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**Title:** Hematological Parameters to Predict Post-COVID-19 Immune Response, and the Development of On-Reaching Herd Immunity. A Cross-Sectional Study

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**Statement and Declarations**

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**Abstract**

**Background:** Here we conducted this study to find the changes in the hematological parameters of healthy individuals that can predict their immune status, also this study aims to find out whether herd immunity against COVID-19 is developing or not in the Pakistani population.

**Methods:** A comparative cross-sectional study of 210 healthy individuals was conducted. All individuals were divided into three groups i.e IgG positive, IgG negative, and IgG and IgM both positive based on ELISA results. Data analysis was done by SPSS version 25 for windows.  
**Results:**  A statistically significant effect was found among the three groups in terms of mean hemoglobin level, mean hematocrit, mean corpuscular hemoglobin concentration, mean red blood cells count, RDW-CV, mean lymphocyte, mean neutrophil, mean eosinophils, and mean basophil. The study also showed that 52.8% (n=74) had neither taken vaccination nor had any history of previous covid infection but still their antibodies were positive.

**Conclusion:** There was a statistically significant difference among hematological parameters between immune and nonimmune groups and can predict the covid immune status also the study predicted that herd immunity against COVID-19 is developing in Pakistan.

**Keywords:** COVID-19, Hematological parameters, Herd Immunity, Full blood count, Antibodies, Immunity.

**Introduction:**

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), initially began in Wuhan, China in December 2019, as an outbreak of a series of unusual pneumonia cases, and now has become a global pandemic [1]. The World Health Organization (WHO) announced that COVID-19 reached pandemic status on 30 January 2020 and subsequently, declared a global pandemic in March 2020.[2 ,3] As of 26 December 2020, there were approximately 80,500,000 confirmed COVID-19 cases worldwide, including 1,700,000 related deaths with a case fatality rate of 2.2%.[3 , 4] Having currently affected more than 210 countries and territories, Pakistan owing to its high population of 204.65 million and its struggling health care and economic systems, has been greatly vulnerable to this outbreak, reported 4601 confirmed cases within 45 days with a death toll approaching 66 individuals by April 2020.[5]

Primarily coronaviruses are documented as respiratory tract infections ranging from mild to moderate upper respiratory tract infections. While the SARS-CoV-2 virus also negatively impacts the hematological, immune, neurological, gastrointestinal, and cardiovascular systems, resulting in severe systemic diseases. [6] One of the major impacts generated by the spread of covid 19 is mainly related to the high number of patients requiring medical assistance in a short time, overloading the health systems and causing it to collapse. In this sense, several public health measures have been employed such as social distancing, quarantine of the infected patients, and the massive testing of the population, representing crucial strategies for mitigating. However, these methods are insufficient to end the COVID-19 pandemic [7] Countries across the world shifted their attention to more reliable approach of administering vaccines to an enormous number of the population to attain herd immunity thereby decreasing hospitalization, the severity of illness, fatality rate. [8] Several challenges arise when this type of immunity is required since numerous social and economic factors can cause hindrances.

In immunity against SARS-CoV-2, the role of B cells is extremely important ranging, from cytokine production, antigen presentation, to antibody secretion including isotypes predominantly immunoglobulin G (IgG) and immunoglobulin M (IgM). Previous studies conducted show high plasma levels of proinflammatory cytokines including interleukins and tumor necrosis factor-α in affected patients. [9] Vaccination coverage may be used to approximate population immunity levels, but inaccuracies result because vaccinated individuals can remain susceptible and unvaccinated individuals can be immune following infection [10] Monitoring population immunity to Covid-19 can help identify populations at risk of outbreaks and determine whether targeted vaccination efforts are needed. Diagnostic test used for Covid, the reverse transcription-polymerase chain reaction (RT-PCR), Enzyme-Linked. Immunosorbent Assays ELISA hybridization microarray assays, and chest CT scan. [11] These technique requires complex laboratory infrastructure, specialized professionals and long time to the results, and economically not suitable for developing countries thus causes great challenges in limited resources settings.

This study aims to determine the changes in hematological parameters of healthy individuals to predict their immunity status and intends to find out whether herd immunity against covid 19 is developing or not in the Pakistani population.

**Materials and Methods:**

**Study design:** A comparative cross-sectional study was conducted from July 2022 to November 2022 on healthy individuals. All the participants were recruited from District Kohat of Pakistan.

**Sample size:**

The world health organization (WHO) sample size calculator was used for the estimation of sample size. A total of 210 healthy individuals participated in the study out of which 140 participants were immune to COVID-19, 64 were nonimmune and 6 were having asymptomatic active covid infection.

**Data collection and management:**

The individual characteristics including the age, gender, vaccination status, information about the previous covid infection, family history, current or past systemic disorder and drug history were collected through a questionnaire after taking informed written consent. Each individual is then tested for covid antibodies testing through rapid antibodies testing kit (------) which was then confirmed by ELISA. Based on ELISA the participants were classified in three groups i.e IgG positive, IgG negative and IgG and IgM both positive. A complete blood count test was done using CBC analyzer machine for all the participants in each group was then done by a single CBC analyzer. Various hematological parameters were then compared.

**Inclusion and exclusion criteria:**

Only healthy individuals of age 20 to 60 years were included in the study. Informed consent including the purpose of the study was taken from all the individuals. Those who have active infection, systemic disorders, hematological malignancies, anemias, or autoimmune conditions were excluded from the study. Individuals who smokes or using any medication routinely or recreationally and those who donated blood or history of blood transfusions in the last three months were also excluded from the study. No restrictions of gender, occupation and education were applied for conducting this study.

**Data analysis**

Statistical analysis used the One Way-ANOVA test to compare numerical data that was normally distributed and that involved more than two unpaired groups. Distribution of the data was analyzed using SPSS version 25 for windows in which Kolmogorov Smirnov was used for the number of samples greater than or equal to 50 while Shapiro Wilk was used for the number of samples less than 50.

**Ethical Consideration**

The ethical clearance was granted from DHQ Teaching Hospital KDA Kohat on 22 July 2022 (Ethics number: 3223).

**Results**

A total of 210 patients were included in this study which consisted of 54.3% male (n = 114) and 45.7% female (n = 96). Among these 210 patients, 66.7% were only immunoglobulin G (IgG) positive (n = 140), 30.5% were antibody negative (n = 64), and 2.8% were positive for IgG and immunoglobulin M (IgM). The mean age of the total patients in this study was 32.48 ± 14.63 years. No subjects in the study had comorbidity. Regarding vaccination history, about 47.1% of patients in the only IgG-positive group had been vaccinated and most of them had been vaccinated within one year. In addition, 39% of patients in the antibody-negative group had been vaccinated within less than a year (n = 3), within a year (n = 5), more than a year ago (n = 4), and unknown date of vaccination (n = 13). No patients in the IgG and IgM-positive groups had been vaccinated previously. The vaccines used during vaccination in the only IgG-positive group were varied, dominated by Sinovac (92.4%), followed by Pfizer (3.0%), Sinopharm, Moderna, and Sputnik. All the patients in the antibody-negative group were vaccinated by Sinovac. A history of COVID-19 infection among the patients was also recorded. About 95.2% of patients never acquired a COVID-19 infection. Of those who had COVID-19 infections, 9 of them were in the IgG-positive group while only 1 patient was from the antibody-negative group. The summary of patient demographic and vaccination history is given in Table.1.

**Table 1. Participant’s demographic information and vaccination history.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Only IgG Positive | IgG Negative | Both IgG and IgM Positive |
| Number of patients | 140 | 64 | 6 |
| Male | 84 | 27 | 3 |
| Female | 56 | 37 | 3 |
| Mean age (range) | 33.5 (20-60) | 30.1 (20-60) | 34.7 (27-56) |
| Currently Vaccinated   * Less than 1 year * 1 year back * More than 1 year back * Vaccination date unknown | 1  65  0  0 | 3  5  4  13 | 0 |
| No vaccination done | 74 | 39 | 6 |
| Sinovac | 61 | 25 | 0 |
| Sinopharm | 1 | 0 | 0 |
| Moderna | 1 | 0 | 0 |
| Pfizer | 2 | 0 | 0 |
| Sputnik | 1 | 0 | 0 |
| Previous COVID-19 infection  < 1 year  1 year back  2 years back | 1  7  1 | 1  0  0 | 0  0  0 |
| No history of previous COVID-19 infection | 131 | 63 | 6 |

**Hematological Parameters**

The summary of hematological parameter variations among three immunological groups is given in the Table. 2. The detail of each parameter is given below.

**Table 2. Variations in hematological parameters among the immunological groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mean Hematological Parameters | Cut off values | Only IgG Positive | Antibody Negative | IgG and IgM Positive |
| Hemoglobin (g/dL) | 11.5 – 17.3 | 14.4 ± 1.8 | 13.5 ± 1.5 | 12.9 ± 1.3 |
| Hematocrit (%) | 34 – 53.90 | 41.1 ± 5.1 | 38.6 ± 4.7 | 39.8 ± 3.3 |
| MCV (fL) | 84 – 98 | 79.7 ± 8.1 | 77.8 ± 7.4 | 84.4 ± 4.7 |
| MCH (pg) | 27.5 – 32.4 | 28.0 ± 3.6 | 26.8 ± 3.7 | 27.4 ± 1.9 |
| MCHC (g/dL) | 31.7 – 34.2 | 35.0 ± 2.3 | 34.9 ± 2.0 | 32.4 ± 0.6 |
| RBC (cells/mm3) | 4.50 – 5.50 x 106 | (5.20 ± 0.7) x 106 | (5.00 ± 0.6) x 106 | (4.7 ± 0.3) x 106 |
| RDW-CV (%) | 11.1 – 14 | 15.1 ± 2.1 | 15.8 ± 2.2 | 13.7 ± 0.9 |
| Leukocyte (/mm3) | 5 – 11 x 103 | (12.9 ± 44.7) x 103 | (8.1 ± 2.4) x 103 | (9.9 ± 3.3) x 103 |
| Neutrophil (%) | 40% - 60% | 60.0 ± 8.9 | 56.4 ± 8.1 | 58.5 ± 8.3 |
| Lymphocyte (%) | 1.19 – 40 | 33.8 ± 8.6 | 37.3 ± 8.3 | 36.8 ± 7.4 |
| Monocyte (%) | 2% - 8% | 2.5 ± 1.1 | 4.0 ± 0.7 | 3.2 ± 1.2 |
| Eosinophil (%) | 1% - 4% | 3.5 ± 2.0 | 2.0 ± 0.5 | 1.5 ± 0.5 |
| Basophil (%) | 0.5% - 1% | 1.5 ± 1.9 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Platelet (cells/mm3) | 150 - 450 | 267,364.3 ± 74,104.1 | 256,703.1 ± 60,179.5 | 266,666.7 ± 94,326.4 |
| NLR | 1 – 3 | 2.1 ± 1.6 | 1.6 ± 0.5 | 1.7 ± 0.6 |

*MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RBC: red blood cell; RDW-CV: red cell distribution width; NLR: Neutrophil-Lymphocyte Ratio; g/dL: grams per deciliter; fL: femtoliter; pg; picogram; cells/mm2 : cells per square millimeter;*

**Hemoglobin.** There was a significant effect of mean hemoglobin at *p* < 0.05 for three immunological groups [F (2, 207) = 7.85, *p* = 0.027]. The Post Hoc Tamhane test indicated that the mean score for hemoglobin level in the IgG-positive group was significantly different from those in the negative antibody group in terms of statistics (M = 0.92, SE = 0.25, *p* = 0.001). However, the mean hemoglobin level in the IgG-positive group did not significantly differ from IgG and IgM-positive groups (M = 1.51, SE = 0.55, *p* = 0.100). Similarly, the mean hemoglobin level in the antibody-negative group did not significantly differ from IgG and IgM-positive groups (M = 0.59, SE = 0.56, *p* = 0.706).

**Hematocrit.** A significant effect of mean hematocrit was seen among three immunological groups (F(2, 207) = 5.70, *p* = 0.004]. The Post Hoc Tamhane test for the mean hematocrit level in the IgG-positive group was significantly different from the mean hematocrit level in the antibody-negative group (M = 2.51, SE = 0.73, *p* = 0.002). Mean hematocrit level was not different statistically in the IgG-positive only group and IgG and IgM-positive groups (M = 1.34, SE = 1.41, *p* = 0.757), as well as negative antibody group and IgG and IgM-positive groups (M = 1.2, SE = 1.46, *p* = 0.835).

**Mean Corpuscular Volume.** There were no significant effect of mean corpuscular volume among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F(2, 207) = 2.61, *p* = 0.076].

**Mean Corpuscular Hemoglobin.** There were no significant effect of mean corpuscular hemoglobin among the three immunological groups [F (2, 207) = 2.22, *p* = 0.111].

**Mean Corpuscular Hemoglobin Concentration.** There was a significant effect of mean corpuscular hemoglobin concentration among the IgG-positive group, negative antibody group, and IgG and IgM-positive groups [F (2, 207) = 4.10, *p* = 0.018]. The Post Hoc Tamhane test demonstrated that the mean score for mean corpuscular hemoglobin concentration level in the IgG-positive group was significantly different from those in the IgG and IgM-positive groups in terms of statistics (M = 2.61, SE = 0.30, *p* = 0.000). Moreover, a significant difference in mean corpuscular hemoglobin concentration was also seen between the negative antibody group and IgM and IgG-positive groups (M = 2.52, SE = 0.34, *p* = 0.000).

**Red Blood Cells**. A significant effect of mean red blood cells was observed among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F(2, 207) = 3.12, *p* = 0.046]. The Post Hoc Tamhane test for mean red blood cells in the IgG-positive group was significantly different from mean red blood cells in IgM and IgG-positive groups (M = 0.49, SE = 0.24, *p* = 0.028).

**Red cell distribution width (RDW-CV)**. There was a significant effect of RDW-CV among the three immunological groups [F (2, 207) = 4.3, *p* = 0.015]. The Post Hoc Tamhane test for the mean RDW-CV in IgG-positive group was significantly different from the mean RDW-CV in the IgM and IgG-positive groups (M = 1.34, SE = 0.39, *p* = 0.028). In addition, a significant difference in RDW-CV was also seen between the negative antibody group and IgM and IgG-positive groups (M = 2.06, SE = 0.44, *p* = 0.002).

**Total leukocyte count.** There was no significant effect of mean leukocyte count among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F(2, 207) = 0.378, *p* = 0.686].

**Neutrophil.** A significant effect of mean neutrophil among the three immunological groups [F(2, 207) = 3.83, p = 0.023]. At least two groups had significant differences in terms of mean neutrophils (*p* = 0.023). The Post Hoc Tamhane test demonstrated a significant difference in mean neutrophils between the IgG-positive group and negative antibody group (*p* = 0.015). However, it was not clinically significant (mean difference = 3.6).

**Lymphocyte percentage.** There was a significant effect of mean lymphocyte percentage among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F (2, 207 = 3.76, *p* = 0.025]. Also, there were at least two groups with significant differences in mean lymphocyte percentage (*p* = 0.025). According to the Post Hoc Tamhane result, there was a significant difference in mean lymphocyte percentage between the IgG-positive group and negative antibody group (*p* = 0.023) which did not differ clinically (mean difference = 3.44).

**Lymphocyte count** There was no significant effect of mean lymphocyte count among the three immunological groups [F (2, 207) = 0.27, *p* = 0.767].

**Monocytes**. There was a significant effect of mean monocytes among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F(2, 207) = 47.83, *p* = 0.000]. At least two groups had a significant difference in mean monocytes in terms of statistics (*p* = 0.000). The Post Hoc Tamhane test showed a statistically significant difference in mean monocytes among the IgG-positive group and negative antibody group (*p* = 0.000). However, it was not clinically significant (mean difference = 1.43).

**Eosinophils.** A significant effect of mean eosinophils was observed among the three groups [F (2, 207) = 20.25, *p* = 0.000]. Post Hoc Tamhane test demonstrated a statistically significant difference in mean eosinophils between the IgG group and negative antibody group (*p* = 0.000) as well as between the IgG-positive group and IgM and IgG-positive groups (*p* = 0.000) with a mean difference was 1.51 and 1.99, respectively.

**Basophils**. There was a significant effect of mean basophil among the IgG-positive group, negative antibody group, and IgM and IgG-positive groups [F (2, 207) = 21.17, *p* = 0.000]. The Post Hoc Tamhane test showed a significant difference in mean basophil between the IgG-positive group and IgM and IgG group (*p* = 0.000). However, the difference was not clinically significant (mean difference = 1.52).

**Platelet count**. There was no significant effect of mean platelet count among the three immunological groups [F (2, 207) = 0.50, *p* = 0.606].

**NLR**: There was no significant effect of mean NLR among the three immunological groups [F (2, 207) = 2.56, *p* = 0.080].

**Herd immunity:**

The study also showed that out of 140 immune participants (IgG positive) 47% percent (n= 74) were vaccinated, 1% percent (n=1) had a history of previous COVID-19 infection, and 52 % percent n=73) were not vaccinated nor had any previous COVID-19 infection but still their IgG were positive and they were immune to COVID-19. This data predicts that herd immunity against COVID-19 is developing in the Pakistani Population. The detail has been given in Table 3.

**Table 3. Vaccination status in comparison to previous infection**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **IgG positive** | **Total (n=140)** | **Percentage** | **Division** | | |
| Vaccinated | n=66 | 47% | Without previous infection | 87% (n=58) | |
| Previous infection | 8% (n=12) | |
| Non vaccinated | n=74 | 52% | Without previous infection | | 98% (n=73) |
| Previous infection | | 1% (n=1) |

**Discussion**

The identification of biomarkers for immunological efficacy through several longitudinal research on immune system responses around the world may aid in combating this infectious disease that is spread all over the world. [12]

The novel scope of this research included the investigation of various hematological parameters to predict immune response in healthy individuals against covid 19 These parameters included hemoglobin (Hb), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBcs), and its subtypes.

In our findings a statistically significant effect was found among the three groups in terms of mean Hb level, mean HCT, MCHC, mean RBC count, RDW-CV, mean lymphocyte, mean neutrophil, mean eosinophils and mean basophil but the mean values of these parameters are in normal It shows that although the difference is not clinically significant, it is statistically significant. Similarly in another study COVID-19 cases were found to have significantly lower levels of Hb concentration MCH, MCHC, and MCV in comparison with negative patients, this result being quite similar to that of previously reported studies [13] This low Hb level could be associated with the action of the virus against the development or degradation of RBC or the existence of comorbidities however previous research, RBC count had no significant indication in COVID-19 patients. [14 ] Moreover for each parameter, there is a specific life span so its number changes with time also external factors also have an impact on these parameters such as smoking, alcohol, dehydration, systemic illnesses, and various medications. In this study, we only recruited healthy individuals with and proper history in terms of past illnesses, medication intake, and malignancies with thorough examinations done before recruitment to the study.

Studies have been done to evaluate the value of hematological markers in determining the severity of the COVID-19 disease. However, no research has been done regarding the hematological markers as indicators of immunity. Liao D et al. also found elevated neutrophil to lymphocyte ratio as a useful predictor for severity and mortality of SARS-CoV-2 infection [14] Leukocytosis, neutrophilia and increased neutrophil to lymphocyte ratio (NLR), which might be due to inflammatory response, have a significant association with the disease severity. NLR was highest in patients with critical disease. [15] Tan et al. have reported that lymphopenia may be used as an indicator for detecting the severity of COVID-19, and thus, it acts as an adjunctive aid for identifying prognostic indicators for differentiating between early and severe cases of COVID-19. [ 16] Hematological parameters can contribute in disease monitoring by changing in value when the disease is detected. Moreover, a combination of hematological characteristics can be used to estimate severity or outcome, but these parameters cannot directly predict the post covid immune response, but a physician can make a rough estimate that whether the person is immune or not to COVID-19. These parameters are variable but further research is needed to estimate the ratios among various parameters that would be constant and will be individual specific.

The study also predicted that on reaching herd immunity is developing in the Pakistani population because most of the immune individuals do not have a history of previous COVID-19 infection, nor they were vaccinated against this lethal virus, but their IgG antibodies were positive. Herd immunity is an important concept for epidemic control. [17] It can be acquired in two ways, either by infection with the pathogen or vaccination. Using safe and effective vaccines inducing herd immunity reduces disease prevalence and decreases mortality and morbidity. [18] This type of immunity is necessary, but it presents a number of difficulties because it can be delayed by a variety of social and economic variables and vaccine hesitancy may threaten communities to develop herd immunity. [19] To date, Pakistan has faced a significant obstacle mostly in terms of vaccine hesitancy, which is a major roadblock to the eradication of vaccine-preventable diseases, but it was found that awareness of vaccines is one of the most prominent reasons for vaccinations [20]. In Pakistan, the COVID-19 vaccine has been introduced gradually to immunize people in order to achieve herd immunity. [21] A total of five vaccines (AstraZeneca, Sinopharm, CanSino, Sputnik V, and Gamaleya) and the phased rollout method of mass immunization have been approved for use in Pakistan as of June 29, 2021. [22]. Currently, Pakistan's healthcare system has not been subjected to the worst of the pandemic's effects, but large vaccination campaigns, with an estimated 84% of Pakistan's population aged 12 or older receiving all recommended vaccinations. [23] These measures might soon aid the country achieve herd immunity.

The study results cannot be applicable to those who have an active infection, are immune deficient, and took vaccination long time ago; taking medication such as steroids, antiplatelet, anticoagulants, antiepileptics, etc., family history of hematological disorders or has any chronic systemic disorders. The limitation of the study also includes the study sample size, further research with increased sample size is needed to support this hypothesis.

**Conclusion**

The study concluded that some hematological parameters in the complete blood picture were statistically different in immune people than non-in immune. These parameters includes Hb level, HCT, MCHC, RBC count, RDW-CV, lymphocyte, neutrophil, eosinophil and basophil that can predict immunity against covid-19. However, these parameters are not clinically different because the study has been conducted on normal healthy individuals. Further research is needed to find out the ratios of these hematological parameters which are individual specific that can further support this hypothesis. Furthermore, it has been concluded from the study that herd immunity against COVID-19 is developing in Pakistan.

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