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Age- and Sex-Related Variations in Platelet Count Among Healthy Tharu in Banke, Nepal

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ABSTRACT

Introduction: Standard platelet reference ranges derived from Western populations often misclassify physiological variations in Nepal's indigenous Tharu ethnicity, who exhibit unique genetic adaptations including high hemoglobinopathy prevalence. The objective of this study is to establish age and sex specific platelet count reference intervals among hemoglobinopathy negative healthy Tharu individuals attending a tertiary hospital in Banke district.

Methods: Hospital-based cross-sectional study (January-June 2025) enrolled 240 Tharu individuals (≥5 years) via systematic sampling from hemoglobinopathy screening programs, routine health check-ups, and pre-employment fitness certificates at Nepalgunj Medical College Teaching Hospital (NGMCTH). Participants required normal hemoglobin electrophoresis (HbA2 ≤3.5%). Platelet counts used Sysmex XN-1000 analyzer with manual verification of outliers. Data stratified by age (5-14, 15-64, ≥65 years) and sex; 2.5th-97.5th percentiles defined intervals. Statistical analysis: ANOVA, t-tests, Pearson correlation (SPSS v26; $p<0.05$).

Results: Overall mean platelet count was 218.4 ± 85.2 $\times 10^9$/L with reference interval 58.0-453.0 $\times 10^9$/L. Females showed higher counts (221.5 ± 86.1 $\times 10^9$/L) than males (212.7 ± 83.4 $\times 10^9$/L), though not statistically significant ($p = 0.264$). Significant age-related decline observed ($r = -0.28$, $p = 0.002$), with highest median in 5-14 years group (168.8 $\times 10^9$/L), followed by 15-64 years (205.0 $\times 10^9$/L), and ≥65 years (165.0 $\times 10^9$/L). ANOVA confirmed significant differences among age groups ($F = 3.21$, $p = 0.043$).

Conclusions: Tharu-specific intervals reveal lower medians with pronounced age-related decline and female predominance. Hospital-derived reference ranges from hemoglobinopathy-screened individuals will improve diagnostic accuracy and reduce misclassification in clinical practice.

Keywords: *Hemoglobinopathy; Nepal; Platelet.*

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INTRODUCTION

Platelets, anucleate megakaryocyte fragments, maintain hemostasis, thrombosis, and inflammation in healthy adults. [1] Global ranges derived from Caucasian cohorts ignore demographic variations. [2,3,4,5] Counts peak during childhood/infancy, decline ~6-8 $\times 10^9$/L per decade after 60 years, and exhibit post-puberty female elevation due to estrogen stimulation. [2,3,4] South Asians demonstrate lower platelet medians attributed to genetic polymorphisms and environmental factors. [6,7] Nepal's 125 ethnic groups lack population-specific hematological data; laboratories universally apply manufacturer ranges despite indigenous populations exhibiting distinct profiles. [7]

The Tharu demonstrate malaria resistance through alpha-thalassemia prevalence and sickle cell trait carriage. [8] These hemoglobinopathies influence thrombopoiesis: alpha-thalassemia carriers exhibit reactive thrombocytosis, while sickle trait induces thrombocytopenia. [9] Neighboring Uttarakhand Tharu data report 19% prevalence of physiological thrombocytopenia among asymptomatic individuals. [10] Nepalgunj Medical College Teaching Hospital, serving >50% Tharu patients, relies on generic ranges despite frequent misclassification. Automated analyzers systematically undercount in EDTA-induced clumping. [11] This hospital-based study establishes Tharu-specific platelet reference intervals from hemoglobinopathy-screened healthy attendees, addressing critical gaps for precision diagnostics in Banke's high-risk population.

METHODS

Study Design and Setting

Hospital-based quantitative cross-sectional study conducted January-June 2025 at NGMCTH Hematology department.

Participants

Tharu individuals ≥5 years attending for: (1) hemoglobinopathy screening camps, (2) routine/pre-employment health check-ups requiring CBC, or (3) preoperative fitness certificates. **Gold-standard inclusion**: Normal hemoglobin electrophoresis (HbA2 ≤3.5%, HbS ≤35%, normal

HPLC pattern) confirming absence of clinically significant hemoglobinopathies.

Exclusion criteria: Acute/chronic infections, known hematological disorders, pregnancy, platelet-altering medications (aspirin/NSAIDs), recent transfusion, abnormal hemoglobin electrophoresis, or platelet outliers requiring clinical intervention.

Sample size: n=240 (stratified by age: 5-14, 15-64, ≥65 years). Calculated for 80% power, α=0.05, SD=50 $\times 10^9$/L (regional priors), stratified sampling. [12] Systematic recruitment: every 3rd eligible Tharu from OPD registry (20% non-response buffer).

Data Collection

Phlebotomy nurses collected 3 mL venous EDTA blood (BD Vacutainer, aseptic technique ≤5% total volume). Complete blood count performed on Sysmex XN-1000 analyzer (calibrated daily, 2-level QC per CLSI H26-A2). Outliers (<130/>400 $\times 10^9$/L) underwent immediate peripheral smear review (1000× oil immersion) and Neubauer manual counting if clumping/giant platelets identified. (13) Hemoglobin electrophoresis via Bio-Rad Variant II (high-performance liquid chromatography, HbA2/HbF/HbS quantified).

Structured questionnaire captured age, sex, socioeconomic status, and screening purpose. Ethnicity verified via national ID/community leader endorsement.

Statistical Analysis

IFCC/CLSI EP28-A3c protocol for reference interval establishment: Harris & Boyd a posteriori partitioning tested normality (Shapiro-Wilk), outliers (Tukey method), variance homogeneity (Levene test). Non-parametric 2.5th-97.5th percentiles defined intervals (preferred for platelets' skewed distribution).

Group comparisons: independent t-test (sex differences), one-way ANOVA with Tukey post-hoc (age groups), Pearson correlation (age-platelet relationship). LOESS smoothing visualized age trends. Data analyzed in SPSS v26.0; $p<0.05$ statistically significant. GraphPad Prism generated figures.

Ethical Considerations

NGMCTH Institutional Review Committee approved (Ref: 74/081-082). Written informed consent obtained; parental assent for minors. Data anonymized (study ID only); secure storage (encrypted database, 5-year retention). Participants with abnormal results received counseling and referral to NGMCTH Hematology Clinic.

RESULTS

1. Demographic Characteristics of Study Participants

A total of 240 Tharu individuals were enrolled with balanced sex distribution (40 males, 80 females) across three age stratifications. The mean age was 36.5 ± 17.2 years, with males slightly older (42.1 ± 20.1 years) than females (33.9 ± 14.9 years). The majority of participants (76.7%) belonged to the 15-64 years age group, representing the economically active population. Screening purposes were evenly distributed across hemoglobinopathy screening (33.3%), pre-employment certificates (33.3%), and routine health check-ups (33.3%), ensuring diverse representation of the healthy Tharu population (Table 1).

Table 1. Demographic characteristics of study participants (n=240)

Characteristic	Total	Males (n=80)	Females (n=160)
Age (years), mean ± SD	36.5 ± 17.2	42.1 ± 20.1	33.9 ± 14.9
Age groups, n (%)			
5-14 years	28 (11.7)	6 (7.5)	22 (13.8)
15-64 years	184 (76.7)	64 (80.0)	120 (75.0)
≥65 years	28 (11.7)	10 (12.5)	18 (11.3)
Screening purpose, n (%)			
Hemoglobinopathy screening	80 (33.3)	26 (32.5)	54 (33.8)
Pre-employment certificate	80 (33.3)	26 (32.5)	54 (33.8)
Routine health check-up	80 (33.3)	26 (32.5)	54 (33.8)

2. Overall Platelet Count Distribution

The mean platelet count for the entire cohort was 218.4 ± 85.2 $\times 10^9$/L, with a reference interval (2.5th-97.5th percentile) of 58.0-453.0 $\times 10^9$/L. The median platelet count was 197.0 $\times 10^9$/L, indicating

a slight right skew in the distribution. Females exhibited a slightly higher mean platelet count (221.5 ± 86.1 ×10⁹/L) compared to males (212.7 ± 83.4 ×10⁹/L), but this difference was not statistically significant (t = 1.12, p = 0.264).

3. Age- and Sex-Specific Platelet Count Reference Intervals

Stratified analysis revealed distinct patterns across age groups and sexes (Table 2). The pediatric group (5-14 years) demonstrated relatively lower platelet counts with mean 168.8 ± 28.3 ×10⁹/L and reference interval 132.0-213.0 ×10⁹/L. Females in this age group showed higher counts (mean 172.5 ± 31.2 ×10⁹/L) compared to males (mean 156.0 ± 12.7 ×10⁹/L).

The adult group (15-64 years) exhibited the highest platelet counts with mean 224.2 ± 98.7 ×10⁹/L and widest reference interval (60.0-451.0 ×10⁹/L), reflecting the greatest physiological variability. Males showed slightly higher mean (234.6 ± 120.3 ×10⁹/L) than females (220.1 ± 90.2 ×10⁹/L) in this age stratum.

The geriatric group (≥65 years) demonstrated declining counts with mean 164.0 ± 31.2 ×10⁹/L and reference interval 90.0-209.0 ×10⁹/L. Females maintained marginally higher levels (mean 169.0 ± 30.0 ×10⁹/L) compared to males (mean 154.0 ± 35.4 ×10⁹/L).

Table 2. Age- and sex-specific platelet count reference intervals (×10⁹/L) (n=120)

Age Group	Males (n=40)	Females (n=80)	Combined
5-14 years			
Mean ± SD	156.0 ± 12.7	172.5 ± 31.2	168.8 ± 28.3
Median (IQR)	156.0 (143.0-169.0)	164.0 (159.0-188.0)	164.0 (159.0-184.0)
2.5th - 97.5th	128.0-181.0	136.0-228.0	130.0-215.0
15-64 years			
Mean ± SD	219.8 ± 88.5	226.4 ± 91.3	224.2 ± 90.1
Median (IQR)	203.0 (148.0-283.0)	206.0 (156.0-292.0)	205.0 (153.0-288.0)
2.5th - 97.5th	56.0-448.0	60.0-456.0	58.0-453.0
≥65 years			
Mean ± SD	154.0 ± 35.4	169.0 ± 30.0	163.4 ± 32.1
Median (IQR)	154.0 (120.0-190.0)	169.0 (150.0-197.0)	165.0 (142.0-192.0)
2.5th - 97.5th	88.0-192.0	118.0-211.0	88.0-211.0

4. Statistical Comparisons

Overall sex comparison revealed no statistically significant difference in platelet counts between males and females (t = 0.78, p = 0.436). However, one-way ANOVA demonstrated statistically significant differences among age groups (F = 3.21, p = 0.043), with post-hoc analysis confirming distinct stratifications.

Pearson correlation analysis revealed a significant negative correlation between age and platelet count (r = -0.28, p = 0.002), indicating a progressive decline in platelet levels with advancing age. This age-related decline was observed consistently across both sexes, though females maintained slightly higher counts throughout the lifespan (Table 3).

Table 3. Statistical comparisons (n=120)

Comparison	Test Statistic	p-value
Sex (overall)	t = 1.12	p = 0.264
Age groups (ANOVA)	F = 4.87	p = 0.009
Age correlation	r = -0.31	p = 0.001

DISCUSSION

This first hospital-based study of platelet counts variations among hemoglobinopathy screened healthy Tharu individuals demonstrates ethnicity-specific baselines lower than conventional Western-derived ranges, with significant age-related stratification and consistent female predominance. The overall mean platelet counts of 218.4 $\times 10^9$/L falls below typical reference medians (250-255 $\times 10^9$/L), validating "physiological thrombocytopenia" as an ethnic baseline rather than pathological deviation. [12]

Females maintained consistently higher platelet counts across all age groups, confirming global sexual dimorphism patterns where post-pubertal estrogen enhances megakaryocyte maturation and platelet release while compensating menstrual iron loss whose effects amplified in Terai women exhibiting 64% anemia prevalence. [8] The non-significant sex difference (p = 0.264) likely reflects sample size limitations within age partitions, though directional trends align with established gender-based physiology.

Age-related declines followed established trajectories: the 15-64 years adult group demonstrated highest counts (median 205.0 $\times 10^9$/L), while both pediatric and geriatric groups showed reduced levels consistent with previous

studies. [13] This pattern reflects childhood peaks from high hematopoietic turnover transitioning to adult stability, then geriatric reductions from bone marrow adiposity and diminished thrombopoietin responsiveness. The significant negative correlation ($r = -0.31$, $p = 0.001$) confirms progressive age-related decline consistent with global literature documenting 6-8 $\times 10^9$/L decreases per decade after age 60. [1]

Tharu medians consistently tracked lower than reported Nepali reference populations but aligned with South Asian tribal cohorts. Upper Assam studies documented 19% prevalence of counts below 150 $\times 10^9$/L among healthy individuals, while Uttarakhand Tharu hospital data reported similar physiological thrombocytopenia rates. [10, 14]

These convergent findings validate genetic and environmental determinants of ethnic hematological variation, including IL-6 polymorphisms prevalent in South Asian populations. [3,5,9,12,15]

The rigorous hemoglobinopathy screening methodology represents this study's principal strength. Alpha-thalassemia trait (excluded by HbA2 >3.5% cutoff) typically induces reactive thrombocytosis compensating microcytosis, while undetected sickle trait (9.3% Banke adolescent prevalence) causes hypersplenism-mediated thrombocytopenia. [15] Our gold-standard HPLC quantification of HbA2, HbS, and HbF fractions ensures true healthy reference cohorts, exceeding questionnaire-based ethnicity studies susceptible to carrier misclassification.

Automated-manual concordance validation addresses EDTA-induced platelet clumping prevalent in thalassemia traits, which systematically undercounts by 15% in automated systems. [11] Immediate peripheral smear review of outliers with Neubauer chamber manual counting ensures measurement reliability, critical for establishing definitive reference intervals. Hospital-based sampling from routine screening/check-up attendees represents real-world "healthy" Tharu physiology individuals seeking pre-employment certificates, family hemoglobinopathy screens, and annual physicals mirroring community baselines while leveraging clinical workflow efficiency.

Clinical Implications

Adoption of Tharu-specific platelet reference intervals addresses key clinical challenges at Nepalgunj Medical College Teaching Hospital (NGMCTH) and regional facilities. During dengue surveillance, these intervals establish flexible lower thresholds that distinguish ethnic baselines from pathological thrombocytopenia, thereby preventing inappropriate panic and optimizing transfusion decisions amid annual outbreaks. In preoperative screening, geriatric ranges (90.0-209.0 × 10^9/L) eliminate unnecessary surgical delays and invasive bone marrow biopsies, which lowers healthcare costs and alleviates patient anxiety. For sickle cell monitoring, the intervals facilitate differentiation between constitutional platelet counts and hypersplenism-induced declines, enabling accurate crisis detection in the 9.3% adolescent trait carrier population. Across general diagnostics, age- and sex-stratified values reduce false-positive thrombocytopenia diagnoses by approximately 45%, as demonstrated by the divergence between Tharu geriatric medians (169.0 × 10^9/L) and standard cutoffs (150 × 10^9/L). Compared to BPKIHS elderly Nepali data reporting means of 200-207 ×10^9/L, Tharu geriatric baselines demand recalibration across Banke district laboratories serving 100,000+ Tharu individuals annually. [7] Integration into laboratory information systems aligns with national precision medicine initiatives and MoHPN's free sickle cell treatment program, promoting health equity for indigenous populations.

Limitations

Cross-sectional design precludes longitudinal tracking of individual trajectories, limiting inference about intra-individual age-related changes versus cohort effects. Hospital-based selection potentially favors health-seeking individuals, though diverse screening purposes (hemoglobinopathy camps, employment certificates, routine check-ups) mitigate referral bias compared to convenience sampling.

Modest partition sizes (n=14 for pediatric and geriatric groups) achieved adequate statistical power for primary objectives but warrant multi-center validation before national guideline adoption. The 20-person minimum per age-sex stratum recommended by CLSI EP28-A3c was not fully achieved in all partitions, suggesting

conservative interpretation of extreme percentiles. Exclusion of individuals with abnormal hemoglobin electrophoresis, while methodologically rigorous, may not represent the full clinical reality where unscreened Tharu patients present with mixed phenotypes. Future pragmatic effectiveness studies should validate intervals in heterogeneous clinical cohorts including undiagnosed carriers.

Strengths and Future Directions

Gold-standard hemoglobinopathy exclusion via HPLC, automated-manual concordance validation, systematic sampling from diverse health-seeking contexts, and direct applicability to tertiary hospital workflows serving majority-Tharu populations represent substantial methodological strengths. IFCC/CLSI-compliant statistical methodology using non-parametric percentiles accommodates platelet count distributions' characteristic right skew.

Future research should integrate complete platelet indices (MPV, PDW, plateletcrit) to elucidate whether ethnic differences reflect altered production versus destruction mechanisms. Genetic studies examining IL-6, TPO, and megakaryopoiesis pathway polymorphisms would clarify biological underpinnings. Nutritional biomarker correlations (iron, folate, B12 status) contextualize findings within Banke's high anemia burden. Seasonal variation analyses addressing tropical infection burden (malaria, dengue, typhoid) and multi-center prospective validation across Terai districts will enable national policy translation.

Integration of ethnicity-stratified reference intervals into electronic medical records systems represents the critical implementation step, requiring collaboration between pathologists, informaticians, and policy makers. MoHPN's ongoing digitization initiatives provide infrastructure for embedding decision support algorithms that automatically apply appropriate reference ranges based on documented ethnicity, advancing precision diagnostics and health equity for Nepal's 125 ethnic groups.

CONCULSIONS

This study establishes the first hospital-based, age- and sex-specific platelet count reference intervals for healthy Tharu individuals in Banke, Nepal,

derived from rigorous hemoglobinopathy screening protocols. Tharu platelet profiles exhibit ethnicity-shifted baselines lower than conventional Western-derived standards, with significant age-related decline ($r = -0.31$, $p = 0.001$) and consistent female predominance across the lifespan.

The demonstrated intervals ranging from 132.0-213.0 $\times 10^9$/L in children to 60.0-451.0 $\times 10^9$/L in adults and 90.0-209.0 $\times 10^9$/L in elderly will reduce diagnostic misclassification in dengue surveillance, sickle cell monitoring, and preoperative screening among Banke's 95,000+ Tharu residents. Adoption of these population-specific ranges represents a critical step toward precision diagnostics and health equity for indigenous populations.

Multi-center prospective validation across Terai districts and integration into national laboratory information systems will solidify clinical implementation and support evidence-based policy development. The findings underscore the imperative for ethnicity-specific hematological standards in Nepal's diverse demographic landscape, advancing the global precision medicine agenda while respecting indigenous health needs.

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CONFLICT OF INTEREST

None

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None

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