

## Plasma Heparin Levels in Healthy Children from Chandigarh, Northern India

Heparin is a key molecule involved in iron homeostasis. We measured heparin levels in 50 healthy children from Chandigarh, Northern India for establishing normal ranges. Heparin ranges (19.96-36.6 ng/mL; 0-2 years) and (9.54-36.15 ng/mL; 2-6 years) with mean (SD) of 32.5 (4.84) ng/mL, and 31.13 (6.62) ng/mL respectively were noted in study participants. The mean (SD) and ranges for plasma heparin in boys and girls in the study was 31.01 (6.71) ng/mL (9.54-36.6 ng/mL) and 32.7 (4.14) ng/mL (19-36.2 ng/mL), respectively.

**Keywords:** Anemia, Diagnosis, Hemoglobin, Iron deficiency.

Heparin is a 25-amino acid peptide that regulates systemic iron flow by binding to ferroportin and inducing its internalization and degradation [1-3]. Heparin can be detected in both serum and urine, and is being increasingly utilized as a marker for detecting iron-refractory iron deficiency anaemia (IRIDA). However, before the same can be utilized in the clinical context, it is important to establish normal ranges in different age groups and populations. Median (ranges) for reference human serum heparin levels in adults based on immunoassay analysis are 112 (29-254) ng/mL for men and 65 (17-286) ng/mL for women [4]. The literature highlights a relatively lower reference range in children as compared to adults (**Table I**).

We screened children from OPD/Vaccinology clinic, and those with normal hemogram, RBC indices, ESR and/or CRP were enrolled. 2ml peripheral blood sample in EDTA vacutainers was taken after informed consent; plasma

was separated after centrifugation at 1500rpm for 10 minutes and stored at -20°C for ferritin and heparin analysis. Ferritin was performed by Chemiluminescence assay (Siemens Immulite Ferritin kit) and heparin by sandwich ELISA assay (Human Hep 25 ELISA Kit; Sincere Biotech). Standards were run in duplicate, and readings were read at 450 nm in an ELISA reader.

For group comparison of continuous variables with normal distribution, Student's t test was applied. Data analysis was done on SPSS version 22.0. The study was approved by the Institute's Ethics committee.

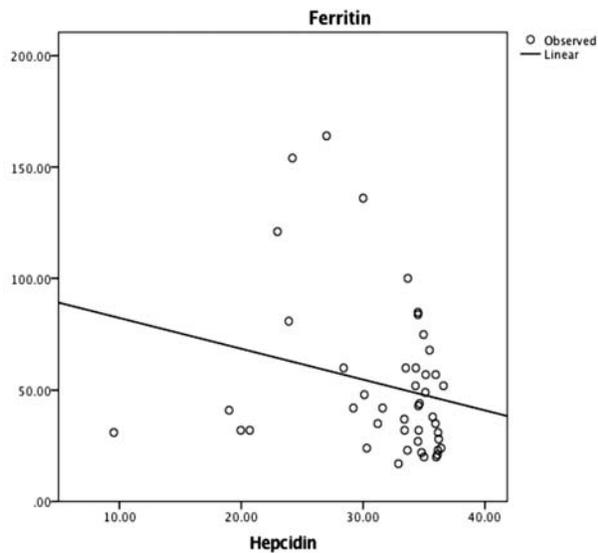
We enrolled 50 cases and divided them into two age groups (0-2 years-group I) and (2-6 years-group II). However, 5/50 (10%) cases were found to have a low ferritin value (range 0.2-5.75 ug/L), and hence were not included considering early iron deficiency state. Of remaining 45 cases, 21 (47%) were in group I and 24 (53%) in group II. The mean (SD) ferritin in study was 59.9 (35.22) ug/L with a range of 17-164 ug/L. The mean (SD) heparin levels in our study were 31.7 (5.83) ng/mL with range 9.5-36.6 ng/mL. In group I, the mean (SD) heparin levels were 32.5 (4.84) ng/mL (range 19.96-36.6 ng/mL) and in group II, mean (SD) were 31.1 (6.62) ng/mL (range 9.54-36.4 ng/mL). There was no statistically significant difference in serum heparin ranges between groups I and II. The mean (SD) and ranges for plasma heparin in boys and girls in the study was 31.0 (6.71) ng/mL (9.54-36.6 ng/mL) and 32.7 (4.14) ng/mL (19-36.2 ng/mL), respectively and this difference was also not statistically significant. The correlation between plasma heparin and ferritin levels was not statistically significant (**Fig. 1**) ( $r=0.054$ ).

The mean heparin levels and ranges in our pilot study in pediatric age were low as compared to adult reference ranges, but were in accordance with other pediatric

**TABLE I** HEPARIN REFERENCE RANGES/MEANS IN DIFFERENT STUDIES ON CHILDREN

Study	Number of children (age range)	Heparin Mean (SD), (ranges) ng/mL
Choi HS, <i>et al.</i> [6]	24 (5 months-17 yrs)	16.71 (14.74), (3.24- 66.86)
Jaeggi T, <i>et al.</i> [5]	29 boys and 39 girls (Infants)	6.4 (3.4), (0.2- 50.6)
Cangemi G, <i>et al.</i> [7]	86 (1-18 yrs)	40.8 (13.9), (13.6-68)
Sdogou T, <i>et al.</i> [8]*	180 (2-12 yrs)	Median- 46.94 for boys and 46.79 for girls
Chauhan R, <i>et al.</i> [9]	70 (0-12 yrs)	14.47 (11.68), (0.9-60)
Current Study	45 (0-6 yrs)	31.69 (5.83), (9.54- 36.6)

\*data on mean (SD) and ranges not available.



**FIG. 1** Scatter plot of correlation between hepcidin and ferritin ( $r= 0.054$ ).

studies. It has been seen that infants around 2-4 months have lowest hepcidin values due to onset of physiological anaemia and values are more so lower in pre-term infants [5]. However, in our study there was no significant difference in levels across the 0-6 year age group. This variability needs to be assessed in large studies.

Result of this pilot study is likely to be helpful in interpreting hepcidin results in clinical context in future studies on the biomarker, as it provides with normal pediatric ranges for plasma hepcidin in our population. However, the study is limited by a small sample size, and stresses on the need for further larger prospective multicentric or community-based studies in different pediatric age groups.

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