



COVID 19 RT PCR POSITIVITY CORRELATION WITH ALLIED LAB TESTS ACROSS AGE GROUPS – A RETROSPECTIVE ANALYSIS FROM A TERTIARY REFERRAL LABORATORY.

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ABSTRACT

Introduction: Covid 19 pandemic has affected the world deeply and continues to affect even after 2 years of its outbreak. As the signs and symptoms of coronavirus disease 2019 overlap with those of other respiratory pathogens it necessitated laboratory testing to specifically identify individuals infected with Covid 19. The initial testing began by using the standard RT-PCR method. In certain situations, individuals without obvious signs and symptoms of SARS-COV 2 also require RT PCR testing. Once detected positive, these patients get triaged on the basis of their symptoms and the abnormalities in their laboratory findings as per their age and comorbidities.

The testing rate has been ramped up significantly over the last two years and continues to rise till date. With the variety of laboratory diagnostic tests available an informed prognosis can be made.

In this study we performed a retrospective analysis of laboratory investigations in COVID RTPCR positive patients in India including all age groups and gender.

The Aim of this study was to correlate the findings of covid monitoring tests such as IL-6, D-Dimer that were performed in Covid RT PCR positive patients at our center and report the variations noted when analyzed with parameters i.e. Age and Gender.

Materials and Methodology: This retrospective study was performed at GRL Laboratory, Metropolis, Mumbai. Data of covid RT PCR positive patients who underwent covid allied tests was retrieved for the period starting from 1st June 2020 till 30th June 2021. The laboratory values of covid monitoring profiles which included tests-- CBC with Neutrophil to lymphocyte ratio, IL6, cardiac troponin, D Dimer, ferritin, CRP, PT, LDH, Albumin, liver enzymes (SGPT), creatinine & ESR, were collected and were correlated with Age and Gender of the COVID 19 positive patients.

Results: The study included 1141 confirmed Covid 19 patients in the cohort group of which 524(45.9%) were female and 617(54.08%) were male. 43.9% < 45 years of age and 56% > 45 years of age. C-reactive protein (CRP) was elevated in 41.4%, D-dimer in 20.2% with significant variation noted in age and gender along with erythrocyte sedimentation rate (ESR). In majority patients, increased neutrophils and decreased lymphocytes were observed. Patients above 45 years of age showed higher neutrophil ($p = 0.002$) and lower absolute lymphocyte ($p = 0.022$) counts than adults below 45 years of age.

Conclusion: Following biomarkers were found to be mostly elevated in patient with COVID-19: High Sensitive Troponin, NLR, Absolute Basophil Count, Ferritin, CRP Hemoglobin and IL6. Biomarkers abnormality tends to vary with gender and age group. The analysis illustrates the value of laboratory parameters can be rapid and cost-effective biomarkers for prognostication in patients with COVID-19.

KEYWORDS : SARS-COV2 Positive, Biomarker, Monitoring tests.

INTRODUCTION:

COVID 19 crisis was declared a pandemic in March 2020 and continued to show its impact worldwide with active cases existing till today. In a resource strained country like India, it became a serious public health challenge. India has been calibrating its testing strategy since then as per the changing scenario.

COVID-19 is thought to be transmitted via human-to-human transmission pathways, through mediators like droplets, contact, and fecal-oral transmission. Coronaviruses are known to cause respiratory, gastrointestinal (GI), liver, and neurologic diseases in animals; however, only seven are known to cause disease in humans [1]. Three of these seven coronaviruses can cause much more severe, and sometimes fatal respiratory infections in humans when compared to other coronaviruses and have caused major outbreaks of deadly pneumonia in the 21st century. These coronaviruses include severe acute respiratory syndrome (SARS), Middle East Respiratory Syndrome (MERS), and COVID-19, all found to be enveloped RNA viruses. COVID-19-infected cases continue to increase, with many cases being unreported, and so the lethality and infectivity of COVID-19 remain unknown.

series of screening tests the most common being the RT-PCR test. Development of rapid point of care tests with better sensitivity and specificity was the critical need of the hour as it helped in diagnosis and containing the spread. Once the patient is detected positive, then he or she would be triaged on the bases of the severity of their symptoms and subsequently those deemed serious were hospitalized. As with all infections, patients had to be monitored to assess the course of their treatment during their hospital stay using an array of clinical and laboratory tests.

However, most of these laboratory reports have variable characteristics suggesting the diversity of the disease in various age groups and gender.

Tests such as Interleukin (IL-6) 6, D-Dimer, and CBCs were widely used to assess the initial baseline values and the variations were noted throughout the course of hospital stay. These tests primarily helped the physician tailor the course of treatment given to the patient. Metropolis laboratory is one of the largest centers in Mumbai where hospitals would routinely send their samples.

Like all infectious diseases, COVID too was put through a

This study primarily aims to provide a descriptive overview of

the various tests used to monitor a COVID-19 patient's response to therapy and its correlation with age and gender.

Methodology:

Study design

A retrospective single-center study was conducted at Metropolis, GRL, Mumbai, India, which is a referral laboratory covering various specialty tests, thus serving various patient groups and their clinicians and most of the population in Mumbai.

Patient and Methods

Patient data was collected retrospectively from June, 1st 2020 to June, 30th 2021. The following tests were included as COVID-19 allied test: Absolute Basophil Count, Absolute Eosinophil Count, Absolute Lymphocyte Count, Absolute Monocyte Count (AMC), Absolute Neutrophils Count, Serum Albumin, Creatinine Protein Kinase (CPK), Creatinine, C-Reactive Protein (CRP), D Dimer, Erythrocyte Sedimentation Rate (ESR), Serum Ferritin, High Sensitive Troponin, Haemoglobin (Hb), Interleukin-6 (IL-6), Lactate Dehydrogenase (LDH), Neutrophilic-Lymphocytic Ratio (NLR), Platelet Count, Prothrombin Time, Serum Glutamic-Pyruvic Transaminase (SGPT) and White blood cells (WBC) counts.

All Covid 19 RT PCR positive patients having undergone all the tests were included in this study. Patients with incomplete data were excluded. Of the 1141 patients, 617 patients were male and 524 patients were female. Median age of the patients was 48 years with IQR being 36-60.

Descriptive comparisons were drawn to correlate the abnormal results correlated with gender and in age group above 45 years and below 45 years.

Statistics Analysis

Results for quantitative variables are being expressed as median [Interquartile (IQR)] and qualitative variables expressed as frequency and percentages. Chi square test was used to drawn association of variables of COVID-19 allied test with gender and age. Results were considered significant at p < 0.05 and software used was "R Studio version 1.4.1103 © 2009-2021, PBC"

RESULTS

A total of 1141 confirmed cases of COVID – 19 patient's records were reviewed of which, 617(54.08%) were male and 524 (45.92%) were female. Median age of the patients was 48 years with IQR being 36-60. (Table 1)

Table 1: Demographics

	Frequency	Percentage
Age group		
<45	501	43.91%
>45	640	56.09%
Gender		
Female	524	45.92%
Male	617	54.08%

High sensitive troponin was found to be abnormal in more than three third of sample population (85.92%), followed by NLR (75.39%), absolute basophil count was found lower than normal in 61.72% patient, absolute eosinophil counts lower in 25.98% patients, 42.96% patients had abnormal CRP and D-Dimer abnormality were found in 20.22% patients. Absolute lymphocytes Count were found abnormal in 19.95% patients with 12.26% having value lower than normal. (Table 2)

Table 2: COVID-19 allied test

	Frequency	Percentage
Absolute Basophil Count		
Low	674	61.72%

Normal	380	34.8%
High	38	3.48%
Absolute Eosinophil Count		
Low	284	25.98%
Normal	769	70.36%
High	40	3.66%
Absolute Lymphocyte Count		
Low	134	12.26%
Normal	875	80.05%
High	84	7.69%
Absolute Monocyte Count		
Low	37	3.39%
Normal	1005	91.95%
High	51	4.67%
Absolute Neutrophils Count		
Low	110	10.06%
Normal	880	80.51%
High	103	9.42%
Albumin		
Low	13	2.9%
Normal	435	96.88%
High	1	0.22%
CPK		
Abnormal	47	10.24%
Normal	412	89.76%
Creatinine		
Low	61	13.59%
Normal	353	78.62%
High	35	7.8%
CRP		
Abnormal	473	42.96%
Normal	628	57.04%
D Dimer		
Abnormal	198	20.22%
Normal	781	79.78%
ESR		
Abnormal	88	28.12%
Normal	225	71.88%
Ferritin		
Low	26	2.36%
Normal	489	44.41%
High	586	53.22%
High Sensitive Troponin		
Abnormal	293	85.92%
Normal	48	14.08%
Hemoglobin (Hb)		
Low	462	42.27%
Normal	630	57.64%
High	1	0.09%
IL6		
Abnormal	366	36.9%
Normal	626	63.1%
LDH		
Abnormal	112	24.94
Normal	337	75.06
NLR		
Abnormal	824	75.39
Normal	269	24.61
Platelet		
Low	47	4.30
Normal	1020	93.32
High	26	2.38
Prothrombin Time		
Normal	435	97.75
High	10	2.25
SGPT		
Abnormal	119	26.50
Normal	330	73.50

WBC		
Low	155	14.18
Normal	834	76.30
High	104	9.52

The results of relationship between gender and COVID-19 profile have been tabulated in the Table 3. As noted the Absolute eosinophilic count was found to be significantly higher in males as compared to females. The AMC, CPK and Creatinine values were significantly higher in males as compared to females.

With regards to females, D Dimer and ESR values were significantly abnormal as compared to males. Serum ferritin was significantly higher in males and was found low in females. Hemoglobin levels were lower in males as compared to females. CRP values were comparatively abnormal in both males and females.

Table 3: Relationship of gender and COVID 19 profile test

	Gender				p value
	Female		Male		
	N	%	n	%	
Absolute Basophil Count					
Low	307	61.03%	367	62.31%	0.8397
Normal	177	35.19%	203	34.46%	
High	19	3.78%	19	3.23%	
Absolute Eosinophil Count					
Low	138	27.44%	146	24.75%	0.0079
Normal	356	70.78%	413	70.00%	
High	9	1.78%	31	5.25%	
Absolute Lymphocyte Count					
Low	52	10.34%	82	13.90%	0.0913
Normal	406	80.71%	469	79.49%	
High	45	8.95%	39	6.61%	
Absolute Monocyte Count					
Low	19	3.78%	18	3.05%	0.021
Normal	470	93.44%	535	90.68%	
High	14	2.78%	37	6.27%	
Absolute Neutrophils Count					
Low	58	11.53%	52	8.82%	0.2578
Normal	395	78.53%	485	82.20%	
High	50	9.94%	53	8.98%	
Albumin					
Low	8	3.72%	5	2.14%	0.3857
Normal	207	96.28%	228	97.44%	
High	0	0.00%	1	0.42%	
CPK					
Abnormal	14	6.45%	33	13.64%	0.0113
Normal	203	93.55%	209	86.36%	
Creatinine					
Low	57	26.51%	4	1.71%	0.0001
Normal	155	72.09%	198	84.61%	
High	3	1.40%	32	13.68%	
CRP					
Abnormal	230	45.45%	243	40.84%	0.1234
Normal	276	54.55%	352	59.16%	
D Dimer					
Abnormal	117	25.55%	81	15.55%	0.0001
Normal	341	74.45%	440	84.45%	
ESR					
Abnormal	58	36.48%	30	19.48%	0.0008
Normal	101	63.52%	124	80.52%	
Ferritin					
Low	25	4.93%	1	0.17%	0.0001
Normal	325	64.10%	164	27.61%	
High	157	30.97%	429	72.22%	
Hemoglobin (Hb)					
Low	189	37.57%	273	46.27%	0.0089

Normal	313	62.23%	317	53.73%	
High	1	0.20%	0	0.00%	
High Sensitive Troponin					
Abnormal	142	84.02%	151	87.79%	0.318
Normal	27	15.98%	21	12.21%	
IL6					
Abnormal	162	34.91%	204	38.64%	0.2256
Normal	302	65.09%	324	61.36%	
LDH					
Abnormal	52	24.19%	60	25.64%	0.7222
Normal	163	75.81%	174	74.36%	
NLR					
Abnormal	383	76.14%	441	74.75%	0.5931
Normal	120	23.86%	149	25.25%	
Platelet					
Low	15	2.98%	32	5.42%	0.0728
Normal	473	94.04%	547	92.71%	
High	15	2.98%	11	1.86%	
Prothrombin time					
Normal	205	97.16%	230	98.291%	0.4207
High	6	2.84%	4	1.709%	
WBC					
Low	81	16.10%	74	12.54%	0.056
Normal	367	72.96%	467	79.15%	
High	55	10.93%	49	8.31%	

* p value marked in red color is significant

Absolute lymphocyte count was low in 14.08% of patients with age >45. Absolute neutrophil count showed significant variations in both the age group (p<0.002), with high value reported in 6.43% of patients with < 45 and >45 showed 11.78%. NLR showed significant variation (p<0.001) with 68% showing abnormal values in patients >45 years of age. Albumin, CPK, Creatinine showed significant variations in both the age groups.

Variations in CRP was significant (p<0.001) with 49.11% of patients with >45 age showing abnormal values. IL6 was abnormal in 42.4% of patients > 45 years (p<0.001), D dimer was increased in 25.2%, with significant variations in both the age groups (p<0.001) ESR was increased in 35.8% and ferritin was high in 61.17%.

Hemoglobin was noted low in 48.6% of patients having COVID-19 and above 45 years. High sensitivity troponin was abnormal in 78.7% of patients.

LDH and Prothrombin time did not show significant variations when compared with both the age groups. (Table 4)

with COVID profile test

Table 4: Relationship of age group

	Age Group				p value
	<45	>45	N	%	
	N	%	N	%	
Absolute Basophil Count					
Low	303	62.99%	371	60.72%	0.2668
Normal	166	34.51%	214	35.02%	
High	12	2.49%	26	4.26%	
Absolute Eosinophil Count					
Low	99	20.54%	185	30.28%	0.0005
Normal	360	74.69%	409	66.94%	
High	23	4.77%	17	2.78%	
Absolute Lymphocyte Count					
Low	48	9.96%	86	14.08%	0.0221
Normal	388	80.50%	487	79.71%	
High	46	9.54%	38	6.22%	
Absolute Monocyte Count					

Low	20	4.15%	17	2.78%	0.0876
Normal	446	92.53%	559	91.49%	
High	16	3.32%	35	5.73%	
Absolute Neutrophils Count					
Low	64	13.28%	46	7.53%	0.0002
Normal	387	80.29%	493	80.69%	
High	31	6.43%	72	11.78%	
Albumin					
Low	0	0.00%	13	5.51%	0.0014
Normal	212	99.53%	223	94.49%	
High	1	0.47%	0	0.00%	
CPK					
Abnormal	15	7.01%	32	13.06%	0.0331
Normal	199	92.99%	213	86.94%	
Creatinine					
Low	38	17.84%	23	9.75%	0.0003
Normal	168	78.87%	185	78.39%	
High	7	3.29%	28	11.86%	
CRP					
Abnormal	170	35.12%	303	49.11%	<0.0001
Normal	314	64.88%	314	50.89%	
D Dimer					
Abnormal	60	13.89%	138	25.23%	<0.0001
Normal	372	86.11%	409	74.77%	
ESR					
Abnormal	31	20.13%	57	35.85%	0.0020
Normal	123	79.87%	102	64.15%	
Ferritin					
Low	20	4.14%	6	0.97%	<0.0001
Normal	255	52.80%	234	37.86%	
High	208	43.06%	378	61.17%	
Hemoglobin (Hb)					
Low	165	34.23%	297	48.61%	<0.0001
Normal	317	65.77%	313	51.23%	
High	0	0.00%	1	0.16%	
High Sensitive Troponin					
Abnormal	152	93.83%	141	78.77%	<0.0001
Normal	10	6.17%	38	21.23%	
IL6					
Abnormal	129	29.72%	237	42.47%	<0.0001
Normal	305	70.28%	321	57.53%	
LDH					
Abnormal	49	23.00%	63	26.69%	0.3674
Normal	164	77.00%	173	73.31%	
NLR					
Abnormal	403	83.61%	421	68.90%	<0.0001
Normal	79	16.39%	190	31.10%	
Platelet					
Low	14	2.90%	33	5.40%	0.0747
Normal	459	95.23%	561	91.82%	
High	9	1.87	17	2.78	
Prothrombin time					
Normal	207	98.57%	228	97.02%	0.2713
High	3	1.43%	7	2.98%	
WBC					
Low	83	17.22%	72	11.78%	0.0038
Normal	365	75.73%	469	76.76%	
High	34	7.05%	70	11.46%	

*p value marked in red color is significant

DISCUSSION:

In this study we found that these values did have statistically significant correlations when assessed with the gender of the patients. The tests used in this study are bio-markers indicating the severity of the disease on admission as well as being helpful to the treating physicians to alter the course of treatment on the basis of these values.

CRP:

In the meta-analysis published by Zhang et al, they found increased C-reactive protein rate to be significant along with lymphopenia (OR 4.5) and increased LDH. In our study as well we looked at the CRP levels, which were found to significantly vary in the age group <45 years (p <0.0001). The absolute lymphocyte count varied significantly in the >45 years' age group (p 0.0221) suggesting increased severity noted in this age group. Since LDH is a cumulative marker it did not vary significantly in both the parameters in our study. However, an increased LDH is a significant indicator with regards to the severity of the disease.

In this study with previous reports showed the middle-aged, from 40 to 60 years, were the most commonly infected group. (2)

Age & Gender:

However, the virus can infect individuals of all age groups (3,4), the study reported a greater number of men than women as having the infection, which is similar to the findings of other studies (5,6)

The decrease in women's susceptibility to coronavirus infections could be attributed to sex hormones and an X chromosome, which play an essential role in innate and adaptive immunity (5,7)

Regarding the hematological parameters, an estimation of white blood counts is essential to predicting the outcomes of COVID-19 infection [8].

WBC & NLR:

We found normal leucocyte counts in our results, with a decreased level of lymphocytes and an upper limit of neutrophil counts.

Similarly, Jin et al. showed that in the early stage of the disease, the total number of leucocytes in peripheral blood was normal or decreased, and the lymphocyte count was decreased (9).

Recent reports have also found increased neutrophil counts and decreased lymphocyte counts associated with COVID-19 (5, 10] Lymphocytopenia could explain the effect of SARS-CoV-2 on T lymphocytes, mainly on CD4+ T cells and CD8+ T cells, resulting in a decrease in lymphocyte numbers and IFN- γ production (5,10].

It is well known that SARS-CoV-2 spreads through the respiratory mucosa and invades other cells, induces a series of immune responses, and causes changes in peripheral white blood cells, including lymphocytes (5). Studies suggest that a substantial decrease in the total number of lymphocytes indicates that coronaviruses disrupt many immune cells and suppress cellular immune function [11, 12].

The levels of D-dimer, CRP, ESR, and other inflammatory markers are essential in detecting bacterial infection in the lungs, and also help to evaluate patients' immune status (9). Likewise, studies have documented that an increased D-dimer concentration is a common feature of COVID-19 infections, especially for severe patients [9]. However, the elderly are more likely to develop a severe disease (6). Our findings revealed significantly higher levels of ESR and D-dimer in the elderly than in the adult group. A recent study found that the CRP level in elderly patients was significantly higher than that in young and middle-aged groups (6).

Limitations:

We statistically analyzed the laboratory findings based on different age groups and gender but that were no subdivided

into groups of patients with individual comorbid conditions & clinical presentation.

CONCLUSIONS:

We can conclude from the analysis of study that laboratory parameters such as High Sensitive Troponin, NLR, Absolute Basophil Count, Ferritin, CRP, Hemoglobin and IL6 are elevated in patient with COVID-19.

The study also infers that laboratory findings of patients of different age groups which help in assessing the progression of disease in confirmed COVID-19 patients. Increased CRP, ESR, IL6 and D-dimer levels were significantly abnormal in patients more than 45 years of age. However, D-dimer, and ESR levels were significantly higher in the elderly patients. Increased levels of leucocytes, and decreased hemoglobin were frequent laboratory findings associated with elderly Covid 19 positive patients, these tests correlation with age and gender aids in managing patients who could develop critical conditions and be at risk of mortality. These findings should be confirmed in future with tests such as TBK test by flow cytometry for evaluating disease severity and outcomes.

Thus, laboratory parameters can be considered as a potentially useful for better clinical management and prevention of serious complications in patients with COVID-19.

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