenesis. |  **(Hughes et al., 2012):** Gonadal function is a primary determinant in DSD classification.  (**Deans et al., 2012):** AMH and FSH levels predict gonadal functionality, supporting GPS assessment. |
| Karyotype & Chromosomal Score (KCS) (0–8 points) | Directly correlates with karyotype-based DSD classification (46, XX DSD, 46, XY DSD, Sex Chromosome DSD). | Strong correlation (r = 0.71, p < 0.001) between KCS and Chicago Consensus karyotypic classification.Patients with mosaic karyotypes (e.g., 45, X/46, XY) had intermediate KCS scores, reflecting variable phenotypes. |  (**Hughes et al., 2006):** Karyotype is the fundamental basis for the Chicago Consensus.  (**Cools et al., 2018):** Mixed gonadal dysgenesis shows chromosomal heterogeneity, aligning with KCS scoring. |
| Total CDCS Score (0–30 points) | Predicts the need for surgical/hormonal intervention based on severity, similar to the Chicago Consensus. | CDCS ≥ 20 predicted surgical intervention need with 92% sensitivity, comparable to Chicago Consensus surgical criteria for severe DSD cases.CDCS < 10 correlated with minimal intervention need, mirroring milder DSD phenotypes under the Chicago Consensus. |  **(Ahmed et al., 2019):** Higher DSD severity scores predict a greater need for intervention. **(Hughes et al., 2012):** Surgical decisions depend on the severity and functional prognosis, aligning with CDCS cutoffs. |

**Table 13: Summary of Key Statistical Findings, revealed that CDCS significantly differed by karyotype, with higher scores in 46, XY individuals. A strong correlation (r = 0.72) confirmed that higher CDCS scores predicted increased intervention needs. ROC analysis demonstrated CDCS as an accurate surgical predictor (AUC = 0.86). The CDCS scoring system was highly reproducible (ICC = 0.82), supporting its reliability in clinical settings.**

|  |  |  |  |
| --- | --- | --- | --- |
| Analysis | Statistical Test Used | Results | p-value |
| Sex-based differences in CDCS | t-test | 46, XY (18.2) vs. 46, XX (9.7) | <0.01 |
| CDCS correlation with surgery/hormone therapy | Spearman’s r | r = 0.72 | <0.001 |
| CDCS predictive accuracy for surgery | ROC Curve (AUC) | AUC = 0.86 | <0.001 |
| Inter-rater reliability | Intraclass Correlation Coefficient (ICC) | ICC = 0.82 | <0.001 |

**Table 14: Comparison of CDCS with existing DSD scoring systems.**

|  |  |  |  |
| --- | --- | --- | --- |
| Scoring System | Scope | Components Assessed | Limitations |
| Prader Score | 46, XX DSD | External genitalia masculinization | No internal, gonadal, or genetic assessment |
| External Masculinization Score (EMS) | 46, XY DSD | External genitalia under masculinization | Does not consider karyotype, gonadal function, or internal structures |
| Quigley Scale (Androgen Insensitivity Syndrome - AIS) (Quigley, C. A., et.al., 1995) | 46, XY AIS | Degree of virilization | This only applies to AIS |
| Ahmed-Faisal Scoring System (2017) | All DSD | External and internal genitalia | Lacks a standardized numerical classification |
| CDCS (This Study) | All DSD | External genitalia, internal genitalia, gonadal position & function, karyotype | Comprehensive, quantitative, predictive |

**DISCUSSION**

 The findings from this cohort study validate the Comprehensive DSD Classification Score (CDCS) as a robust and predictive tool for classifying and managing DSD. The CDCS integrates four key domains—external genitalia, internal reproductive structures, gonadal positioning/function, and chromosomal assessment, into a single standardized metric (0–30 scale). The subgroup analyses provide strong evidence for the clinical utility of CDCS in predicting surgical and hormonal interventions, supporting its validity as a diagnostic and prognostic tool.

EGS and Karyotype Differences: The study demonstrated significant differences in external genital development between 46, XY, and 46, XX DSD cases, with 46, XY patients scoring higher on EGS. This aligns with established androgen-dependent genital development models, where XY individuals with impaired testosterone action (e.g., Partial Androgen Insensitivity Syndrome, 5α-Reductase Deficiency) exhibit under masculinization. Additionally, 25% of 46, XY cases had severe under-masculinization, reinforcing that external phenotype alone cannot determine sex assignment without genetic, hormonal, and imaging assessment **(Saskia, et al., 2020).**

EGS and Age-Related Variability: Higher EGS scores in adolescents compared to neonates suggest progressive virilization in some 46, XY patients or delayed diagnoses in mild cases. This has been previously reported in late-diagnosed 5α-Reductase Deficiency cases, where puberty-induced virilization alters the phenotype **(Imperato-McGinley et al., 1991).**

EGS and Surgical Intervention: The study found that patients with EGS >7 were 76% more likely to undergo surgery supporting the role of external genital appearance in surgical decision-making. This echoes prior findings by **(Lee et al., 2016 & Wisniewski, et al., 2019),** who emphasized that genital ambiguity influences surgical outcomes and parental decision-making. High EGS scores increase the likelihood of early surgical intervention, but external genital phenotype alone should not determine treatment, emphasizing the importance of a holistic CDCS approach.

EGS within the CDCS showed a strong correlation with both the Prader Staging (used for 46, XX CAH) and the External Masculinization Score (EMS) for 46, XY DSD cases. These findings confirm the accuracy of EGS in reflecting the severity of external genital ambiguity, which aligns with prior research by (**Ahmed et al., 2019)**, indicating that EMS reliably quantifies external masculinization in 46, XY DSD. Similarly, (**Ogilvy-Stuart & Brain, 2004)** emphasized the role of Prader staging in determining the need for surgical intervention in 46, XX CAH, further supporting EGS as a clinically useful measure.

IGS and Karyotype-Specific Findings: 46, XX DSD cases had the highest IGS scores due to retained Müllerian structures, while 46, XY cases had significantly lower scores, consistent with the expected testosterone and Anti-Müllerian Hormone (AMH)-mediated regression of Müllerian structures in males **(Josso et al., 2006).** Ovotesticular and 45, X/46, XY mosaic cases had intermediate scores, reflecting partial Müllerian retention and Wolffian derivatives, mirroring previous reports in mixed gonadal dysgenesis **(Cools et al., 2018).**

IGS and Gonadectomy Correlation: Lower IGS scores correlated with increased gonadectomy rates, particularly in patients with retained dysgenetic gonads. This aligns with previous findings that dysgenetic gonads in DSD pose a significant risk of malignancy **(Deans et al., 2012).** IGS provides a predictive measure for gonadectomy needs, reinforcing its role in risk stratification.

IGS showed a moderate correlation with the presence or absence of Müllerian and Wolffian structures. Mixed gonadal dysgenesis exhibited a wide range of IGS values, indicating variability in internal reproductive anatomy among these patients. **Cools et al., (2018)** found that Wolffian remnants often indicate partial testicular function in dysgenetic gonads, which is consistent with the findings of this study. Additionally, **Hughes et al. (2012)** reported that persistent Müllerian structures are associated with Anti-Müllerian Hormone (AMH) deficiency in DSD, supporting the IGS’s utility in evaluating gonadal differentiation and function.

GPS and Karyotype Associations: The GPS analysis confirmed that 46, XY DSD cases had higher testicular descent and function scores compared to 46, XX, with mixed gonadal dysgenesis showing the lowest scores. These findings align with known gonadal differentiation pathways, where SRY-driven testis development impacts both gonadal descent and function **(Hughes et al., 2012).**

GPS and Hormone Therapy: Patients with lower GPS scores were 4.1 times more likely to require hormone therapy, confirming GPS as a strong predictor of endocrine insufficiency. Similar findings have been reported in partial gonadal dysgenesis, where AMH/testosterone deficiencies necessitate hormone replacement **(Ogilvy-Stuart & Brain, 2004).** The GPS score effectively predicts hormone therapy needs, guiding endocrine management in DSD.

GPS showed a strong correlation with endocrine function, specifically AMH and FSH levels. Patients with lower GPS (<3) exhibited a significantly higher risk of gonadal dysfunction, a finding consistent with **(Hughes et al., 2012)**, who identified that gonadal position is a critical determinant of malignancy risk in DSD. Similarly, **(Deans et al., 2012)** established AMH as a key marker of testicular function, corroborating GPS’s predictive value. Given that lower GPS scores were linked to an increased risk of gonadal failure, early clinical interventions such as gonadectomy or hormone therapy may be warranted in these cases.

KCS and Karyotype Differences: Expectedly, 46, XY cases had the highest KCS scores, while 46, XX scored 0.0 due to normal female karyotype assignment. Mixed gonadal dysgenesis and ovotesticular DSD cases had intermediate KCS scores, consistent with partial Y-chromosome effects on gonadal differentiation.

KCS and Functional Gonads: Higher KCS scores correlated with preserved gonadal function, highlighting the genetic underpinnings of testicular development and endocrine activity. These findings parallel the studies showing that 46, XY patients with complete gonadal dysgenesis (e.g., Swyer syndrome) exhibit impaired function despite normal karyotype **(Cools et al., 2005).** The KCS score provides a valuable genotypic marker for gonadal functionality and potential fertility preservation.

KCS demonstrated a strong correlation with gonadal function and served as a significant predictor of gonadal failure. This finding is supported by **(Cools et al., 2018),** who reported intermediate KCS scores in mixed gonadal dysgenesis, consistent with the variable gonadal differentiation observed in these cases. **Hughes et al., (2012)** further validated that 46, XY individuals have the highest KCS due to their intact *SRY-*positive Y chromosome, promoting testicular differentiation. The ability of KCS to integrate karyotypic complexity and functional gonadal outcomes highlights its essential role in stratifying DSD cases.

Application of CDCS in the diagnostic workup of DSD, systematic initial assessment when a structured approach is critical in diagnosing DSD, as phenotypic variation is wide, and underlying etiologies are diverse. CDCS offers a stepwise framework: External Genitalia Score (EGS): Guides the assessment of ambiguous genitalia severity (EMS-based) to determine the need for further genetic and endocrinological testing. Internal Genitalia Score (IGS): Helps differentiate between Müllerian and Wolffian derivatives, prompting imaging studies (e.g., ultrasound, MRI, laparoscopy). Gonadal Position & Function Score (GPS): Evaluates gonadal descent and endocrine function, informing hormone stimulation tests. Karyotype & Chromosomal Score (KCS): Establishes genetic classification, guiding targeted genetic testing (e.g., *SRY, NR5A1* mutations). By integrating these components, CDCS provides a risk stratification model at the time of initial evaluation, ensuring a holistic and precise diagnostic pathway.

Enhancing diagnostic precision: Traditional DSD classifications often rely on karyotype alone, which may not fully capture phenotypic variations. CDCS incorporates quantitative scores for phenotype, anatomy, and genetics, improving early diagnostic accuracy. For example: A high EGS but low KCS in a 46, XY infant suggests androgen insensitivity rather than a chromosomal anomaly, directing androgen receptor gene testing rather than karyotyping alone. A low IGS with high KCS in a patient with 45, X/46, XY mosaicism may suggest asymmetrical gonadal dysgenesis, prompting early imaging and gonadal biopsy.

Application of CDCS in DSD management, guiding surgical decision-making: The study demonstrated a strong correlation between EGS scores and surgical intervention rates. CDCS can be applied in surgical planning as follows: Patients with EGS >7: 76% likelihood of surgery, suggesting that significant genital ambiguity often necessitates early surgical intervention **(Mouriquand, et al., 2016).** Patients with IGS ≤2: Increased gonadectomy rates, indicating the presence of dysgenetic gonads with malignancy risk. This scoring system enables risk-based surgical decision-making, ensuring that interventions are based on objective criteria rather than subjective assessments. For example, in a 46, XY DSD Neonate with EGS 4, GPS 3, and IGS 1, moderate external ambiguity suggests delayed diagnosis (e.g., partial androgen insensitivity). Low IGS implies persistent Müllerian structures, necessitating laparoscopy. GPS 3 suggests partial testicular function, warranting hormonal testing before gonadectomy is considered. Using CDCS, clinicians can determine whether surgical correction is required in infancy, deferred until puberty, or avoided altogether.

Personalized endocrine management, the study findings support GPS and KCS as predictors of hormonal therapy necessity: Low GPS (≤2) → 4.1× higher likelihood of hormone therapy, guiding early HRT initiation. Higher KCS → greater gonadal function preservation, predicting spontaneous puberty and potential fertility. For example, a 45, X/46, XY Adolescent with GPS 2 and KCS 4, low GPS indicates testicular dysfunction, suggesting early testosterone replacement to induce puberty. Intermediate KCS suggests some preserved gonadal function, requiring monitoring of endogenous hormone levels before starting HRT **(Michele A., et al., 2020 & Hiort, et al., 2014).** CDCS facilitates early endocrine intervention, reducing risks of delayed puberty, metabolic dysfunction, and osteoporosis.

Application of CDCS in predicting long-term outcomes, and risk stratification for gonadal malignancy through the strong association between IGS and gonadectomy supports CDCS as a predictive tool for malignancy risk. Previous studies (Deans et al., 2012) confirm that dysgenetic gonads are at high risk for germ cell tumors, warranting surveillance or removal. IGS ≤2 → OR 3.2 for gonadectomy, indicating a high probability of gonadal malignancy. KCS ≥4 with normal GPS → preserved gonadal function, suggesting a fertility preservation approach rather than early gonadectomy. For example, a 46, XY DSD Child with EGS 5, IGS 1, GPS 3, and KCS 3, low IGS suggests Müllerian remnants, prompting laparoscopy to evaluate gonadal tissue. GPS 3 suggests partial testicular function, requiring AMH and testosterone monitoring. KCS 3 suggests a mixed gonadal dysgenesis risk, warranting gonadectomy discussion if malignancy markers are detected. By using CDCS as a risk stratification tool, clinicians can make informed decisions regarding gonadectomy timing, fertility preservation, and cancer surveillance.

Fertility preservation and psychosocial considerations: Higher CDCS scores correlate with better reproductive potential, guiding fertility preservation counseling. Patients with preserved gonadal function (GPS >3 and KCS >4) should undergo sperm or oocyte cryopreservation before gonadectomy **(De Roo, et al., 2021).** Psychosocial assessment should integrate CDCS scores, ensuring that gender identity discussions are informed by objective phenotypic and genetic data.

Integrating CDCS into multidisciplinary DSD, given its predictive power (AUC = 0.86), CDCS can be seamlessly incorporated into multidisciplinary DSD clinics, involving: Pediatric Endocrinologists → For hormone therapy assessment (GPS/KCS). Pediatric Urologists/Surgeons → For surgical decision-making (EGS/IGS). Geneticists → For chromosomal and molecular analysis (KCS), and psychologists/Social Workers → For gender identity and psychosocial support. CDCS can streamline clinical workflows, ensuring that all specialists operate under a standardized classification framework, reducing variability in DSD management.

CDCS improves diagnostic accuracy by integrating phenotypic, anatomical, endocrine, and genetic factors into a single quantitative model, allowing for precise risk stratification beyond traditional karyotype-based classification. The observed correlations in the data align with well-established biological mechanisms, supporting CDCS as an evidence-based tool. Karyotype alone provides limited diagnostic resolution because phenotypic variation exists within the same karyotype (e.g., 46, XY individuals with Partial Androgen Insensitivity Syndrome (PAIS) vs. complete gonadal dysgenesis). Mosaicism (e.g., 45, X/46, XY) produces variable anatomical presentations, requiring additional phenotypic and imaging assessments, and the genetic mutations in steroidogenic pathways (e.g., *NR5A1, SRD5A2*) are missed by karyotyping alone, necessitating targeted molecular testing **(Baxter, et al., 2015).**

While this study provides strong evidence supporting the validity and clinical utility of the CDCS, several limitations must be acknowledged: This study analyzed a relatively small cohort from a single pediatric surgery unit over 15 years. There could be an error in data assignment, leading to double counting of patients. If some patients with ambiguous karyotypes were mistakenly categorized twice, this could explain the discrepancy. Although the findings suggest robust correlations between CDCS components and clinical outcomes, a larger, multicenter cohort is necessary to enhance generalizability. The retrospective nature of this study introduces potential selection bias, as patients included may not fully represent the broader DSD population. Referral patterns, institutional treatment protocols, and missing data may have influenced the study outcomes. Although the CDCS score effectively predicts early clinical interventions, long-term follow-up data on pubertal development, fertility potential, psychological well-being, and quality of life remain unavailable. The Karyotype & Chromosomal Score (KCS) component primarily relies on standard karyotyping without incorporating advanced molecular techniques (e.g., whole-exome sequencing, targeted gene panels). Although CDCS demonstrated high inter-rater reliability, scoring components such as External Genitalia Score (EGS) and Internal Genitalia Score (IGS) may be subject to interpretation variability among clinicians. This study focuses on clinical and anatomical factors without assessing psychosocial implications, parental decision-making processes, or patient-reported outcomes.

To address these limitations, future research should expand the study to larger, multicenter cohorts to improve generalizability. Conduct prospective, longitudinal studies to track pubertal development, fertility, and long-term quality of life. Integrate advanced genetic and molecular data to refine the KCS component. Develop standardized assessment protocols to minimize inter-clinician variability. Incorporate patient-reported outcomes and ethical considerations to ensure CDCS is aligned with holistic DSD management.

**CONCLUSION**

The CDCS is the first fully integrative, evidence-based, and predictive scoring system in DSD management. Its ability to correlate with existing classification methods while offering a superior risk-stratification model highlights its novelty. Most DSD scoring systems are static, focusing only on initial diagnosis. CDCS is dynamic, allowing tracking of disease progression, endocrine response, and surgical/hormonal outcomes over time, and reassessment at puberty, guiding future reproductive and endocrine management. By providing clear surgical thresholds, long-term prognostic insights, and individualized patient care, CDCS sets a new standard in DSD diagnosis and management, surpassing previous classification systems in scope and clinical applicability. Future research should focus on validating CDCS across larger, multi-center cohorts and exploring its role in predicting long-term endocrine and reproductive outcomes.

**ETHICAL CONSIDERATIONS**

The institutional ethics committee approved of this study, and all patients or their legal guardians provided written informed consent. For minors, both parental consent and patient assent were obtained. Patient confidentiality was maintained, with personal data anonymized. No experimental treatments were performed beyond standard clinical care.

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