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Original Article

Demographic Factors Influence the Laboratory Parameters in COVID 19 Patients - Experience from Tertiary Care Center in Islamabad

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ABSTRACT

Background: As the pandemic of COVID-19 spread in different part of world, reports show significant differences in disease presentation, clinical symptoms, and progression, treatment-response and intensive care admissions

Objective: The aim is to study demographic parameters and to evaluate and compare various basic laboratory parameters according to age groups of PCR confirmed COVID-19 patients and statistically analyze its significance.

Material and Methods: It is a cross sectional, prospective study of **3** months duration from April 2020 to 2020, Pakistan Institute of Medical Sciences.

Results: 115 COVID-19 positive patients with moderate to severe symptoms were included in the study. Patients were divided into three groups based on their ages, less than 25 years, 25-50 years and more than 50 years. Distribution of SARS-CoV-2 patients showed male predominance and age gradient with only few cases in children. We found that laboratory parameters associated with worse prognosis as lymphopenia and hypoalbuminemia were more common in patients more than 50 years of age. Neutrophilia was more common in young patients. Deranged Neutrophil lymphocyte ratio NLR was observed in all three-age stratified groups

Conclusion: Combining parameters as NLR and age can be a potential indicator of prognosis.

Key words: COVID-19, Lymphopenia, demographics, Neutrophil to Lymphocyte ratio NLR, MuLBSTA Score system, Immune senescence.

Introduction

The upsurge of COVID-19 has essentially startled the humanity with its "novelty". Starting December 2019, when it first appeared , just as few unexplained pneumonia cases in Wuhan city , Hubei province , China, its spread went unbound, whelming the entire city, country and as of August 2020, Johns Hopkins Corona Virus Resource Center, it has spread to 188 countries.[1]As the pandemic spread in different part of world, reports show significant differences in disease presentation, clinical symptoms, and progression, treatment-response and intensive care admissions. Different countries' outbreak and mortality rate (MR). As the older ages deaths toll is the highest, we highlight the important role of demography, particularly, how the age structure of a population may help explain differences in fatality rates across countries and how transmission occurs [2]. This will help in "flattening the curve" of disease spread and prevent health systems from saturating

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The importance of age structure is also emphasized by the fact that clinical COVID -19 pneumonia scoring systems CURB-65 and MuLBASTA incorporate age into its score. CURB -65 includes Confusion, Urea, Blood pressure, Respiratory rate, Age more than 65 years whereas MuLBASTA is based on 6 parameters - multilobular infiltration, lymphopenia, bacteria co-infection, smoking history, hypertension, and age. Both these prognostic scores have a powerful predictive value regarding disease progression and mortality. [3]

Laboratory workup is the initial requisite for every admitted patient. We observed the difference in basic laboratory parameters as Lymphopenia, Complete blood profile, Serum albumin, Liver function test (LFT), Renal function test (RFT), Prothrombin time (PT), Activated partial thromboplastin time (APTT) and Lactate dehydrogenase (LDH) in different age groups. These basic tests are readily available, inexpensive and give an insight in the disease pathogenesis and clinical course. Variation in hemogram significantly contributes to meaningful clinical consequences. Epidemiologic studies suggest that age and gender are the major determinants of hematological indices[4].

The aim is to study demographic parameters and to evaluate and compare various basic laboratory parameters according to age groups of PCR confirmed COVID-19 patients and statistically analyze its significance.

Materials and Methods

This was a cross-sectional prospective study. Duration of this study was 3 months from April 2020 to June 2020. Study was conducted at Pathology Department, Pakistan Institute of Medical Sciences (P.I.M.S) and Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad. PIMS is a tertiary care, government hospital with main influx of patients from twin cities, Rawalpindi and Islamabad as well as approximately 10 cities in near vicinity.

Ethical approval for conducting the study was obtained from institutional Ethical Review Board on and written informed consent was obtained from the participants after explaining the purpose of the study. This study included 115 patients of COVID-19 confirmed by RT-PCR on nasopharyngeal and oro-pharyngeal swab specimens, admitted to isolation ward of PIMS Hospital with moderate to severe symptoms of disease according to National Institute of Health guidelines. This study included patients of both genders over the age of 12 years, who had not received any treatment. At the time of admission, electronic laboratory records of Complete Blood Count, coagulation parameters-Prothrombin time PT/International normalized ratio INR, Activated partial thromboplastin time APTT and biochemical parameters (Liver Function Tests, Renal Function Tests, serum albumin and LDH) were collected and noted on a Proforma. Patients were divided into three groups based on their ages, less than 25 years, 25-50 years and more than 50 years.

Data was entered and analyzed using SPSS version 20. Post-stratification, ANOVA test was applied and p value of <0.05 was considered significant.

Results

This study included 115 adult cases infected with SARS-CoV2 confirmed on RT-PCR on respiratory specimens, over a period of 3 months. Mean age of patients in our study was 48.0 ± 17.6 years. Patients ranged from 13 to 90 years. Males were in vast majority (80%) as compared to female patients (20%). According to age stratification, patients were divided into three groups and the frequency of patients in the three age groups is represented in figure 1. Almost 15% patients belonged to age group less than 25, 39% were between 25 and 50 years, and 46% were more than 50 years.

According to this age stratification, various hematological parameters were compared among the three defined groups; the results along with p value are shown in table 2 and 3. There was statistically significant difference between the three age groups in terms of Lymphopenia, Red cell count, Total leucocyte count TLC, Hemoglobin, Hematocrit, Mean corpuscular volume, Red cell distribution width (RDW), neutrophil%, lymphocyte%, eosinophil%, Prothrombin time (PT), Activated partial thromboplastin time (APTT), INR, Serum albumin, absolute lymphocyte count, absolute neutrophil count, Neutrophil to lymphocyte ratio (NLR) and Platelet to lymphocyte ratio (PLR).

We observed neutrophilic leukocytosis in young population with the average mean of $14.42x10^3$ (normal 3-11). Similarly RDW was also deranged in this group of patients. In patients of more than 25 years of age, liver function test especially ALT (alanine amino transferase) was out of range at the time of presentation. Hypoalbuminemia was a constant feature in all age groups but more frequent in elderly population particularly more than 50 years of age 81.2 % (43/53) .We categorized the patients into lymphopenic groups taking ALC of $1.0x10^3$ as a cut of value and observed that lymphopenia was also more common in patients >50 years of age 30/53.

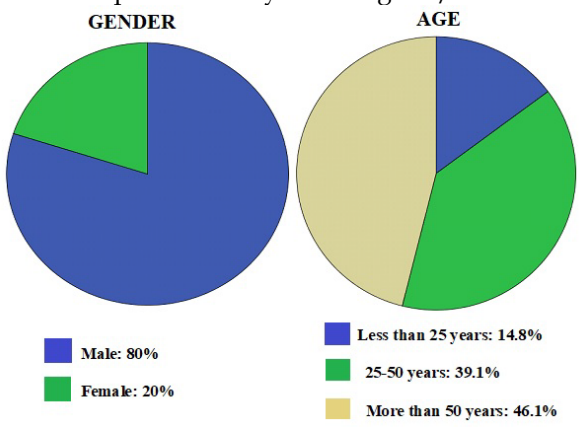

FIGURE 1: Distribution of cases (percentages) based on demographic features

TABLE 1: Association of Lymphopenia and albumin with Age groups

	Lymphopenia | Cut off 1.0x10³/microliter		Albumin (3.5 g/dl cut off)		Total
	No	Yes	Low	Normal	
Less than 25 years	11	6	10	7	17
25-50 years	31	14	31	14	45
More than 50 years	23	30	43	10	53
Total	65	50	84	31	115
P value	**0.031**		0.142		
Male	51	41	67	25	92
Female	14	19	17	6	23
P value	0.23		1.0		

TABLE 2: Correlation between various laboratory parameters and Age groups

AGE GROUPS					p-value
Parameters (mean and sd)	Reference Range	< 25 Years	25-50 Years	> 50 Years	
RED BLOOD CELL COUNT (x10⁶/µl)	3.5-5.6	3.9047 ±1.01790	4.9486± .80469	4.5013 ±.76135	**0.00**
TOTAL LEUCOCYTE COUNT (x10³/µl)	4-11	14.420 ±9.7964	9.486 ±5.0303	8.354 ± 3.2167	**0.002**
HEMOGLOBIN (g/dl)	13.5-17.5-M 12.0-15.5-F	11.587 ±2.5587	14.046 ±2.5098	12.853 ±2.0509	**0.003**
HEMATOCRIT (%)	41-50-M 36-48-F	34.887 ±7.8218	42.363 ±7.1071	38.437 ±6.0734	**0.002**
MEAN CORPUSCULAR VOLUME (fl)	80-100	90.427 ±7.6954	85.686 ±6.5978	85.747 ±6.1327	**0.047**
MEAN CORPUSCULAR HEMOGLOBIN (pg)	27-32	30.080 ±2.9993	28.363 ±2.6777	28.645 ±2.3275	**0.097**
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (g/dl)	31.5-35.5	33.293 ±2.1069	33.060 ±1.2857	33.408 ±1.1842	**0.57**
RED CELL DISTRIBUTION WIDTH (CV %)	11-14	16.800 ±4.0727	15.326 ±3.3576	14.242 ±1.0515	**0.01**
PLATELET COUNT (x10³/µl)	150-450	196.40 ±144.743	230.29 ±89.022	239.58 ±101.167	**0.4**
NEUTROPHIL%	45-70	79.573 ±14.7602	69.446 ±13.7708	78.847 ±10.2320	**0.003**
LYMPHOCYTE%	20-40	14.087 ±11.1000	22.166 ±11.8715	14.292 ±8.6231	**0.003**
EOSINOPHIL%	1-6	1.340 ±2.6186	1.757 ±2.2521	.695 ±.9687	**0.06**
MONOCYTE%	2-10	4.840 ±3.0657	6.349 ±3.8359	5.921 ±2.9874	**0.35**
PROTHROMBIN TIME (sec)	11-13.5	21.5207 ±11.06056	15.5774 ±3.67962	16.3945 ±8.49636	**0.037**
ACTIVATED PARTIAL THROMBOPLASTIN TIME (sec)	30-40	43.3607 ±15.06653	35.5909 ±8.76924	39.8632 ±7.54467	**0.025**
INR	0.43-2.75-M 0.37-2.87-F	1.8433 ±.98602	1.2683 ±.31711	1.3547 ±.79688	**0.026**
SERUM CREATININE (mg/dl)	0.6-1.3	2.107 ±3.5040	0.946 ±.3081	1.292 ±1.8227	**0.139**
SERUM UREA (mg/dl)	13-43	46.20 ±33.145	33.94 ±19.996	48.53 ±35.441	**0.103**
SERUM BILIRUBIN (mg/dl)	0.3-1.2	1.187 ±2.1454	0.67 ±.5179	0.653 ±0.3203	**0.155**
ALT (U/L)	4-42	29.00 ±17.897	55.60 ±68.457	46.55 ±47.727	**0.284**
ALKALINE PHOSPHATASE (U/L)	40-130	157.80 ±93.577	132.20 ±131.709	106.92 ±35.095	**0.189**
SERUM CREATININE PHOSPHOKINASE (U/L)	39-308-M 26-192-F	502.92 ±566.374	215.54 ±318.220	284.45 ±603.660	**0.275**
SERUM ALBUMIN (g/dl)	3.5-4.5	2.827 ±1.1925	3.477 ±.7317	2.984 ±.4565	**0.004**
SERUM LACTATE DEHYDROGENASE (U/L)	40-460	630.20 ±541.487	411.06 ±363.405	436.71 ±276.513	**0.141**
ABSOLUTE NEUTROPHIL COUNT (x10³/µl)	2.0-7.0	12.6913 ±9.38365	6.7957 ±4.24478	6.9242 ±3.11725	**0.001**
ABSOLUTE LYMPHOCYTE COUNT (x10³/µl)	1.0-3.0	1.5161 ±.81376	1.8171 ±.95076	1.0650 ±.53929	**0.000**
NEUTROPHIL/LYMPHO CYTE RATIO	0.43-2.75-M 0.37-2.87-F	17.3407 ±23.33505	6.4262 ±10.98720	7.9823 ±5.64325	**0.016**
PLATELET/LYMPHOCYTE RATIO	47-323	220 ±318	172.76 ±139	282.4 ±195.7	**0.075**

Discussion

In the past few decades, Pakistan has faced many challenges of infectious diseases, as a consequence of various factors, including high population density, increasing environmental pollution, the emergence of new infections as well as the changing lifestyle and behavior of its residents. The upsurge of COVID-19 is definitely a challenge for our country, recent data highlighted the importance of demographic sciences in understanding Corona virus transmission and fatality rates in different parts of world [5].Morbidity and mortality in developed countries has been documented high, attributed to having big proportion of aging population as compared to China-12%[25].A couple of weeks back Pakistan recorded the lowest death toll. An insight into the population pyramid of Pakistan show favorable structure with major concentration of population in young age groups. Pakistani population above 65 years is 4.5% compared to Italy where 22 % population is above 65 years. The average life expectancy of our population is 67.8 years , far less than the developed countries[6].

Numerous studies have reported changes in the basic laboratory parameters as Complete blood picture-hemogram, Serum Albumin and LDH of patients with COVID-19 particularly in adult age group [7]. Predictive implication of critical disease has been described for simple tests as Neutrophil lymphocyte ratio NLR, Lymphopenia and thrombocytopenia [7,8,9]. However there is considerable dearth of data for distinct age stratified groups and the underlying pathophysiology of heterogeneous laboratory profile observed in younger population.

We found that adults > 25 years and males were frequently affected. Similar pattern has been observed from earliest report in pandemic.[8] Davies et al. estimated age specific susceptibility of COVID-19 and found that population under 20 years are half as susceptible to infection as those over 20 years of age and 79% of infections are asymptomatic or subclinical in 10- to 19-year-olds, compared with 31% in those over 70 years of age[9].

In accordance with the published literature, we observed that in addition to increased age, male gender was associated with PCR positivity[10]. Reduced susceptibility of women could be attributed to the protection from X chromosome and sex hormones especially estrogen, which play an important role in innate and adaptive immunity[10, 11].

In terms of basic laboratory parameters, Blood Complete picture CP, Coagulation profile, LFTs, RFTs, we observed significant heterogeneous values in different age's .We stratified the patients into three sub groups. In group lesser than 25 years of age, we observed WBC count was predominantly normal and neutrophilic leukocytosis was observed in 41% of the cases. Lymphocytes were below the normal range in only 35 % of total patients in this group. These findings are consistent with the observation made by Vakili *et al* and pooled analysis conducted by Henry *et al*.[12] who observed that leukocyte indices in children and young adults do not appear to be reliable markers of disease severity. They also observed that lymphopenia is a minor feature in young population. These basic findings are different in elderly patients in whom Lymphopenia picture predominates- in our study 57%.

The observation might be just the "tip of underlying cytokine storm". This result suggests that Corona Virus mainly act on lymphocytes, especially T lymphocytes. T cells are important in body's immune response against any viral infection. Studies suggest that a substantial decrease in the total number of lymphocytes indicates that Corona Virus consumes immune cells which contribute to the exacerbation of clinical symptoms.[13]

Xia *et al* observed similar findings and highlighted the role of procalcitonin in this scenario. Apart from its role in identifying sepsis, procalcitonin is a marker of disease severity in COVID-19 particularly in young patients. They observed that Procalcitonin (PCT) was raised in 80 % of the children regardless of coexisting bacterial infection. In light of the aforementioned points, we propose that procalcitonin should be a part of basic laboratory workup especially in young CoV-2019 patients[14]. In addition , procalcitonin can also help in differentiating COVID-19 alone and other viral infections as SARS , a genetically similar corona virus , in having significant association with leucopenia -low WBCs , neutropenia and lymphopenia in laboratory confirmed admitted young patients.[15]

The laboratory parameters in adult age group followed different trends .we observed lymphopenia in 31% of patients in 25-50 years and 57 % of patients in more than 50 years of age. We also observed hypoalbuminemia as a significant finding in adult population group more than 50years of age (81%).

The findings are considerably similar to the earlier published data.[16] Studies suggest that the combinations of the hypoalbuminema, lymphopenia, and high concentrations of CRP and LDH in CoV-2019 infected patients upon hospital admission may predict more severe acute lung injury.[17] The initial recognition

of these deranged laboratory parameters can help in timely therapeutic intervention that can be crucial to the patient management.

Another marker neutrophil lymphocyte ratio NLR with a cut off of 3.13 has also been discussed as promising predictive factor for critical illness incidence of COVID-19 pneumonia. We observed that 84/115 total admitted COVID 19 cases in our study had NLR greater than 3.13. Liu *et al* observed that patients aged ≥ 50 and having an NLR ≥ 3.13 have a high risk of developing a critical illness and should be prepared for transfer to ICU for invasive respiratory support equipment. They presented guidelines for COVID-19 management depending upon age and NLR. This risk stratification and management guidelines based on simple parameters derived from Complete blood picture can alleviate medical resource shortage, a frequent problem faced by our health systems in these unprecedented situations.[18]

This study is limited as it is blinded of clinical follow-up, progression and outcome of the disease .The study is also limited because of the small sample size specifically in younger/children age group. A future implication of the study is to include radiological details and mortality rate on a much larger sample size. Other demographic details as ethnicity and family size should also be studied to better comprehend virus transmission and break the chain of it spread.

As the pandemic unravels, we realize that time of appropriate action is of utmost importance, especially in resource restricted communities. Policy makers need to stratify their lock down strategy with age groups and population structure. In addition, our study highlighted the difference in laboratory parameters which are far readily available before RT-PCR, considered gold standard for Corona virus detection. The role of Lymphopenia, hypoalbuminema, Neutrophil lymphocyte ratio especially in elderly is of paramount importance. An understanding of these parameters along with a high index of suspicion can help physician to identify and isolate suspected, subclinical cases before they can be a source of transmission to others.

Conclusion

We concluded that laboratory parameters associated with worse prognosis as lymphopenia and hypoalbuminemia were more common in patients more than 50 years of age. Neutrophilia was more common in young patients. Deranged Neutrophil lymphocyte ratio NLR was observed in all three-age stratified group. Combining parameters as NLR and age can be a potential indicator of prognosis

Declarations

Availability of data

Data regarding individual results can be furnished upon request.

Competing interests

The authors of the study have no conflict of interest.

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