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Haematological Parameters in Relation to Peripheral Smear Examination to Evaluate Morphological Types of Anaemia: A Hospital Based Study

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ABSTRACT

Introduction: Although the diagnosis of anaemia no longer remains a significant challenge in Nepal due to the availability of diagnostic technologies, even in rural settings, the accurate classification of anaemia however, remains challenging due to a lack of qualified pathologists across the healthcare facilities.

Objective: To study the morphological types of anaemia in patients visiting Madan Bhandari Academy of Health Sciences Hetauda Hospital.

Methods: Hospital based cross sectional study was used to evaluate anaemia based on morphological characteristics and haematological parameters among patients visiting the hematology lab of Madan Bhandari Academy of Health Sciences, Hetauda Hospital from 2021-05-23 to 2021-12-31. By using MIDAS software 386 patients were recruited. Ethical approval was taken from Nepal Health Research Council. Statistical analysis was performed using SPSS version 16, with categorical variables summarized as frequencies and percentages.

Result: The majority of participants were diagnosed with microcytic hypochromic anaemia, with a notable predominance among females. Additionally, the highest proportion of cases was observed in adults aged 45 years and older.

Conclusion: A concerning finding was that vulnerable groups, women, adults over 45, and children were significantly affected by microcytic hypochromic anaemia. The high prevalence indicates a considerable burden of likely preventable anaemia, underscoring the urgent need for public health action and government-supported dietary interventions.

Keywords: Anaemia; microcytic hypochromic; peripheral blood smear.

INTRODUCTION

Anaemia poses a significant public health problem in developing nations. According to a study by Harding et al.[1], the prevalence of anaemia in children in Nepal is 46%, whereas around one third of women age (15-49) were affected with anemia.[1]

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Accurate identification of the type of anaemia remains challenging due to the lack of sophisticated diagnostic tools.[2] Nevertheless, even when advanced techniques like molecular diagnostics are available, peripheral blood smear (PBS) is still considered the gold standard for anaemia classification.[3] However, as PBS requires skilled manpower for its interpretation, its usage in smaller towns in Nepal is less. Blood cell indices on PBS remain the most reliable method for characterizing anaemia. However, the data on the types and distribution of anaemia in Nepal remains limited, thereby a major gap exists in delivering targeted healthcare interventions despite the high burden of diseases. Hence, Nepal faces a lack of proper treatment and prevention strategies leading to significant anaemia related morbidity and mortality.[4] Therefore this study aims to find out the haematological parameters in relation with peripheral smear examination to evaluate anaemia of various types of anaemia in Hetauda Hospital.

METHODS

This hospital-based cross-sectional study evaluated anaemia based on morphological characteristics and haematological parameters among patients visiting the haematology lab of Madan Bhandari Academy of Health Sciences, Hetauda Hospital from 2021-05-23 to 2021-12-31. Ethical approval was taken from the Ethical Review Board, Nepal Health Research Council with approval number 298/2021P. Permission letter

was taken in written from hospital administration for the purpose of data collection of the patients.

Sample size was calculated using the formula: n = (Z2 x P x (1 - P))/e2 Where: - Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI) - P is expected true proportion = 24.8% from de Benoist et al., we get 287 and then using 10 percent non response rates and 1.2 as design effect, we computed the sample size as 386. For the purpose of data collection census sampling was done.[5] All the patients that came for anaemia testing or who had their haemoglobin levels tested were included in the sample. The study included demographic variables such as age and sex, along with clinical data, including haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell (RBC) count, packed cell volume (PCV), white blood cell (WBC) count, platelet count, and the grades and types of anaemia.

The study population comprised patients presenting with clinical features suggestive of anaemia and referred for complete blood count (CBC) and peripheral smear examination. Inclusion criteria were patients of all age groups and both sexes diagnosed with anaemia (as per WHO criteria) [2], patients with available CBC and peripheral smear reports, and those patients (or guardians, in case of minors) who gave informed consent. Exclusion Criteria were patients who received a blood transfusion in the last 3 months, haemolysed or clotted blood samples, and incomplete clinical or laboratory data. A simple random sampling technique was employed, where each eligible patient was assigned a number, and participants were selected using a random number generator in MS Excel.

Haematological Parameters analysed using automated haematology analyser included in the study are: Haemoglobin (Hb), Red Blood Cell Count (RBC), Haematocrit (HCT/PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Red Cell Distribution Width (RDW). Peripheral Blood Smear (PBS), smears were made using EDTA (Ethyl-Dimethyl Tetraenoic Acid) blood for the PBS examination and allowed to air dry. A Wright stain was applied to the entire slide and examined.

The PBS was reported by one of the pathologists involved in this study and his/her effort was verified by a different pathologist for RBC morphology, presence of nucleated RBCs, and white blood cell and platelet abnormalities, if any. Based on the findings, the anaemia was classified based on WHO criteria on severity as mild, moderate, and severe. We also classified anaemia based on morphology as per MCV values as: Microcytic (<80 FL), Normocytic (80–100 FL), and Macrocytic (>100 fL). Further, using peripheral blood smear findings, anaemia was finally classified as: microcytic hypochromic, normocytic normochromic, and macrocytic normochromic. Data collection was carried out using a self-developed proforma, which was partially filled by the data collector for clinical data and partially extracted from the MIDAS software for demographic details. In our proforma, the collected variables included Patient ID, Sample Number, Patient Name, Age, Sex, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), and Haemoglobin (Hb) and peripheral blood smear finding. The collected data were first entered into Microsoft Excel for organization and preliminary review. Following data entry, statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (version 16). The categorical variables were presented in the form of frequencies and corresponding percentages to summarize the distribution of data across different groups.

RESULTS

A total of 386 individuals were analysed in this study and most participants were from the Makwanpur district, particularly Hetauda, with fewer from Bara district of Madhesh province and other neighbouring districts. They were categorized based on age group, gender and anaemia severity. (Table 1). The old aged adults (>45yr) category is the largest, making up more than half of the sample (55.7%) whereas children make up the smallest portion, with 34 individuals (8.8%) of the total. There was significant female preponderance (71.2%) with Female to male ratio as 2.48:1. In total, 323 (83.7%) patients were found to be anaemic and 63 (16.82%) had no anaemia.

Table 1: Characteristics of Participants.

Characteristics	Frequency (%)
Age	
< 16 years	34 (8.8%)
17-30	58 (15%)
31-45	79 (20.5%)
>45	215 (55.7%)
Gender	
Female	275 (71.2%)
Male	111 (28.8%)
Anaemic (Yes)	**323 (83.67%)**
Mild anaemia	49(15.17)

Moderate anaemia	191(59.13)
Severe anaemia	71(29.18)
Very severe	12(3.72)

Most patients presented with microcytic hypochromic anaemia (Table 2). The findings also suggested a transparent gender and age disparity, with females and individuals over 45 years affected disproportionately.

Most (n=190, 58.82%) of our study participants presented with microcytic hypochromic anaemia. In terms of percentage 59.01% of females and 58.22 % of male who underwent haematological evaluation had microcytic hypochromic anaemia. Likewise, 36.06% female and 30.37% of male were found to have normocytic normochromic anaemia. Around 4.91% of females and 11.39% of males were found to have macrocytic normochromic anaemia. The majority of the younger population (<16 years) presented with microcytic hypochromic anaemia (66.67%), followed by normochromic normocytic anaemia (30%).

In the middle age population, the majority had microcytic hypochromic anaemia (60.27%), followed by normocytic normochromic anaemia (34.24%). Similar findings were observed in age groups above 45 years, where microcytic hypochromic anaemia predominated (55.78%), followed by normocytic normochromic anaemia (36.05%).But, macrocytic normocytic anaemia had more prevalence in the age group above 45 years (8.16%).

Table 2: Classification of Anaemia Based on Peripheral Blood Smear (PBS) Analysis by Sex and Age group (years).

Anaemia Type	Total	Female	Male	0-16	17-45	>45
Macrocytic Normochromic Anaemia	21 (6.50%)	12	9	1	8	12
Microcytic Hypochromic Anaemia	190 (58.82%)	144	46	20	88	82
Normocytic Normochromic Anaemia	112 (34.67%)	88	24	9	50	53
Total	323	244	79	30	146	147

DISCUSSION

Among 386 participants who were evaluated for anaemia, most of the sample were anaemic. Moderate anaemia is the most commonly seen severity. Severity of anaemia was seen more among female patients and those aged more than 31 years. The most common anaemia observed was microcytic hypochromic anaemia followed by normocytic normochromic anaemia and macrocytic normochromic anaemia. The most common anaemia among the male population was normocytic normochromic anaemia whereas, microcytic hypochromic anaemia among females. Among the age categories the most common anaemia was microcytic anaemia whereas, patients more than 45 years mostly had normocytic normochromic anaemia.

The most common type of anaemia in our study is microcytic hypochromic anaemia with a significant gender disparity. Most commonly affected age groups were over 45 years, highlighting the importance of addressing anaemia in the older population, specifically females. On the other hand, macrocytic normochromic anaemia is the least common and the 0 to 16 age group shows the fewest cases. In our study we found that there is a significant association between anaemia type and age group (Chi-Square Test, p = 0.012), and between anaemia type and sex (Chi-Square Test, p = 0.001) indicating that age and sex influences the type of anaemia. The high prevalence of anaemia, particularly among females and older adults as seen in our study highlights the need for targeted public health interventions like dietary interventions, iron supplementation, and improved healthcare accessibility. Key findings show older and middle-aged adults are most affected by anaemia, and females are disproportionately impacted. Males are significantly less likely to have anaemia (OR = 0.40, p=0.0014) with 30 male and 33 females having no anaemia.

Demographic and Health Survey, 2022 in Nepal reported 34% of women of reproductive age group have anemia.[5] Similarly, majority of the patients in this study were females. Factors like education, economic status, size of the family, exposure to media and residing location have been associated with anaemia among females.[6] The lower nutritional status, pregnancy, multiparity, lactation, domestic violence, tropical infection like malaria and helminth infection in this region may contribute to the higher frequency of anaemic female patients seen in this study.[7] A study conducted by Baral et al. In Morang district of Nepal, 2009 similarly observed microcytic hypochromic

anaemia was the most prevalent anaemia. However, anaemia was mostly observed among male gender and study was conducted among only adolescents.[8]Similarly a study conducted by Joshi et al in 2019, Nepal among 342 anaemic patients observed microcytic hypochromic anaemia was the most common type of anaemia followed by anaemia due to chronic disease.[9]

Parasitic infestation is commonly seen among the population living in the terai belt which may be due to factors like inadequate drinking water source, improper latrine use, walking barefoot, low socio-economic status, humid climate and poor sanitation.[10] The most frequently seen anaemia was microcytic hypochromic in this study which may be due to the similar demographics on the patients recruited in this study. Similar to this study Ni et al. in 2022 also observed older age was positively associated with anemia.[11] Diseases that are undiagnosed disease, impaired functioning of gonads, decreased sensitivity of bone marrow to erythropoietin, chronic inflammation of low grade, vitamin D and iron deficiency seen among elderlies may have shown higher cases of anaemia among old aged population in this study.[12]

As a single centre, hospital-based study, our findings may not fully reflect the broader community. Since we used convenience sampling, there's a chance of selection bias, and the results may not be generalizable to all populations. Also, while we focused on haematological and morphological parameters, we did not explore the underlying causes of anaemia, such as nutritional deficiencies, chronic illnesses, or socioeconomic factors, which could have provided a more complete picture.

CONCLUSION

This study highlights a significant burden of microcytic hypochromic anaemia in patients with suspected anaemia, and those who underwent haematological analysis in Hetauda Hospital, the provincial capital of Bagmati province. Such findings suggest that the type of anaemia prevalent in the Bagmati region is probably correctable, especially given the high prevalence of iron-deficiency-related morphological types with proper programmatic actions in place like Iron folic distribution programs and adolescent girls iron folic acid distribution programs. Although the diagnosis of anaemia based on WHO criteria is now feasible due to the widespread availability of automated instruments, a major challenge remains the lack of specialized manpower to accurately classify the type of anaemia. Hence, this underscores a critical gap in anaemia management. Therefore, it is important to improve healthcare manpower and apply specific public health strategies, especially for women and elderly people, to help reduce anaemia related problems and improve the health condition of the population in this area.

Conflict of Interest: None

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