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Significance of Hematological Biomarkers in Association to Syphilitic Patients

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ABSTRACT

Background: Venereal syphilis is a sexually transmitted disease, involving pathological activities mediating tissue destruction by extensive tissue necrosis. As such, the goal amongst researchers has been set to the identification of effective laboratory biomarkers that can reflect the broad spectrum of disease and ultimately aid in timely diagnosis and effective treatment of syphilis. This research aimed to study the applications of hematological biomarkers associated with syphilitic patients visiting a tertiary care hospital.

Methods: A retrospective cross-sectional study was conducted in the syphilitic patients attending KIST Medical College and Teaching Hospital, Lalitpur, Nepal. A total of 25 syphilitic patients and 41 non-syphilitic participants were included. The rapid plasma reagin test and *Treponema pallidum* hemagglutination assay were used for the screening and confirmation of syphilis respectively. The hematological investigation was performed using a hematology analyzer. Statistical Package for Social Science version 17.0 was used for data analysis. A P value <0.05 was considered significant.

Results: Syphilitic patients showed significantly elevated levels of lymphocytes (39.8±11.5) (p=0.025), monocyte (1.9±0.8) (p=0.002), mean corpuscular volume (MCV) (92.6±12.9) (p=0.005), and mean corpuscular hemoglobin (MCH) (31.9±4.6) (p=0.008) and lowered levels of red blood cell (RBC) (4.2±0.3) (p=0.005) and platelets (237.2±628.6) (p=0.048) as compared to the lymphocytes (32.9±11.9), monocyte (0.6±1.2), MCV (83.9±8.8), MCH (34.3±1.5), RBC (4.6±0.7), and platelets (280.9±113.3) of the non-syphilitic participants.

Conclusions: The results showed that the elevated levels of lymphocyte, monocyte, MCV, and MCH and lowered levels of RBC and platelets are highly specific hematological biomarkers for the diagnosis of patients with syphilis.

Keywords: Hematological biomarkers; sexually transmitted disease; syphilis

INTRODUCTION

Characterized as a multistage infectious disease with a relapsing and remitting course,[1] syphilis is usually transmitted through contact with active primary or secondary lesions of a sexual partner.[2] In 2015, the World Health Organization (WHO) estimated that there were 45.4 million syphilitic people in the world,[3] with around 107,000 deaths.[4,5]

Without treatment, the condition can progress through multiple clinical stages over time, eventually leading to irreversible neurological or cardiovascular consequences.[6] Additionally, infection due to *Treponema pallidum*, an etiological agent of syphilis, can cross the placental barrier of a syphilitic mother from the bloodstream and can infect the fetus resulting in congenital syphilis,[7] which is also a leading cause of spontaneous abortion, stillbirth, and perinatal mortality in many of the countries.[8,9]

This study aimed to provide a comprehensive overview on

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understanding the significance of altered hematological biomarkers to improve the diagnosis of syphilitic patients.

METHODS

This was a hospital-based retrospective study conducted in the Department of Clinical Laboratory, KIST Medical College and Teaching Hospital (KISTMCTH), Lalitpur, Nepal from January 2018 to December 2020. The approval was obtained from the Institutional Review Committee (IRC) of the KISTMCTH (reference number: 2077/078/25). Only syphilitic patients who were tested using rapid plasma regain (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) techniques were included, and individuals without syphilis were excluded.

A total of 66 patients were recruited for the study. There were 25 syphilitic patients and 41 healthy individuals, who were considered as the non-syphilitic participants. Three milliliter whole blood in EDTA was collected for hematological testing, while 3 milliliter whole blood in a gel tube was collected for RPR and TPHA assays. The flocculation card test was used to detect antibodies produced against antigens released by damaged host cells in syphilitic patients. In the test, the serum was mixed with the RPR antigen. If antibodies were present, they combined with the lipid particles of the antigen, causing them to agglutinate with showing up as black clumps against the white card. A rapid diagnostic test kit, TPHA was used for the diagnosis of syphilis from each serum sample. In this test, one drop of serum (25 microliters) followed by one drop of buffer were added to the sample port. After 15 minutes, the result was detected. Positive results were indicated by the appearance of two distinct pinks to deep purple color bands on the device.

The hematology analyzer (Sysmax XN-550, Japan) was used for the testing of complete blood cell count (CBC). The parameters investigated include white blood cell (WBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil, red blood cell (RBC), hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and platelet.

The relevant demographic data and detailed medical history of the syphilitic patients were collected from the hospital record section. Any missing or ambiguous records were correctly obtained through communication with involved health-care workers or patients and patient parties. After complete and detailed information was obtained, it was recorded in a customized data collection form. Statistical analysis was performed by the SPSS program for Windows, version 17.0. Categorical variables were presented as absolute numbers (*n*) percentage (%), mean ± Standard Deviation (SD). The independent *t*-test was used for data analysis. A *P*-value less than 0.05 was considered as a significant difference.

RESULTS

Of the total of 66 patients, 37.8% (*n* = 25) were syphilitic patients with the mean age ± Standard deviation of 29.1±7.7 years and 62.1% (*n* = 41) were non-syphilitic participants with the mean age of 42.9±24.1. While 52% (*n* = 13) patients were male from the syphilitic patients, 53.6% (*n* = 22) patients were male from the non-syphilitic participants (Figure 1).

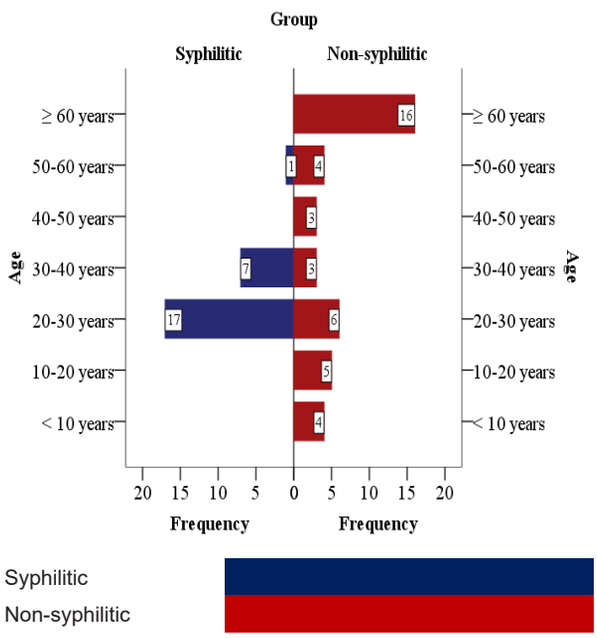

Figure 1. Showing age groups of syphilis subjects and the non-syphilitic participants.

Higher incidence of syphilis infection (*n* =17, 68.0%) was seen in age group of 20-30 years, whereas age groups of 30-40 years and 50-60 years showed an incidence of 28.0% (*n* = 7) and 4.0% (*n* = 1) respectively. The youngest syphilitic patient found to be positive in this study was 21 years and the oldest was 56 years (Figure 1).

Table1. Hematological biomarkers of the syphilitic patients and the non-syphilitic participants.

Parameters	Group		P-value
	Syphilis	Non-syphilitic cases	
WBC (X 10^9 cells/l)	13.3±22.0	9.8±5.6	0.340
Neutrophil (%)	57.4±12.0	62.2±13.7	0.158
Lymphocyte (%)	39.8±11.5	32.9±11.9	0.025
Monocyte (%)	1.9± 0.8	0.6±1.2	0.002
Eosinophil (%)	0.7±2.0	2.4±2.4	0.000
Basophil (%)	0.0±0.0	0.03± 0.1	0.439
RBC (X 10^{12} cells/l)	4.2± 0.3	4.6± 0.7	0.005
Hemoglobin (g/dl)	13.7± 2.5	13.7± 2.4	0.996
PCV (%)	39.6±7.1	38.8±6.7	0.674
MCV (fl)	92.6±12.9	83.9±8.8	0.005
MCH (pg)	31.9±4.6	28.9±3.5	0.008
MCHC (g/l)	34.2±2.0	34.3±1.5	0.767
Platelets (X 10^9 cells/l)	237.2±628.6	280.9±113.3	0.048

WBC = White Blood Cell, RBC = Red Blood Cell, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration.

The results showed a decrease in the neutrophil (57.4%±12.0) (P>0.05), eosinophil (0.7%±2.0) (P<0.05), and basophil (0.0±0.0) (P>0.05) of the syphilis subjects as compared to the neutrophil (62.2%±13.7), eosinophil (2.4%±2.4), and basophil (0.03%±0.1) of the non-syphilitic participants. However, there was a substantial increase (P<0.05) in lymphocytes (39.8%±11.5) (P<0.05), monocyte (1.9%±0.8) (P<0.05), and PCV (39.6%±7.1) (P>0.05) of the syphilis subjects as compared to lymphocytes (32.9%±11.9), monocyte (0.6%±1.2), and PCV (38.8%±6.7) of the non-syphilitic participants (Table 1).

Besides, there was a decrease in the value of RBC ($4.2X10^{12}$ cells/L±0.3) (P<0.05) and platelets ($237.2X10^9$cells/l±628.6) (P<0.05) of the syphilis subjects as compared to the RBC ($4.6X10^{12}$cells/L±0.7) and platelets ($280.9X10^9$cells/l±113.3) of the non-syphilitic participants. On the other hand, there was an increase in WBC ($13.3X10^9$cells/l±22.0) (P>0.05) of the syphilitic subjects as compared to WBC ($9.8X10^9$cells/l±5.5) of the non-syphilitic participants (Table 1).

Moreover, the results showed a moderate decrease in the MCHC (34.2g/l±2.0) (P>0.05) and a moderate increase in hemoglobin (13.7g/dl±2.5) (P>0.05) of the syphilis subjects as compared to the MCHC (34.3g/l±1.5) and hemoglobin (13.7g/dl±2.4) of the non-syphilitic participants. Additionally, there was a significant increase in MCV (92.6%±12.9) and MCH (31.9%±4.6) of the syphilis subjects as compared to MCV (83.9%±8.8) and MCH (28.9% ±3.5) of the non-syphilitic participants (Table 1).

DISCUSSION

Despite the availability of new diagnostic tests and antibiotic therapy, syphilis has re-emerged in several developed and developing countries, including Nepal.[10] Efforts to eliminate syphilis have met with only modest success.[11] Alternatively, a quantitative measurement of disease severity with the use of clinical biomarkers reflecting pathological development can be of use to clinicians for understanding disease prognosis, outcomes, and enabling finesse to decision making.[12] Therefore, a study concerning the laboratory biomarkers of a cohort of 25 patients with syphilis visiting a tertiary care hospital was conducted. We studied a clear pattern of 13 hematological biomarkers. To the best of our knowledge, this is the first report concerning the significance of hematological biomarkers and their usefulness in the diagnosis of patients with syphilis from Nepal.

In this study, the majority of syphilitic patients belonged to the age group of 20-30 years (68.0%), further preceded by the age group of 30-40 years (28.0%) and 50-60 years (4.0%).The finding was in consensus with the report of the Center for Disease Control and Prevention (CDC),[13] which mentioned the highest incidence of primary and secondary cases of syphilis among persons aged 25–29 years followed by 30–34 years. A higher incidence of syphilis infection in such an age group could be attributed to not being accustomed to using a condom, not thinking of themselves or a partner as being at high risk for syphilis, or not considering oral or anal sex as a way of contracting or transmitting syphilis.[14] In contrast to the study by Obeagu et al.,[15] this study was predominated by males (52.0%) in the incidence of syphilis infection. Similarly, CDC discussed the highest rates of syphilis among men (18.7 cases per 100,000 males) as compared to the rate among women (3.0 cases per 100,000 females).[13] Higher incidence of syphilis in men could be attributed to the increased practice of men having sex with men and/or practice of men going to health care providers to be tested for HIV.[16]

The mean values for hematological parameters such as neutrophil (57.4%±12.0), hemoglobin (13.7g/dl±2.5), PCV (39.6%±7.1), MCV (92.6%±12.9), and MCH (31.9%±4.6) from this study were in correspondence with the study done by Obeagu et al.,[15] which reported the mean values of neutrophil, hemoglobin, PCV, MCV, and MCH as 4.5%±0.6, 14.0g/dl±0.5, 42.0%±3.2, 89.9fl±10, and 29.9pg±5.6 respectively. Similarly, decreased levels of hemoglobin (1.9g/dL) and neutrophils (23%) were also reported by a study by Lee et al. Besides, the mean values for the parameters such as WBC ($13.3X10^9$cells/l±22.0), monocyte (1.9%±0.8), eosinophil (0.7%±2.0), and RBC ($4.2X10^{12}$cells/L±0.3) from the study were in contrast with the result of the research conducted by Obeagu et al.,[15] which reported the mean values for WBC, monocyte, eosinophil, basophil, and RBC as 4.5cells/l±$0.6X10^9$, 30.6%±7.2%, 1.0%±0.1, 0.1%±0.1, and RBC 4.7cells/l±0.3 X 10^{12} respectively. The findings of the study coincide with the report of Hira et al.,[18] and Kim et al.,[18] which mentioned that almost 90% of children with early congenital syphilis diagnosed were anemic. Additionally, the findings from our study were in contrast to the study of Lee et al.,[17] who reported the substantially altered value of WBC (53.7K/μL ≈ 0.0537 cell/liter), neutrophils (23%), lymphocytes (65%), and monocytes (7%) in congenital syphilis. Moreover, the mean values of lymphocytes (39.8%±11.5) and platelets ($237.2X10^9$cells/l ± 628.6) from this study also varied to the findings of Kim et al.,[19] and Obeagu et al.[15] Conclusively, our study revealed that the hematological parameters such as lymphocyte, monocyte, eosinophil, RBC, MCV, MCH, and platelets showed significant role ($P<0.05$) in the diagnosis and prognosis of syphilitic patients, which is similar to the conclusion from the study of Obeagu et al.[15]

Syphilis remains a concerned re-emerged disease worldwide, as it causes considerable morbidity. The highly destructive nature of the late stages of syphilis and the recognition that syphilis infection greatly increases the transmission and acquisition of HIV, make syphilis an important public health concern.[7] The risk of sexual transmission of syphilis can be reduced by using a latex condom[20,21] Because *Treponema pallidum* shows the presence of several penicillin-binding proteins responsible for cell wall synthesis pathways,[22] penicillin kills susceptible bacteria by interfering with the production of cell walls and remains the drug of choice in syphilis treatment. Besides, guidelines published by the Centers for Disease Control and Prevention specify oral doxycycline or tetracycline as alternative treatments in the case of penicillin allergy (except for pregnant women).[10] The global persistence of syphilis and the emergence and rapid spread of macrolide-resistant *Treponema pallidum* are reminders that there is no room for complacency. Lack of ability to study other hematological biomarkers such as cytokines, immunoglobulin, and involvement of fewer study subjects were the major limitations of the present study.

CONCLUSIONS

The result showed that syphilis and the ensuing immune response can have a tremendous impact on the hematopoietic process and the persistence of such responses may result in chronic and detrimental inflammatory feedback to the hematopoietic system. The parameters such as lymphocytes, monocytes, eosinophil, RBC, MCH, and platelets are significant hematological biomarkers for syphilis prognosis and diagnosis as compared to other parameters.

CONFLICT OF INTEREST

None

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