Original Article

Morphology of WBCs on Peripheral Blood Smear of COVID 19 Positive Patients

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ABSTRACT

Introduction: Covid-19 pandemic has affected almost all the inhabited areas on our planet. Researchers are working hard to bring this disease to its resolution. There is emerging evidence every day to point towards the altered parameters in our body. Complete blood picture and peripheral blood smears are also showing considerable changes. These changes can help to raise suspicion of Covid-19 infection at the earliest. This can help us in future to differentiate from diseases with similar findings on peripheral blood smear.

Materials and Patients: This is a prospective observational study in which 500 peripheral smears of Covid-19 infected patients were observed and the findings were recorded. All the morphological changes in WBCs, total WBC count and neutrophilic lymphocyte ratio were recorded over a span of six months from June2020-December 2020. This data was analyzed statistically.

Results: Our study reveals that there is not much change in WBC counts in initial stage of the disease. Increased Neutrophil Lymphocyte ratio is seen in almost all the patients as the disease progresses. Presence of toxic granules in the cytoplasm of cells of neutrophilic series was the earliest noticeable change in the morphology of WBCs. Earliest response is seen in lymphocytes as azurophilic granules in cytoplasm and large indented nuclei.

Discussion and Conclusion: With the above study, we can conclude that there are significant and specific changes observed in the morphology of WBCs of the affected patients. This simple and easily available test can be an effective tool in helping to raise suspicion of the infection. This in turn will lead to early diagnosis and treatment.

*Keywords: Covid-19, toxic granules, neutrophil lymphocyte ratio*

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. A novel coronavirus, designated as 2019-nCoV, emerged in Wuhan, China, at the end of 2019. [1] And as the disease is progressing and spreading to more and more countries, there is a lot of emerging data. At present, whole of medical fraternity all over the world is trying its best to unravel its secrets. They are relating to its origin, spread, pathogenesis, behavior, diagnosis, prognostic markers, complications and treatment. It is a daunting task. Our institute has been declared as a dedicated COVID hospital since last one month. We are seeing a steady stream of patients daily. Patients' samples are received in our lab for various investigations like CBC and many specific laboratory investigations which are emerging as peculiar to this disease. This is a study involving findings about morphology of WBCs on peripheral smear which are seen in the affected patients. These findings may aid in diagnosing COVID-19 cases in asymptomatic and in suspected cases in future.

Material and Methods

This is a prospective observational study done in Central Laboratory of Terna Hospital and Research Centre and Medical College, Nerul, Navi Mumbai, Maharashtra, India. 500 peripheral blood smears of COVID-19 positive cases were examined during June 2020- December 2020. 3ml of blood was collected in K2EDTA vacutainers per sample. Smears were prepared by technicians and stained with Leishman's stain. The samples were run on a five-part cell counter. These samples belonged to the patients that were admitted in our hospital after they tested positive for Covid-19 by RT-PCR. These were the samples received in our
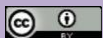
laboratory for testing on day 1 of admission in the hospital. The parameters recorded from the cell counter were total WBC count. Neutrophilic lymphocytic ratio was calculated using standard formula.

All the peripheral blood smears were examined in details. 100 WBCs were counted for the differential count for every smear. Presence of toxic granules, vacuolations in neutrophils, immature forms of neutrophils i.e., band forms, myelocytes, metamyelocytes were noted. Morphological changes in lymphocytes were also noted i.e., large granular cells and presence of nucleoli and any abnormal nuclear features. Ethical committee approval had been obtained for this study prior to its start.

All of these samples were referred to our laboratory for investigations. There has been absolute secrecy maintained. No personal details were revealed of the patients. After the samples were processed, they were discarded with all precautions and following our waste management policy. All the data was recorded in an excel sheet. The data was analyzed with the help of a statistician.

Results

Our study reveals that there is not much change in WBC counts in initial stage of the disease. Increased Neutrophil Lymphocyte ratio is seen in almost all the patients as the disease progresses. Presence of toxic granules in the cytoplasm of cells of neutrophilic series [1,2] (Figure-1) was the earliest noticeable change in the morphology of WBCs. Earliest response is seen in lymphocytes as azurophilic granules in cytoplasm (Figure-2) and large indented nuclei. Correlation between total WBC counts and NLR were statistically significant. (Tables 1 and 2).

Association between total WBC counts and NLR were also found to be highly significant (Tables 3 and 4). Association between Total WBC count and immature forms of neutrophils, presence of toxic granules, activated lymphocytes were also found to be statistically significant by applying Chi square test (Tables 5 and 6). There were no significant morphological changes seen in eosinophils and monocytes. As the disease progressed, there were increasingly a greater number of neutrophils seen as determined with increased neutrophil lymphocyte ratio. The neutrophilic series showed cytoplasm packed with toxic granules. Correlation with other biochemical and serological parameters like CRP levels was not a part of this study.
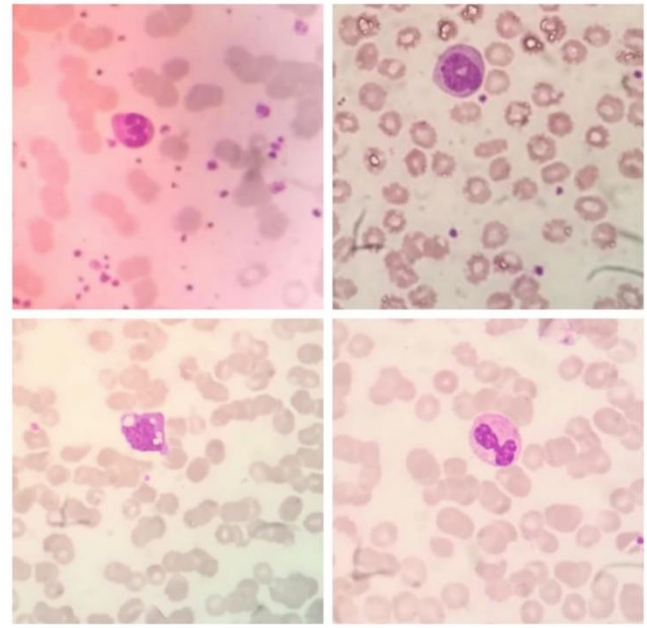

Figure 1: A composite image of neutrophils showing morphological changes i.e., Abnormal nuclear forms, toxic granules and vacuolations. Leishman's stain .100x
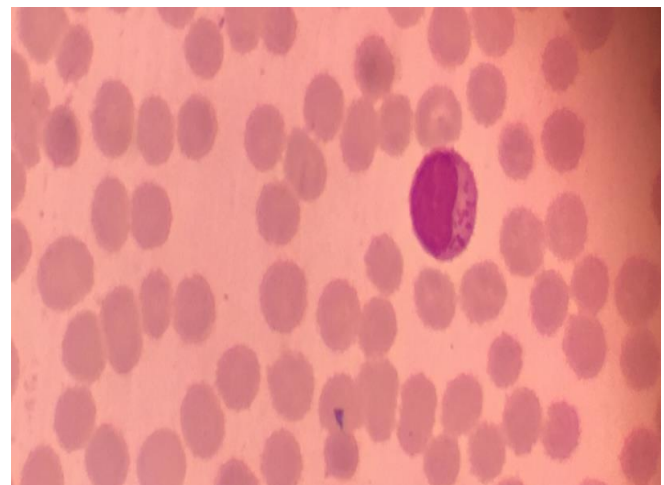

Figure 2: Large granular lymphocyte. Leishman's stain 100x

However, we found that only 27% of cases had CRP levels within normal range. Rest all showed elevated levels.

Discussion

Table -1 shows that normal total WBC counts are seen in 64.4% of patients. 6.8% showed below normal total WBC Count and 28.8% of patients showed WBC count above normal range. This tells us that there is not much change in total WBC count in the initial phase of the disease.

NLR was more than 3.3 in 56.4% of cases (Table-2). The normal range being 1-3 as normal, 6-9 indicates mild stress, 9-18 as moderate stress and more than 18 as severe stress.

Table 1: Total WBC Count

Total WBC Count	Group	N	%
Low Range	<4000	34	6.8
Normal Range	4000 – 11000	322	64.4
High Range	>11000	144	28.8
Total		500	100.0

Table 2: Neutrophil/Lymphocyte Ratio (NLR)

NLR	N	%
<3.3	218	43.6
≥3.3	282	56.4
Total	500	100.0

Table 3: Other Clinical parameters

Parameters	Yes		No	
	N	%	N	%
Immature WBCs i.e., myelocytes, metamyelocytes, band forms	220	44.0	280	56.0
Toxic granules	237	47.4	263	52.6
Vacuolations	19	3.8	481	96.2
Abnormal nuclear features	3	0.6	497	99.4
Activated lymphocytes	73	14.6	427	85.4
LGL	27	5.4	473	94.6
Nucleoli in lymphocytes	3	0.6	497	99.4

Table 2: Correlation between Total WBC Vs NLR

Parameters	Correlation Coefficient (r)	P-Value	Remark
Correlation between Total WBC Vs NLR	0.634**	<0.001	Positive Correlation

** Statistically highly significant at 0.1% level i.e., P<0.001.

Table – 5: Association between Total WBC Vs NLR

Parameters	NLR		Total	Chi Square Test	P-Value	Sig. at 5% level
Total WBC	<3.3	≥3.3				
Low Range	29	5	34	99.276**	<0.001	Yes
Normal Range	173	149	322			
High Range	16	128	144			
Total	218	282	500			

** Statistically highly significant at 0.1% level i.e., P<0.001.

Increase in this ratio is seen in almost all the patients as the disease progressed. There are significant changes seen in the morphology of WBCs on the peripheral smears. These samples are the first ones collected from patients after their admission in hospital after confirmation of the disease. 44%of cases show immature neutrophilic forms i.e., band forms, metamyelocytes and myelocytes.47.4% of cases show toxic granules in their cytoplasm. This makes presence of toxic granules in the cells of neutrophilic series, the earliest noticeable change in the morphology of WBCs.

Activated lymphocytes are seen in 14.6% of cases with large granular lymphocytes in the early phase of the disease. Cytoplasmic vacuolations in neutrophils are seen in only 3.8% cases (Table-3). This leads us to believe that the earliest response seen in lymphocytes [3,4]is azurophilic granules in cytoplasm and large, indented nuclei. These cells get replaced by neutrophils and their precursors rapidly as the disease progresses. This is also revealed by increasing Neutrophilic: Lymphocytic ratio.

Increase in total WBC count is positively correlated with increased NLR ratio and presence of toxic granules in the neutrophilic cell line. Also, statistically highly significant at 0.1% i.e., P<0.001. Activated lymphocytes were seen in smears with normal range of total WBC count is associated with NLR values i.e., statistically highly significant at 0.1% i.e., P<0.001(Table-4 and 5). Similarly, WBC count also be associated with Immature WBCs, myelocytes,

Table – 6: Association between Total WBC count Vs Other Clinical Parameters

Parameters	Total WBC			Total	Chi Square Test	P-Value	Sig. at 5% level
Immature WBCs, myelocytes, metamyelocytes, band forms	Low Range	Normal Range	High Range				
Yes	5	95	120	220	129.721**	<0.001	Yes
No	29	227	24	280			
Total	34	322	144	500			
Toxic granules							
Yes	6	128	103	237	53.250**	<0.001	Yes
No	28	194	41	263			
Total	34	322	144	500			
Vacuolations							
Yes	1	8	10	19	5.488	0.064	Not
No	33	314	134	481			
Total	34	322	144	500			
Abnormal nuclear features							
Yes	0	3	0	3	1.668	0.434	Not
No	34	319	144	497			
Total	34	322	144	500			
Activated lymphocytes							
Yes	9	57	7	73	17.281**	<0.001	Yes
No	25	265	137	427			
Total	34	322	144	500			
LGL							
Yes	2	22	3	27	4.409	0.110	Not
No	32	300	141	473			
Total	34	322	144	500			
Nucleoli in lymphocytes							
Yes	0	3	0	3	1.668	0.434	Not
No	34	319	144	497			
Total	34	322	144	500			

metamyelocytes, band forms, toxic granules, activated lymphocytes [5,6]. These findings also show that the WBC count influences the above parameters (Table-6).

We have studied 500 peripheral smears for this study. However, since the pandemic is still ongoing and we are a COVID-19 hospital, we continue to get more cases and we are still getting the same findings. There have been cases which were suspected to have other illnesses like Dengue. But their tests did not reveal positive results. In such cases we found toxic granules with absence of any suggestion of a bacterial infection. Some of them turn out to be COVID -19 positive.

We believe that even when this pandemic is over and COVID-19 remains as just any other viral disease, these findings will help us to exclude or at least help us to point to

our physicians that it just might be a COVID -19 infection.

We would also like to point out that there may be subjective variation in observations made by different Pathologists. But at the same time, the findings mentioned in this study are fairly routine in examining any peripheral blood smear. This can help to catch the infection in patients who are not even suspected or are asymptomatic carriers and have been advised routine CBC for any other purpose. All of the patients included in this study were COVID-19 positive by RTPCR. Some of them may have had other coexisting diseases without our knowledge and may have gone undetected. But they were treated for COVID-19 infection according to the accepted guidelines. We were not asked to look for any coexisting bacterial or other viral infection like Dengue in these selected patients by the treating physicians

Conclusion

With the above study, we can conclude that there are significant and specific changes observed in the morphology of WBCs in COVID-19 affected patients. This simple and easily available test can be an effective tool in helping to raise suspicion of the infection. This in turn will lead to early diagnosis and treatment. These findings can also help to exclude other diseases.

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