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ORIGINAL ARTICLE

IMMUNOLOGICAL FEATURES BETWEEN URBAN AND RURAL COVID-19 PATIENTS OF KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Background: Cell-mediated immunity T-cells plays an essential role in efficient antiviral responses against coronavirus disease-2019 (COVID-19). Objective of the study was to compare the mean percentage of CD4 and CD8 T-cells between urban and rural COVID-19 patients and to assess the correlation between lymphocyte characteristics (CD4%, CD8%, CD4/CD8 ratio) and demographic factors (age, gender, location) in COVID-19 patients.

Materials & Methods: A cross-sectional study with duration of 6 months in 2021 was conducted in Peshawar's major hospitals with a total of 71 COVID-19 patients. Sample were collected via convenience sampling technique. The study excluded individuals with active viral, autoimmune, or oncological conditions and those with a pre-existing history of chronic diseases or prior treatment with immunosuppressive drugs before contracting COVID-19. Flow cytometry analysis on a Beckman-Coulter Cytoflex, utilizing anti-human directly conjugated antibodies, assessed CD4 and CD8 percentages and ratios in T cells. The study calculated means, frequencies, and performed Point-Biserial correlation and Eta-squared was used.

Results: The results showed that the mean age of patients was 46.07±15.87, with the highest frequency observed in the 60-75 age group (29.58%) and the 30-44 age group (28.17%). Rural patients were founded higher than urban residing patients. Urban COVID-19 patients, except for CD8%, exhibited higher CD4% and CD4/CD8 ratio compared to rural patients. The CD4%, CD8%, and ratio CD4/CD8 was 48.01±11.10, 49.05±10.58, and 1.09±0.53, respectively, and the Pearson correlation analysis showed highly significantly (P value<0.01) strong negative association between CD4% and CD8% whereas strong positive association was found for CD4% and CD8% with CD4/CD8 ratio.

Conclusion: COVID-19 minimally impacted CD4%, but markedly increased CD8% and decreased CD4/CD8 ratio. The CD4% and CD8% were closely associated, suggesting heightened CD8% expression and reduced CD4/CD8 ratio in COVID-19 immune responses.

KEY WORDS: COVID-19; T cells; CD4%; CD8%; CD4/CD8 ratio.

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INTRODUCTION

The emergence of COVID-19 in December 2019 posed a significant global health challenge, leading to a pandemic with widespread mortality.[1] SARS-CoV-2, sharing 79.6% of its genome with SARS-CoV, presents similar clinical features, notably in its impact on immune responses.[2] Although vaccines have been developed[3], the emergence of variants, such as Omicron, continues to complicate epidemic control.[4] COVID-19's clinical manifestations vary, with older individuals and those with comorbidities experiencing more severe outcomes, often due to hyperactivation of the immune system.[5]

COVID-19 has disproportionately affected disadvantaged populations, exacerbating healthcare challenges in regions with limited resources.[6] While Pakistan has reported a lower prevalence and mortality rate compared to other countries, the complexity of the disease, from asymptomatic cases to severe

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pneumonia-like symptoms, has complicated both diagnosis and treatment.[7] Phylogenetic analyses confirm that the Pakistani strain is closely related to a bat SARS-like coronavirus, adding to the global puzzle of its origins and spread.[8] Treatment options, including respiratory support, dexamethasone, and Remdesivir, have mitigated some of the disease's impacts, though patients often require continued care post-discharge for full recovery.[9]

Immune system responses, particularly the role of CD4+ and CD8+ T-cells, are critical in determining the progression and severity of COVID-19.[10] Previous studies in SARS patients found that low CD4+ and CD8+ T-cell counts were associated with worse outcomes, and their restoration was linked to symptom improvement.[11] This has prompted research into their role in COVID-19, where both CD4+ and CD8+ counts are considered biomarkers for disease severity. Wang et al. further demonstrated that CD8+T counts could be a more sensitive predictor of recovery than CD4+T in patients showing clinical improvement.[12]

Given the diverse immune responses and regional variations in the severity of COVID-19, this study aimed to compare the mean percentage of CD4 and CD8 T-cells between urban and rural COVID-19 patients and assess the correlation between lymphocyte characteristics (CD4%, CD8%, CD4/CD8 ratio) and demographic factors (age, gender, location). Understanding these immune profiles across different populations will provide insight into how geography and demographic factors influence immune responses to COVID-19. Thus the hypothesis of the study were:

1. Null Hypothesis (H_0): There is no significant difference in the mean percentage of CD4 and CD8 T-cells between urban and rural COVID-19 patients.

   Alternative Hypothesis (H_1): There is a significant difference in the mean percentage of CD4 and CD8 T-cells between urban and rural COVID-19 patients.

2. Null Hypothesis (H_0): There is no significant correlation between lymphocyte characteristics (CD4%, CD8%, CD4/CD8 ratio) and demographic factors (age, gender, location) in COVID-19 patients.

   Alternative Hypothesis (H_1): There is a significant correlation between lymphocyte characteristics (CD4%, CD8%, CD4/CD8 ratio) and demographic factors (age, gender, location) in COVID-19 patients.

MATERIALS AND METHODS

A cross sectional study with duration of six months was conducted in 2021 in two major hospitals in Peshawar i.e. Hayatabad Medical Complex (HMC) and Rehman Medical Institute (RMI). Double blind convenient sampling technique was used for collecting data of total 71 COVID-19 patients. Sample size was determine using raosoft online sammple size calculator, where margin of error was 5%, confidence level of 95%, population size 340 and with 50% response distribution gave an estimate of 181 patients. Unfortunately, 72 patients were found within that duration that surpass inclusion criteria. Inclusion criteria included patients that were registered as COVID-19 patients in hospital wards. The study excluded individuals with active viral, autoimmune, or oncological conditions, along with those possessing a pre-existing history of chronic diseases, especially those who had previously undergone treatment with immunosuppressive drugs before contracting a COVID-19 infection. Approval for the study was granted by the Hospital Research and Ethical Committee of HMC Peshawar, with the reference number 421/HEC/B&PSC/2020. Subsequently, sample collection adhered to the WHO criteria for obtaining coronavirus samples from human subjects

Flow cytometry analysis was conducted on a Beckman-Coulter Cytoflex (Beckman-Coulter, MA, USA) using anti-human directly conjugated antibodies. For each individual, 50 µl of blood sample was transferred to a round-bottom tube for CD4+ and CD8+ T cell counting. Following the addition of 3µl of cell surface antibodies (anti CD4-FITC, anti CD8-PE), the sample was incubated in a dark place for 15 minutes. Subsequently, 1ml of lysing solution was added, and the sample was incubated again in a dark place. After centrifugation for 5 minutes at 2000 rpm, the supernatant was discarded. The sediment was then treated with 2ml of PBS, re-centrifuged, and the supernatant was discarded. Finally, after adding 500 µl of PBS, the sample was analysed using Beckman Coulter Navios software. The CD4 and CD8 percentages and ratio, along with the absolute lymphocyte count calculated.

SPSS Ver. 26 was used to analyse the data. The demographic (independent/control) variables data was taken as binominal (gender: male, female; location: urban, rural) and age group being ordinal variable. The dependent variables i.e. CD4%, CD8% and CD4/CD8 ratio were taken as continous variables. Descriptive statistics were used to summarize the demographic data (age, gender, and location) and lymphocyte characteristics (CD4%, CD8%, CD4/CD8 ratio) of COVID-19 patients, categorized by urban and rural groups. The Shapiro-Wilk test was applied to assess normality, followed by independent samples t-tests to compare lymphocyte characteristics between urban and rural patients. Point-Biserial correlation was used for binominal variables i.e. gender and location, whereas Eta-squared was used for age categorical independent variable against continous dependent variables i.e. CD4%, CD8%, CD4/CD8 ratio.P-values under 0.05 were considered significant.

RESULTS

The results showed that the mean±S.D for age was 46.07±15.87 (table 1). Among the age groups, the highest frequency was observed for elder aged COVID-19 patients i.e. 60-75 years old age group (29.58%), followed by 30-44 age groups patients (28.17%). Male comparative to female were slightly higher, and most of the patients were residing in rural area.

Results of skewness statistic showed to be highly significant (P value<0.01) indicating a normal distrubation data set (table 2). As indicated in table 3, the highest CD4% and ratio of CD4/CD8 was recorded for urban patients and CD8% was found higher in rural COVID-19 patients.

The mean±S.D for lymphocyte characteristic CD4%, CD8% and ratio CD4/CD8 was 48.01±11.10, 49.05±10.58 and 1.09±0.53, respectively. The Pearson correlation of the demographic characteristic and lymphocyte characteristics showed non-significant (P value>0.05) association. Lymphocyte characteristics showed that CD4% was highly significant (P value<0.01) strong negative association with CD8%, and strong positive association with CD4/CD8 ratio.

Table 1: Demographic and lymphocyte distribution (Mean±S.D and frequency %) among the study COVID-19 patients

Characteristics		Mean ±S.D	Frequency (%)
Age	15-29	46.07 ±15.87	13 (18.31%)
	30-44		20 (28.17%)
	45-59		17 (23.94%)
	60-75		21 (29.58%)
Gender	Female		35 (49.30%)
	Male		36 (50.70%)
Location	Rural		38 (53.52%)
	Urban		33 (46.48%)

Table 2: Shapiro-Wilk statistic of normality test for lymphocyte characteristics

Variables	Statistic	Df	Sig.
CD4%	0.827	71	0.000
CD8%	0.923		0.000
CD4:CD8	0.827		0.000

Table 3: Comparison of rural and urban lymphocytes characteristics (mean±S.D)

	CD4%	CD8	CD4/CD8
Rural	45.16±9.98	51.74±9.53	0.96±0.50
Urban	51.29±11.42	45.96±10.89	1.24±0.53

Table 4. Pearson and Eta squared statistics for determine association strength of lymphocyte characteristics against age groups.

Variables	Pearson correlation	Eta squared
CD4%	-0.201^{ns}	0.038
CD8%	0.220^{ns}	0.047
CD4/CD8 ratio	-0.175^{ns}	0.033

ns = non significant (p value>0.05)

The both association strength statistic including pearson (continuous control age group) and ETA square (ordinal age groups variable) against dependent variable i.e. CD4%, CD8% and CD4/CD8 ratio is shown in table 4. Pearson correlation association showed that CD%4 and CD4/CD8 ratio showed negative weak non significant (p value >0.05) association with age, and a weak positive non significant (p value>0.05) association observed for CD8%. As for Eta squared analysis, results showed a weak association of the resultant dependent variables against ordinal control age variable.

Table 5: Association of lymphocyte characteristics against location (urban/rural) and gender of COVID-19 patients.

Variables	Association strength	Location	Gender
CD4%	Pearson Correlation	-.275*	0.045^{ns}
	Sig. (2-tailed)	0.02	0.709
CD8%	Pearson Correlation	.273*	-0.015^{ns}
	Sig. (2-tailed)	0.021	0.904
CD4/CD8 ratio	Pearson Correlation	-.261*	-0.025^{ns}
	Sig. (2-tailed)	0.028	0.838

* = significant (p value<0.05)

The pearson point-biserial correlation between the binominal independent variable location (urban/rural) and gender (male/female) against dependent variables (CD4%, CD8%, CD4/CD8 ratio) is shown in table 5. Results showed weak negative significant (p value<0.05) association of CD4% and CD4/CD8 ratio towards rural development, indicating a slight increase of these factors in urban area patients than rural belonging patients. Whereas, CD8% showed weak (pearson=0.273) positive significant (p value<0.05) association towards rural patients compared to urban area patients. As for gender, there was a non-significant (p value>0.05) association found with the dependent variables i.e. CD4%, CD8% and CD4/CD8 ratio.

DISCUSSION

The results of this study showed that significant differences in immune responses of COVID-19 patients based on their demographic characteristics. Ganji et al. [13] suggested that the cellular immune responses triggered by COVID-19 infection were developed through overexpression of CD8 and hyperactivation of cytotoxic T lymphocytes. Although, there was no significant difference of CD4/CD8 ratio between COVID-19 patients and control (normal) group. Many COVID-19 patients experience a notable decrease in lymphocyte counts, leading to immunosuppression. [14] The prognosis depends on the resolution of lymphocytopenia, especially T-cell counts.[15] Recovery is more likely when lymphocytopenia is addressed, but persistent cases can lead to severe complications and death.[16] Peripheral lymphocyte subset alteration was associated with clinical characteristics and treatment efficacy of COVID-19. CD8+ T cells tended to be an independent predictor for COVID-19 severity and treatment efficacy.[12] He et al.[17] showed correlation between the reduction in CD3+, CD4+, and CD8+ T lymphocyte levels and the progression of COVID-19 pneumonia, particularly in severe patients. Caution is advised in the use of glucocorticoids for severe cases based on these observations.

As for our study, the male patients slightly by pass female patients. Kushwaha et al. [18] findings were in line with the present study results, which showed that mean age of COVID-19 patients was 39.47 ± 17.59 years. Females had higher infection odds in younger age groups and recovery (O.R. = 1.779), with the 5–17 years age category exhibiting the highest recovery odds (O.R. = 88.286), regardless of gender. Another study revealed that 58.4% of COVID-19 cases were among males, 41.6% among females, with a predominant presence in rural areas (73%). Disease severity, leading to hospitalization, was observed in 67.4% of cases, particularly among the elderly. The mortality rate was noteworthy, accounting for 14.5% of total cases, with 66.4% of fatalities occurring in individuals aged 65 and older.[19] Zheng et al. [20] emphasizes that elderly patients, especially those with pre-existing cardiovascular conditions, exhibit certain biomarkers that can aid in the early detection and identification of critically ill individuals. These indicators include elevated C-reactive protein, heightened myocardial damage markers, increased brain natriuretic peptide levels, and decreased counts of white blood cells, neutrophils, lymphocytes, CD4, and CD8.

The three-tier rural healthcare system in India faces deficits in infrastructure and personnel at various levels. Insufficient government hospital beds, combined with challenges such as inadequate surveillance and the reverse migration of workers during lockdowns, have complicated the management of the pandemic in rural India, creating a significant gap in understanding its true statistics.[21] There low mortality rate in the Indian population, possibly due to a majority living in rural areas, contrasts with the formidable challenge posed by COVID-19 in these regions. BahaaEldin et al.[22] suggested that women aged over 30, living in rural areas, and having underlying medical conditions face an increased risk of severe COVID-19. Additionally, studies indicate that residing in socioeconomically deprived areas is associated with a higher likelihood of severe infections, potentially due to limited healthcare facilities in rural settings.[23,24]

A prospective cohort study at Indus Hospital in Karachi, Pakistan, from March to June 2020, found that 39% of 170 adult COVID-19 patients predominantly aged over 60, exhibited factors such as lower oxygen saturation, critical disease on arrival, and increased use of non-invasive ventilation.[25]

Overall, the study highlighted critical demographic and immunological differences between urban and rural COVID-19 patients. Further studies focusing on the healthcare disparities and access challenges in rural settings may provide valuable insights into the differential immune responses and outcomes for COVID-19 patients across different regions.[8]

CONCLUSION

A conspicuous elevation in CD8+ 48.01% signified a substantial expansion during the antiviral immune response. The discernible reduction in the CD4/CD8 ratio was attributed to the combination of unremarkable alterations in CD4% and a pronounced surge in CD8%, resulting in a notable shift towards an augmented CD8+ T cell predominance relative to CD4+ T cells. The association between CD4% and CD8% hinted at a concerted response, suggesting a synchronized modulation of these T cell subsets. The heightened expression of CD8% and the diminished CD4/CD8 ratio bear implications for the efficaciousness and characteristics of the broader immune response against the virus, underscoring the intricate and multifaceted dynamics within the

immune system during COVID-19 infection.

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REFERENCES

3. Baloch S, Baloch MA, Zheng T, Pei X. The coronavirus disease 2019 (COVID-19) pandemic. Tohoku J Exp Med. 2020;250(4):271-8. https://doi.org/10.1620/tjem.250.271
4. He S, Han J, Lichtfouse E. Backward transmission of COVID-19 from humans to animals may propagate reinfections and induce vaccine failure. Environ Chem Lett. 2021;763-8. https://doi.org/10.1007/s10311-020-01140-4
5. Tripathi D, Yi G, Vankayalapati R. COVID-19: an update on vaccine development. Indian J Rheumatol. 2020;15(2):70-2. https://doi.org/10.4103/0973-3698.285394
6. Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, et al. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) variant. N Engl J Med. 2022;386(16):1532-46. https://doi.org/10.1056/NEJMoa2119451
7. Shahid Z, Kalayanamitra R, McClafferty B, Kepko D, Ramgobin D, Patel R, et al. COVID-19 and older adults: what we know. J Am Geriatr Soc. 2020;68(5):926-9. https://doi.org/10.1111/jgs.16472
8. Brüssow H. COVID-19: vaccination problems. Environ Microbiol. 2021;23(6):2878-90. https://doi.org/10.1111/1462-2920.15549
9. Tsikala Vafea M, Atalla E, Georgakas J, Shehadeh F, Mylona EK, Kalligeros M, et al. Emerging technologies for use in the study, diagnosis, and treatment of patients with COVID-19. Cell Mol Bioeng. 2020;13:249-57. https://doi.org/10.1007/s12195-020-00629-w
10. Ali K, Nawaz KH, Hassan WU, Waqar RM. Absolute lymphocyte count; the most sensitive indicator of the risk of progression and complications in COVID-19. Pak Armed Forces Med J. 2023;73(4):1116-9. https://doi.org/10.51253/pafmj.v73i4.6705
11. Altaf A, Iqbal W, Younis S, Deeba F. Current scenario of COVID-19 with epidemiological and phylogenetic analysis of Pakistani coronavirus: a review. J Bioresour Manag. 2020;7(3):4. https://doi.org/10.35691/JBM.0202.0139
12. Kalpakci Y, Hacibekiroglu T, Trak G, Karacaer C, Demirci T, Kocayigit H, et al. Comparative evaluation of memory T cells in COVID-19 patients and the predictive role of CD4+CD8+ double positive T lymphocytes as a new marker. Rev Assoc Med Bras. 2020;66(12):1666-72. https://doi.org/10.1590/1806-9282.66.12.1666
13. Liu R, Wang Y, Li J, Han H, Xia Z, Liu F, et al. Decreased T cell populations contribute to the increased severity of COVID-19. Clin Chim Acta. 2020;508:110-4. https://doi.org/10.1016/j.cca.2020.05.019
14. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis. 2020;221(11):1762-9. https://doi.org/10.1093/infdis/jiaa150
15. Ganji A, Farahani I, Khansarinejad B, Ghazavi A, Mosayebi G. Increased expression of CD8 marker on T-cells in COVID-19 patients. Blood Cells Mol Dis. 2020;83:102437. https://doi.org/10.1016/j.bcmd.2020.102437
16. Liu Y, Li Y, Xu D, Zhang J, Peng Z. Severe COVID-19: immunosuppression or hyperinflammation? Shock. 2021;56(2):188-99. https://doi.org/10.1097/SHK.0000000000001724
17. Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M. Lymphopenia during the COVID-19 infection: what it shows and what can be learned. Immunol Lett. 2020;225:31. https://doi.org/10.1016/j.imlet.2020.06.013
18. Erdinc B, Sahni S, Gotlieb V. Hematological manifestations and complications of COVID-19. Adv Clin Exp Med. 2021;30(1):101-7. https://doi.org/10.17219/acem/130604
19. He R, Lu Z, Zhang L, Fan T, Xiong R, Shen X, et al. The clinical course and its correlated immune status in COVID-19 pneumonia. J Clin Virol. 2020;127:104361. https://doi.org/10.1016/j.jcv.2020.104361
20. Kushwaha S, Khanna P, Rajagopal V, Kiran T. Biological attributes of age and gender variations in Indian COVID-19 cases: a retrospective data analysis. Clin Epidemiol Glob Health. 2021;11:100788. https://doi.org/10.1016/j.cegh.2021.100788
21. Al-Tameemi K, Nassour R, Sleman RA, Daoud MS, Murad LS. A study on the epidemiology of COVID-19 in the Banias and Alkadmos areas of Syria during 2021. Malays J Microbiol. 2022;18(6). https://doi.org/10.21161/mjm.221505
22. Zheng Y, Xu H, Yang M, Zeng Y, Chen H, Liu R, et al. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. J Clin Virol. 2020;127:104366. https://doi.org/10.1016/j.jcv.2020.104366
23. Al-Dayan N, Venugopal D, Dhanasekaran S. Footprint of the COVID-19 pandemic in India: a study of immune landscape and other factors shielding mortality. Anal Cell Pathol (Amst). 2020;2020. https://doi.org/10.1155/2020/6692739
24. BahaaEldin H, El Sood HA, Samy S, Khader Y, AbdelFatah M, Hassany M, et al. COVID-19 outcomes among pregnant and nonpregnant women at reproductive age in Egypt. J Public Health. 2021;43(Suppl_3):iii12-8. https://doi.org/10.1093/pubmed/fdab376
25. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFE-

LY. Nature. 2020;584(7821):430-6. https://doi.org/10.1038/s41586-020-2521-4

26. Paul R, Arif AA, Adeyemi O, Ghosh S, Han D. Progression of COVID-19 from urban to rural areas in the United States: a spatiotemporal analysis of prevalence rates. J Rural Health. 2020;36(4):591-601. https://doi.org/10.1111/jrh.12486

27. Sarfaraz S, Shaikh Q, Saleem SG, Rahim A, Herekar FF, Junejo S, et al. Determinants of in-hospital mortality in COVID-19; a prospective cohort study from Pakistan. PLoS One. 2021;16(5):e0251754. https://doi.org/10.1371/journal.pone.0251754

CONFLICT OF INTEREST
Authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	YI, MOM
Acquisition, Analysis or Interpretation of Data:	YI, MOM, YMY, MQ, IUK, AM
Manuscript Writing & Approval:	YI, MOM, YMY, MQ, IUK, AM

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.