STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN (SAP)

Official Title: Evaluation of Benign Joint Hypermobility in Children Diagnosed with Attention Deficit Hyperactivity Disorder and Comparison of Serum Prolidase Level with Healthy Children Group
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1. Study Protocol Summary

1.1 Objective & Background

Attention-Deficit/Hyperactivity Disorder (ADHD) is increasingly recognized not merely as a localized disorder of executive function, but as a systemic condition with pervasive somatic comorbidities. Children with ADHD frequently exhibit chronic musculoskeletal pain and joint hyper-flexibility somatic burdens routinely misattributed to behavioral hyperactivity. This study investigates the neurodevelopmental and connective tissue overlap in pediatric ADHD by evaluating the prevalence of Benign Joint Hypermobility Syndrome (BJHS) and assessing serum prolidase activity—a critical rate-limiting enzyme in extracellular matrix and collagen remodeling to validate somatic symptomology and identify a systemic biomarker.

1.2 Study Design & Method

A cross-sectional, controlled observational study was conducted with 171 age- and sex-matched pre-adolescent children (ages 6-12 years). The clinical cohort comprised:

- **ADHD Cohort:** 86 children diagnosed using comprehensive DSM-5 criteria.
- **Healthy Control Cohort:** 85 neurotypical healthy controls.

1.3 Clinical & Biochemical Assessments

- **Joint Hypermobility:** Objectively assessed utilizing the Beighton scoring system (clinical threshold ≥ 5).
- **Somatic Burden & Musculoskeletal Discomfort:** Quantified using Visual Analog Scales (VAS) and Likert severity scales.
- **Biochemical Analysis:** Fasting serum prolidase enzyme activity analyzed via Enzyme-Linked Immunosorbent Assay (ELISA).

2. Statistical Analysis Plan (SAP)

Data analysis was conducted using rigorous statistical protocols to ensure reproducibility and transparency for clinical registry reporting.

2.1 Normality and Descriptive Statistics

Normality of continuous variables was assessed via Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics were reported as:

- Means ± standard deviations (SD) or medians with interquartile ranges (IQR) for continuous data.
- Frequencies with percentages for categorical data.

2.2 Group Comparisons & Bivariate Analysis

- **Continuous Variables:** Group comparisons utilized the Independent Samples t-test for normally distributed data and the Mann-Whitney U test for non-parametric data.
- **Categorical Variables:** Categorical variables (e.g., BJHS prevalence) were analyzed using Pearson's Chi-Square or Fisher's Exact tests.
- **Correlations:** Bivariate relationships among Beighton scores, VAS pain severity, and prolidase levels were examined using Pearson or Spearman correlation coefficients.

2.3 Multivariable Modeling

A multivariable linear regression model was constructed to determine the independent predictive value of clinical hypermobility on serum prolidase activity, adjusting for potential confounders. Statistical significance was established a priori at $p < 0.05$.

Parameter / Variable	Statistical Test / Methodology	Clinical Threshold / Significance
Normality Assessment	Kolmogorov-Smirnov & Shapiro-Wilk	Determines parametric vs. non-parametric track
Group Comparisons (Continuous)	Independent Samples t-test / Mann-Whitney U	Alpha set at $p < 0.05$

Parameter / Variable	Statistical Test / Methodology	Clinical Threshold / Significance
Prevalence & Categorical Data	Pearson's Chi-Square / Fisher's Exact	BJHS prevalence verification
Bivariate Correlation	Pearson / Spearman Coefficients	Triad evaluation (Prolidase, BJHS, Beighton)
Independent Predictors	Multivariable Linear Regression	Adjusting for potential baseline confounders