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ORIGINAL ARTICLE

Longitudinal model of infant hemoglobin: evidence of chained mediation in a peruvian cohort

Modelo longitudinal de hemoglobina infantil: evidencia de mediación en cadena en una cohorte peruana

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What do we know about the subject matter of this study?

Hemoglobin in children undergoes dynamic changes influenced by multiple factors, but most studies are based on cross-sectional analyses without considering longitudinal trajectories or mediational models.

What does this study contribute to what is already known?

This study is the first to apply a chain mediation model to describe the sequential trajectory of hemoglobin in children under three years of age in Latin America. It provides a novel methodological perspective for characterizing hematological trajectories. The identification of critical periods, such as the third month, offers a conceptual framework that could be explored in future research aimed at epidemiological surveillance and the validation of pediatric biomarkers.

Abstract

In Peru, the persistence of childhood anemia despite interventions reveals gaps in understanding its longitudinal progression. **Objective:** To evaluate hemoglobin evolution in children under three years of age using a chain mediation model, identifying the role of intermediate measurements on subsequent values. **Patients and Method:** Analytical longitudinal study using secondary data from hemoglobin screenings in children under three years from Junín, Peru. A total of 2,564 children with four consecutive hemoglobin measurements (baseline, 1, 3, and 6 months) were included. Hemoglobin (Hb) concentrations were the primary variables, with age as a covariate. A chain mediation model was applied with 5,000 bootstrap resamples. A directed acyclic graph was developed to represent the proposed causal structure. **Results:** Baseline Hb was significantly associated with subsequent measurements. It strongly predicted Hb at the first month (B = 0.8743; 95%CI: 0.8482–0.9004), and along with this, explained Hb levels at three months (B = 0.3796 and 0.5296; $p < 0.001$). At six months, all three prior measurements were significant predictors, with Hb carrying the greatest weight at three months (B = 0.3672; 95%CI: 0.3296–0.4048). Age showed a positive effect at all stages. The chain mediation model revealed both direct and indirect effects of baseline Hb on Hb levels at six

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months (total effect = 0.4508), highlighting key mediation pathways through Hb at 1 and 3 months. **Conclusions:** Baseline hemoglobin predicts levels at six months through direct and indirect pathways, underscoring the value of sequential monitoring and the third month as a critical point for intervention.

Introduction

Childhood anemia continues to be a public health problem in many low- and middle-income countries, particularly in Latin America[1]. In Peru, the prevalence of anemia in children under three years of age has been persistently high, with prevalences greater than 30% in children under five years of age[2], despite government interventions for its prevention and treatment. Hemoglobin (Hb), an essential biomarker in the evaluation of hematological status, undergoes dynamic changes from the first months of life, influenced by nutritional, infectious, genetic, and socioeconomic factors[3].

Most studies on Hb in childhood have focused on cross-sectional analyses or bivariate comparisons[4,5], without adequately considering the longitudinal trajectory or the cumulative nature of measurements over time. This approach limits our understanding of the mechanisms by which initial Hb values influence future concentrations, a crucial aspect for optimizing timely and effective intervention strategies. Furthermore, no previous studies have been identified that apply chain mediation models to the sequential monitoring of Hb in pediatric cohorts in Latin America, reinforcing the novelty of this analytical approach in the regional context.

In this regard, longitudinal modeling with a chain mediation approach will allow us to evaluate whether the initial Hb value influences subsequent values, transmitted through intermediate measurements at 1 and 3 months. In this context, this study aimed to model the trajectory of Hb in a cohort of Peruvian children under three years of age, examining how these intermediate measurements mediate the relationship between the baseline concentration and that recorded at 6 months. This analysis seeks to identify sequential routes of influence with physiological implications related to the progressive maturation of the hematopoietic system and clinical implications, providing useful evidence to reinforce monitoring and optimize nutritional or therapeutic interventions at critical windows of child development, promoting sustained hematological outcomes in the Peruvian population.

Patients and Method

Design and population

Longitudinal analytical study based on secondary data available on the open data platform of the Peruvian Ministry of Health (MINSA), from the period between January 2022 and June 2024. The database includes systematic screening for childhood anemia in children under 36 months of age in the Junín region, located in the central part of the country at more than 4,100 meters above sea level[6]. In this region, poverty reaches approximately 27%, extreme poverty 4%, and nearly 40% of the population lives in conditions of economic vulnerability. According to the official poverty line in Peru (2023), this equates to a monthly per capita income of around 415 soles (~$110/€100) and, in the case of extreme poverty, around 228 soles (~$60/€55)[7].

Hb screenings were performed in accordance with the national protocol, beginning between 6 and 12 months of age and repeated 30 days, 3 months, and 6 months after each respective check-up. The measurements were carried out in health facilities belonging to nine provinces of the department: Chanchamayo, Chupaca, Concepción, Huancayo, Jauja, Junín, Satipo, Tarma, and Yauli. In Peru, for the prevention of childhood anemia, ferrous sulfate drops are used in children under 6 months of age (full-term from 4 months; premature/low birth weight from 30 days; ~2 mg Fe/kg/day until 6 months) and multiple micronutrient powders are used in children aged 6-35 months (1 sachet/day, ~12.5 mg Fe)[8]. Adherence to population-based programs has been low to moderate (≈ 24% in reports) but improves with follow-up and counseling[9].

For this study, only records of children with four consecutive complete Hb measurements were included: at baseline (initial measurement performed between 6 and 12 months of age according to national protocol), at 1 month, at 3 months, and at 6 months after the initial measurement. Cases with missing data in any of these measurements, records with chronological inconsistencies between measurements, and extreme Hb values (< 5 g/dL or >18 g/dL) were excluded, as they were considered recording errors or clinically implausible values. It should be noted that the database does not have information on prematurity; however, by starting follow-up at 6 months of age, the possible effect of neonatal iron deposits and perinatal conditions is minimized.

The initial total of the database was 12,486 children, and after applying the selection criteria, a final sample of 2,546 with complete and valid Hb trajectories for longitudinal analysis was obtained.

This sample size allowed the application of chain mediation statistical models with robust power, especially when using bootstrap resampling with 5,000 samples. According to the methodological literature, a sample of more than 500 subjects is sufficient to estimate direct and indirect effects in multivariate mediation models with stability[10], so the size achieved in this study guarantees the reliability of the estimates obtained.

Variables and measurements

The variables considered in the study included Hb concentrations measured at four points during follow-up: initial Hb (baseline measurement), Hb at one month, three months, and six months. Hemoglobin was determined using standardized techniques reported in the Health Information System of the Peruvian Ministry of Health (HIS MINSA). The age of the child was defined as the time interval in months between the date of the first Hb measurement and the date of birth and was included as a control covariate in order to consider the heterogeneity in the age of admission of the children, without forming part of the mediation chain.

All measurements were recorded on specific dates corresponding to each follow-up point (initial, 1 month, 3 months, and 6 months), allowing for a detailed analysis of hematological trajectories during early childhood.

Statistical analysis

To evaluate the longitudinal Hb trajectory in children under three years of age, a chain mediation model (model 6) was implemented using Andrew F. Hayes' PROCESS statistical procedure version 4.1 for SPSS, which allows simultaneous examination of direct and indirect sequential effects between variables measured at different points in time[11]. This approach facilitates the identification of cumulative trajectories, where intermediate measurements act as mediators in the relationship between the initial and subsequent measurements.

Successive hierarchical regression analyses were performed to assess the influence of Hb at each time point (1 month, 3 months, and 6 months after the first Hb measurement). At each stage, regression coefficients, standard errors, t-values, significance levels, and coefficients of determination (R^2) were estimated to quantify the proportion of variance explained.

For the chain mediation analysis, a bootstrap approach with 5,000 resamples was applied to obtain robust 95% confidence intervals for indirect effects, considering those whose intervals did not include zero to be statistically significant. This nonparametric method improves the accuracy of the estimate and the inferential validity in longitudinal samples. Additionally, a directed acyclic graph (DAG) was constructed, developed on the Dagitty.net website to graphically represent the causal structure proposed in the model, visualizing the direct and indirect relationships between Hb measurements and age, and facilitating the interpretation of the mediating pathways evaluated. All analyses were performed considering a statistical significance level of $p < 0.05$.

Ethical considerations

This study used anonymized, publicly accessible secondary data from the Junín Regional Health Directorate, available on the National Open Data Portal under the Open Data Commons Attribution License, within the framework of Legislative Decree No. 1412 (Digital Government Law). According to Memorandum No. 001-2023-UDT-OTIC/INS of the National Institute of Health, no additional authorization is required for the use of data published on this platform if confidentiality and personal data protection are respected. Since the data does not contain identifiable information or involve direct intervention on individuals, no informed consent or evaluation by an ethics committee was required. The principles of the Declaration of Helsinki and the Peruvian Personal Data Protection Law (Law No. 29733) were followed.

The database, as well as the supplementary data, are available at the following link: https://datosabiertos.gob.pe/dataset/ni%C3%B1os-menores-de-36-meses-con-dx-de-anemia-en-la-regi%C3%B3n-jun%C3%ADn-direcci%C3%B3n-regional-de-salud.

Results

In the sample, Hb showed a progressive increase from a mean of 11.2 g/dL at baseline to 11.5 g/dL at one month, 11.8 g/dL at three months, and 12.5 g/dL at six months. Variability remained stable (SD 1.0-1.2 g/dL). The minimums rose from 6.8 to 8.6 g/dL and the maximums from 15.0 to 16.3 g/dL, indicating an overall shift towards higher values throughout the follow-up. The mean age at the first measurement was 9.5 months (SD 6.1; range 6.1-33.3 months), reflecting the inclusion of infants and young children (Table 1).

Hierarchical regression analyses showed a strong and progressive association between initial Hb levels and subsequent values. Hb at one month was strongly determined by the baseline value, suggesting early stability of the biomarker. At three months, both initial Hb and Hb at one month significantly predicted the observed values, forming a cumulative and mediating dynamic. At six months, all previous measurements were significant predictors, with Hb at three months standing out as a key mediator. These findings support a structured hematological trajectory, in which intermediate values act as essential links according to the chain mediation model (Table 2).

The chain mediation model showed that initial Hb is associated with Hb at 6 months both directly (B = 0.2686; 95% CI: 0.2233-0.3136; p < 0.001) and indirectly (total effect = 0.451; 95% CI: 0.4096-0.4935). Three significant indirect pathways were identified whose intervals do not include zero: initial Hb→Hb at 1 month→Hb at 6 months (B = 0.142; 95% CI: 0.0979-0.1876), initial Hb→Hb at 3 months→Hb at 6 months (B = 0.1387; 95% CI: 0.1140–0.1655), and the complete chain Initial Hb→Hb at 1 month→Hb at 3 months→Hb at 6 months (B = 0.1703; 95% CI: 0.1463-0.1971), the latter being the largest. Overall, the results highlight the relevance of intermediate measurements, especially at 3 months, in the trajectories leading to the Hb value at 6 months (Table 3).

Successive regression analyses showed that the longitudinal model explained a significant proportion of the variability in Hb levels. In the first month, it explained 60.3% ($R^2 = 0.6027$), increasing to 62.4% at three months ($R^2 = 0.6236$) when including the initial Hb and that of the first month. At six months, it explained 55.0% ($R^2 = 0.5496$), considering the three previous measurements. Although R^2 decreased slightly in this last stage, the model maintained a solid and significant explanatory capacity (p < 0.001), demonstrating the stability and progressive accumulation of the predictive value of Hb and supporting the chain mediation approach (Table 4).

The DAG summarizes the causal structure of the longitudinal chain mediation model, showing how initial Hb directly and indirectly influences Hb at six months in children under three years of age. Hb level at 1 and 3 months acts as a partial mediator, highlighting a complete chain pathway (initial Hb → Hb at 1 month → Hb at 3 months → Hb at 6 months) with clinically relevant indirect effects. Age was incorporated as a covariate, with a direct effect on all Hb measurements, controlling for possible confounders. This DAG visually synthesizes the theoretical model that allowed us to identify key intermediate trajectories and capture the progressive and cumulative nature of pediatric hematological development (Figure 1).

Table 1. Descriptive statistics of the studied sample from the database (n = 2,546)

Variable	Minimum	Maximum	Mean	Standard deviation
Initial Hb (g/dL)	6,8	15	11,2	1,1
Hb at 1 month (g/dL)	7,3	15,4	11,5	1,1
Hb at 3 months (g/dL)	7,9	15,8	11,,8	1,2
Hb at 6 months (g/dL)	8,6	16,3	12,5	1
Age at first Hb measurement (months)	6,1	33,3	9,5	6,1

Hb: Hemoglobin

Table 2. Successive regressions of the serial mediation model for childhood hemoglobin (PROCESS Model 6)

Dependent variable	Predictors	B	SE	t	p value	95% CI
Hb at 1 month after baseline measurement	Initial Hb	0,8753	0,0126	65,609	< 0,001	0,8480 – 0,9001
	Age	0,2288	0,0422	5,349	< 0,001	0,1458 – 0,3123
Hb at 3 months after baseline	Initial Hb	0,3804	0,0216	17,799	< 0,001	0,3368 – 0,4207
	Hb at 1 month	0,5292	0,0186	28,001	< 0,001	0,4934 – 0,5656
	Age	0,1932	0,0439	4,451	< 0,001	0,1088 – 0,2800
Hb at 6 months after baseline	Initial Hb	0,2674	0,0234	11,621	< 0,001	0,2223 – 0,3119
	Hb at 1 month	0,1612	0,0222	7,39	< 0,001	0,1186 – 0,2040
	Hb at 3 months	0,3668	0,0192	19,149	< 0,001	0,3288 – 0,4050
	Age	0,2526	0,0449	5,66	< 0,001	0,1664 – 0,3409

Hb: hemoglobin; B: unstandardized coefficient; SE: standard error; t: t value of the statistical test; 95% CI: 95% confidence interval; PROCESS: statistical macro for mediation and moderation analysis developed by Andrew F. Hayes.

Table 3. Direct and indirect effects of baseline hemoglobin on hemoglobin at 6 months

Type of effect	Effect (B)	Bootstrap SE	95% CI	p value
Direct effect	0,2686	0,0237	0,2233 – 0,3136	< 0,001
Total indirect effect	0,451	0,0218	0,4096 – 0,4935	—
Ind1: Baseline Hb → Hb at 1 month → Hb at 6 months	0,142	0,0225	0,0979 – 0,1876	—
Ind2: Baseline Hb → Hb at 3 months → Hb at 6 months	0,1387	0,0139	0,1140 – 0,1655	—
Ind3: Baseline Hb → Hb at 1 month → Hb at 3 months → Hb at 6 months	0,1703	0,0127	0,1463 – 0,1971	—

Hb: hemoglobin; B: unstandardized coefficient; SE: standard error; 95% CI: 95% confidence interval; Ind: indirect effect; Bootstrap: nonparametric resampling used to estimate standard errors and confidence intervals.

Table 4. Summary of explained variance at each stage of the longitudinal model

Dependent variable	R	R^2	MSE	F	p value
Hb at 1 month	0,7765	0,6027	0,8079	2167,631	< 0,001
Hb at 3 months	0,7892	0,6236	0,8265	1582,801	< 0,001
Hb at 6 months	0,7412	0,5496	0,8674	873,602	< 0,001

Hb: hemoglobin; R: multiple correlation coefficient; R^2: coefficient of determination; MSE: mean squared error; F: Fisher's F statistic; p value: level of statistical significance.

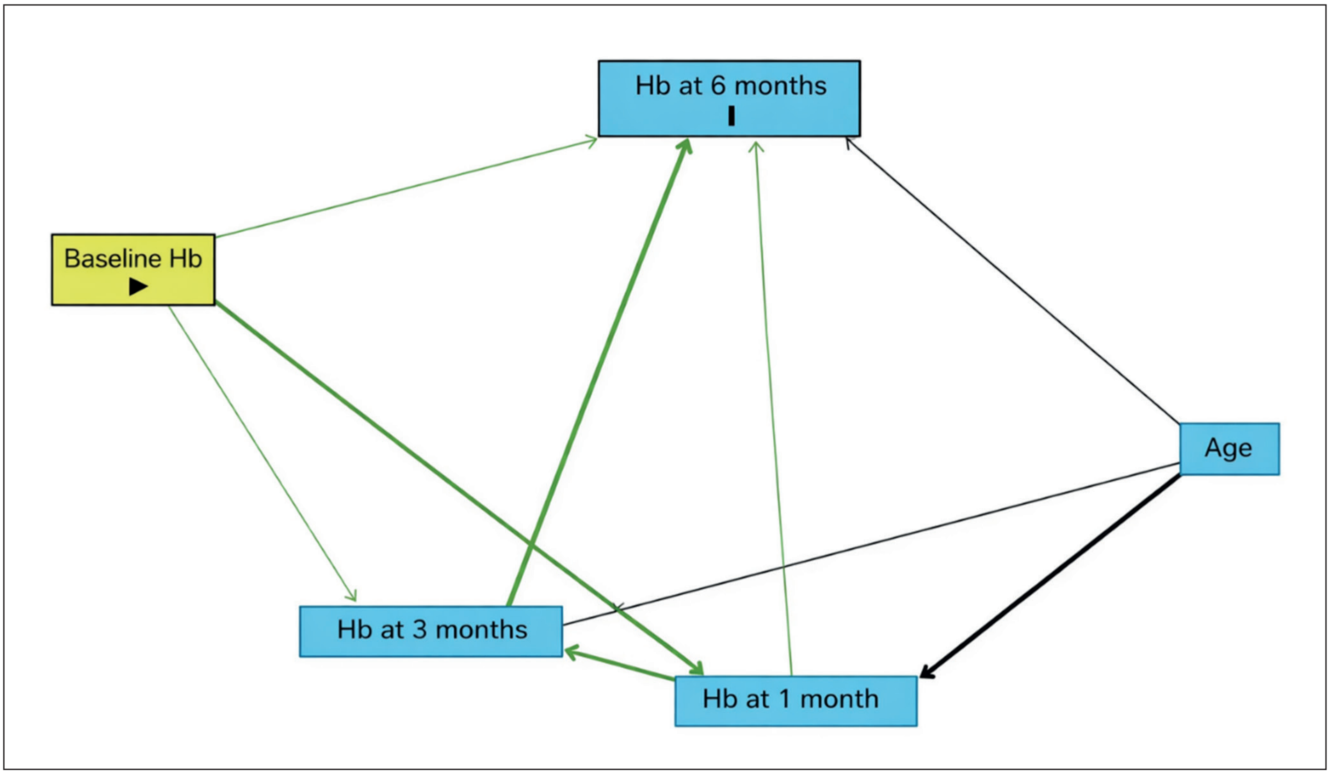

Figure 1. Directed acyclic diagram of the serial mediation model of childhood hemoglobin in a Peruvian cohort under three years of age.

Discussion

The results show a solid longitudinal trend in the trajectory of Hb levels during the months of follow-up in children under three years of age. Hierarchical regression analyses (Table 2) revealed highly significant associations between initial Hb values and subsequent measurements. In particular, the Hb value at one month was strongly determined by its baseline level, indicating remarkable early stability of the biomarker. This stability could reflect not only the physiological persistence of the hematological status at the beginning of child development[12], but also the influence of early biological determinants such as perinatal nutritional status, fetal iron stores, or the duration of exclusive breastfeeding[13,14,15].

At three months, both the initial and first-month Hb values were significant predictors of the observed value, with robust coefficients and narrow confidence intervals. This shows a temporal sequence in which each measurement provides additional information, without necessarily implying an immediate cumulative effect. At six months, the estimated Hb value was influenced by all previous measurements, with greater weight given to the value recorded at three months. Although not immediately after the second measurement, this measurement is positioned as a key intermediate link in the modeled trajectory.

In addition, age as a covariate showed a positive and constant effect in all models, in line with the progressive increase in Hb described in childhood, as in the CALIPER population-based study[16].

From a mediational approach, the findings in Table 3 delve deeper into the sustained effect of initial Hb on the measurement at six months. Although the direct effect was significant and clinically relevant, the total indirect effect was even greater, showing that much of the initial influence is transmitted through the measurements at one and three months. The three mediational pathways identified –including the complete chain (initial Hb → Hb 1 month → Hb 3 months → Hb 6 months) –reinforce the temporal sequence of pediatric hematological development in a clinical follow-up context. The trajectories reflect the expected increase in Hb with age, probably related to physiological processes inherent to childhood growth. In practical terms, intermediate measurements are not redundant: they help to outline the expected course of Hb in childhood and allow for hypothesizing about subgroups with lower-than-expected increases, rather than defining immediate intervention adjustments.

Furthermore, the internal consistency of the model is supported by the explained variance values (Table 4). At each stage of follow-up, the longitudinal model showed considerable explanatory power, exceeding 60% in the first two measurements and remaining at a high level at six months. This high proportion of explained variance validates the relevance of the chain mediation model used (model 6) to capture the trajectory of Hb over time. In addition, the slight reduction in R^2 in the last stage could reflect the emergence of new exogenous influences from the third month of life onwards, such as dietary diversification or environmental exposures[17,18], which raises interesting hypotheses for future studies.

The results obtained have mainly methodological and epidemiological implications, as they provide evidence on patterns of Hb change with age in the pediatric population. This approach complements the cross-sectional findings reported in projects such as CALIPER and KIGGS, confirming from a longitudinal perspective that Hb follows expected trajectories of increase during childhood.

From a clinical point of view, rather than proposing dynamic treatment modifications, which require experimental and controlled designs, these findings may contribute to identifying children whose Hb increase pattern is lower than expected for their age, which could justify closer monitoring or the search for factors associated with anemia risk. In this sense, predictive value continues to be determined mainly by initial Hb levels, but the characterization of trajectories offers a conceptual framework that could be useful in future studies aimed at personalized interventions or validating the role of early biomarkers in specific populations.

This approach complements the universal screening around 12 months recommended by pediatric guidelines and is especially relevant with the new WHO cut-off points for 6–23 months[19], as it allows for early and targeted intervention in children close to the threshold. From a public health perspective, these findings support the design of systematic screening and monitoring programs in the child population, contributing to more effective policies for the prevention and control of anemia at the community level.

Among the limitations of the study is its observational design, which prevents the establishment of causality between Hb measurements. In addition, other relevant factors such as sex, prematurity, nutritional status, infections, iron supplementation, or other clinical conditions were not included due to a lack of information in the secondary database. There was also a lack of information on socioeconomic and environmental variables (such as the distribution of the population by rural and urban areas) that could influence Hb levels. Finally, although the chain mediation model offers a comprehensive view, its validity depends on the quality and frequency of measurements, which could limit its applicability in other contexts or populations.

In conclusion, infant Hb shows structured development, with strong predictive value from early stages, where initial Hb directly and indirectly influences levels at six months, highlighting the key role of the third month as a critical point in the hematological trajectory. This sequential pattern supports the clinical utility of regular monitoring and early interventions against risks such as anemia. In addition, the chain mediation model proved useful for analyzing the trajectory of pediatric biomarkers, with potential for application in other areas of child development.

Given the sequential nature of hematological development in childhood, it is recommended to reinforce Hb screening and monitoring from the first months, especially in contexts with high anemia rates. Early interventions could improve both immediate hematological status and medium-term child health. Future research could apply this approach to other developmental biomarkers to strengthen evidence-based public health policies.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Right to privacy and informed consent: This study used anonymized, publicly available secondary data from the Junín Regional Health Directorate: https://datosabiertos.gob.pe/dataset/ni%C3%B1os-menores-de-36-meses-con-dx-de-anemia-en-la-regi%C3%B3n-jun%C3%ADn-direcci%C3%B3n-regional-de-salud, as recorded in the respective minutes.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

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