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Investigation the role of hematological parameters in pathogenesis of COVID-19 and heart disease

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Abstract

From research in which hematological parameters related to severe COVID-19 patients were tested, adjustments in the unique coagulation index, i.E., elevated D-dimer degree, extended prothrombin time and decreased wide variety of PLTs, emerged. These modifications replicate the hypercoagulable kingdom discovered in intense COVID-19 sufferers, that could promote the development of microthrombosis inside the lungs and different organs.

Introduction

Hemoglobin is the oxygen-carrying pigment in vertebrate red blood cells. The hemoglobin molecule is spherical in shape and consists of two alpha chains and two beta chains, linked to four pigment molecules called hemes. At the center of the heme group is one iron atom in the ferrous form linked to one oxygen molecule (Hillman and Ault 2002). Severe anemia is attributed to the toxicity of the modified drugs used or the formation of dangerous immune complexes for the disease.

Materials and Methods

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Blood

The volume of blood drawn ranged around 10 cm3 from healthy and ill people and turned into divided based on the type of exam. 5 cm3 was located in plastic tubes with covers containing an anticoagulant (EDTA) for the reason of engaging in blood group determination tests, white blood cell count (WBCs cunt), hemoglobin concentration (Hb), percentage of packed red blood cell volume (PCV) and erythrocyte sedimentation rate (ESR) (Powers, 1989).

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Serum Preparation

The serum was received by way of putting the final volume of the drawn blood as in the preceding paragraph in a plastic tube with a decent cover and free of anticoagulant and leaving the blood at a temperature of 25°C until it clotted and then placing it in a centrifuge for 10 minutes. The serum (filter) was withdrawn using a micro pipette and placed in easy, sterile tubes and saved frozen at a temperature of 20°C. The serum was used to conduct hormonal and biochemical checks.

Hematological tests

The first part of the drawn blood was taken and located in tubes containing EDTA for the purpose of conducting the following tests on it (Powers, 1989).

Measuring the percentage of the volume of packed blood cells (PCV)

Capilary tubes were used, 75 mm long and with an internal diameter of about 1 mm, and two-thirds of the capillary tube was filled with blood using the capillary property, then one of its ends was close using manufactured clay. The tube was place in a microcentrifuge at 0.005 rpm for 5 minutes. The separation amid the plasma column and the blood cells was then slow using a special ruler or a hematocrit reader (Heamatocrit Reader) and the percentage of the volume of the packed blood cells was read.

Measurement of Hemoglobin Determination (Hb)

The Hb value was calculate by dividing the result of the PCV value by 3.3, since hemoglobin represents 1/3 of the volume of the red blood cell (Powers, 1989).

Measurement of white blood cell count (WBCs Total Leucocytes Count)

The method of counting white blood cells was used using Turke's Fluid dilution solution, which was prepared by adding 1 cm^3 of a 1% aqueous solution of Gentian Violet dye to 1.5 cm^3 of concentrated glacial acetic acid. The components were mixed well, then 98 cm^3 of distilled water was added to the mixture (Powers, 1989).

Method of work

Put 0.4 ml of Turke's solution in a small tube, then add 0.02 cm^3 of the drawn blood, shake the mixture well, then put a drop of it on the edge of the slide cover placed above the blood cell counter, left for two minutes and examined at the minimum power of 10x, then counted in the four large squares that form the corners of the blood cell counter slide.

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Calculations:

Number of cells = Number of cells counted × 50 = cells per cubic millimeter of blood

Estimation of calcium ion level in blood serum: Determination of Serum Calcium

Basic principle:

The calcium ion in the blood serum was measured according to the method mentioned in the kit prepared by the French company
Biomerieux, which bears the number 1011801.

As calcium reacts with the red complex compound forming a complex in the basic solution O_cresol phtaleine in which we measure the calcium concentration.

Solutions used:-

Reagent(1) 2-Amino -2-methyl -1- 500 mmol/l*

Buffer solution proponol

Reagent(2) Gresolphtaline cowps 0-62mml/l *

69mmol/l - hydroxy quinoleine8

Reagent(3) Calcium standard 2.5 mmol/l*

(10 mg/dc)

(100 mg)

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Preparation of materials used:-

Mix a certain volume of Buffer solutions (R1) with the same volume of color solutions.(R2)

Method of work

Substant	Blank	Standard	sample
Standard	/	20ml	/
sample	/	/	20 ml
Reagent (1)	1 ml	1 ml	1 ml

The tubes were mixed well and left for five minutes at room temperature and read at a wavelength of 570 nm and according to the calcium as Next:-

2.5 x Calcium concentration =

Standard mmol/l reading

Results and Discussion

Data of result study showed there is high significant different ($p<0.05$) between males and females patients, where the males patients scored highest percentage (75.0%) than females (25.0%) (table 1).

Table 1; frequency and percentage of gender of COVID-19 and heart disease patients were calculated by chi-square test.

	Value	Count	Percent	P value
Gender	males	9	75.0%	P<0.001***
	females	3	25.0%	

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The conducted results show high significant different (P<0.05) between PLT, HGB, Granulocytes, and Lymphocytes with study groups .We noticed low levels of PLT, HGB, and Lymphocytes in patients (264.58±83.24, 7.37±2.12, and 20.68± 8.98) respectively, than healthy . In contrast, we noticed high levels of granulocytes in patients (67.08± 8.20) than healthy. Finally, there is no significant different (P>0.05) between MCV, MID, and WBCs with study groups (table 2 and figure 1).

Table 2; comparative mean levels of hematological parameters between study groups were calculated by student t test.

Groups		N	Mean	SD	P value
MCV	Patients	12	84.68	7.56	P>0.05
	Healthy	12	88.33	4.08	
PLT	Patients	12	264.58	83.24	P<0.001***
	Healthy	12	366.42	56.00	
HGB	Patients	12	7.37	2.12	P<0.01**
	Healthy	12	13.67	1.37	
MID	Patients	12	10.96	3.35	P>0.05
	Healthy	12	8.25	2.26	
GRAN	Patients	12	67.08	8.20	P<0.05*
	Healthy	12	46.58	9.49	
LYM	Patients	12	20.68	8.98	P<0.05*
	Healthy	12	31.58	3.48	
WBC	Patients	12	7.02	1.65	P>0.05
	Healthy	12	8.67	1.30	

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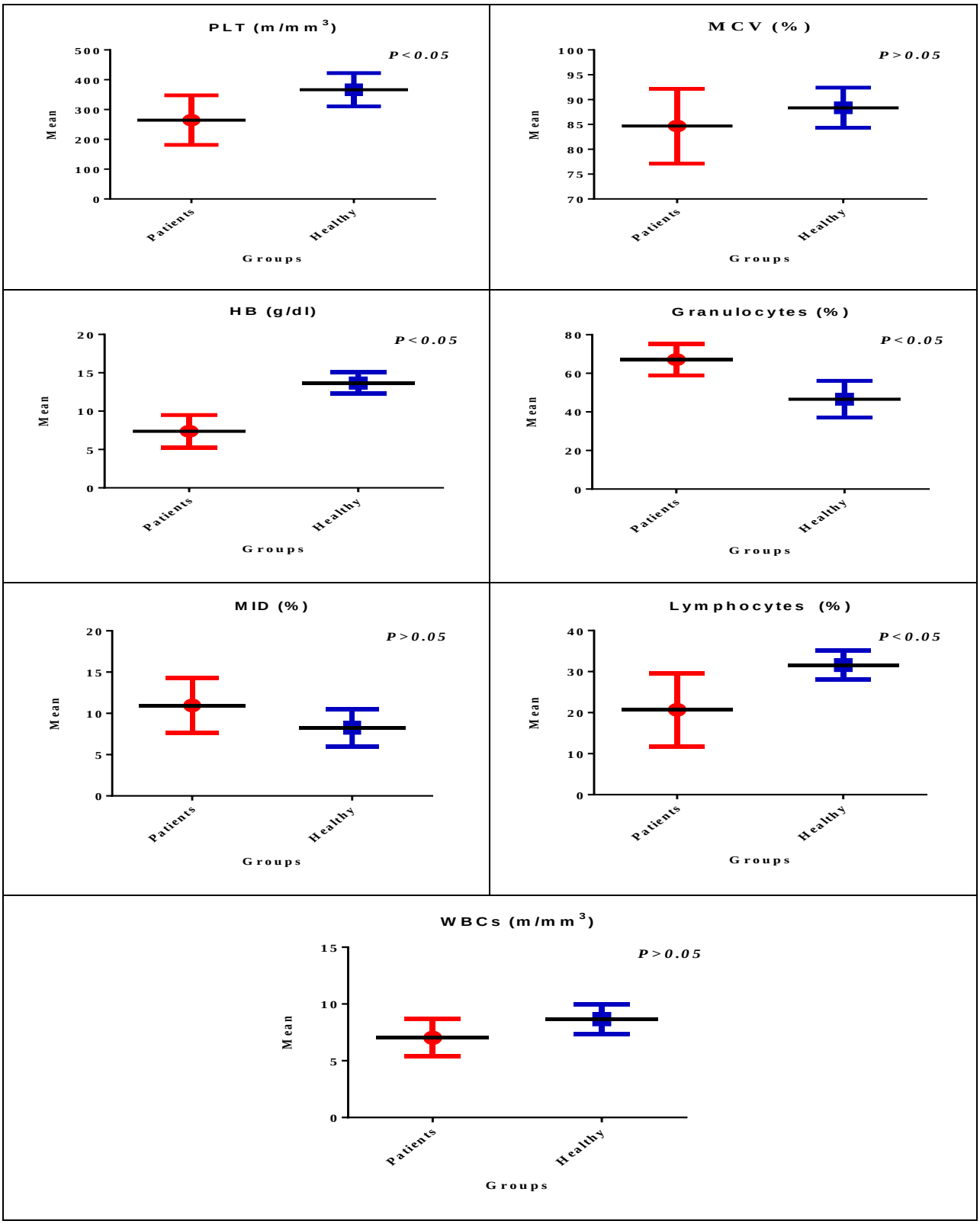

Figure 1; comparative mean levels of hematological parameters between study groups

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Results of present study presented there is positive and negative correlation amongst hematological parameters in patients. Importantly, there negative significant correlation between granulocytes and lymphocytes (r= -0.754** sig.= 0.005) (table 3).

Table 3; correlation relationship among hematological parameters were calculated by Pearson correlation.

		MCV	MID	LYM	WBC
MCV	Pearson coefficient	1	-.248	.024	-.164
	significant		.436	.941	.610
HGB	Pearson coefficient	-.351	.164	-.569	.062
	significant	.263	.609	.053	.849
GRAN	Pearson coefficient	-.151	.116	-.754**	.244
	significant	.639	.719	.005	.444
WBC	Pearson coefficient	-.164	.497	-.544	1
	significant	.610	.100	.067	

Receiver operator characteristic (ROC) curve of parameters

Results of present study showed the MID and granulocytes parameters scored high sensitivity (75%, and 91%) and specificity (50%, and 92%) respectively with significant difference ($p<0.05$) in screening patients with covid-19 and heart diseases (figure 2).

Table 4; ROC curve, sensitivity and specificity of variables

Variable(s)	AUC	P value	C.I. 95%		Sensitivity %	Specificity %
			Lower	Upper		
MCV	.372	.285	.142	.601	50	34
PLT	.153	.004	0.000	.306	25	17
HGB	.021	.000	0.000	.069	8	25
MID	.760	.030	.558	.963	75	50
GRAN	.951	.000	.872	1.000	91	92
LYM	.132	.002	0.000	.299	25	17

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WBC	.215	.018	.031	.400	58	18

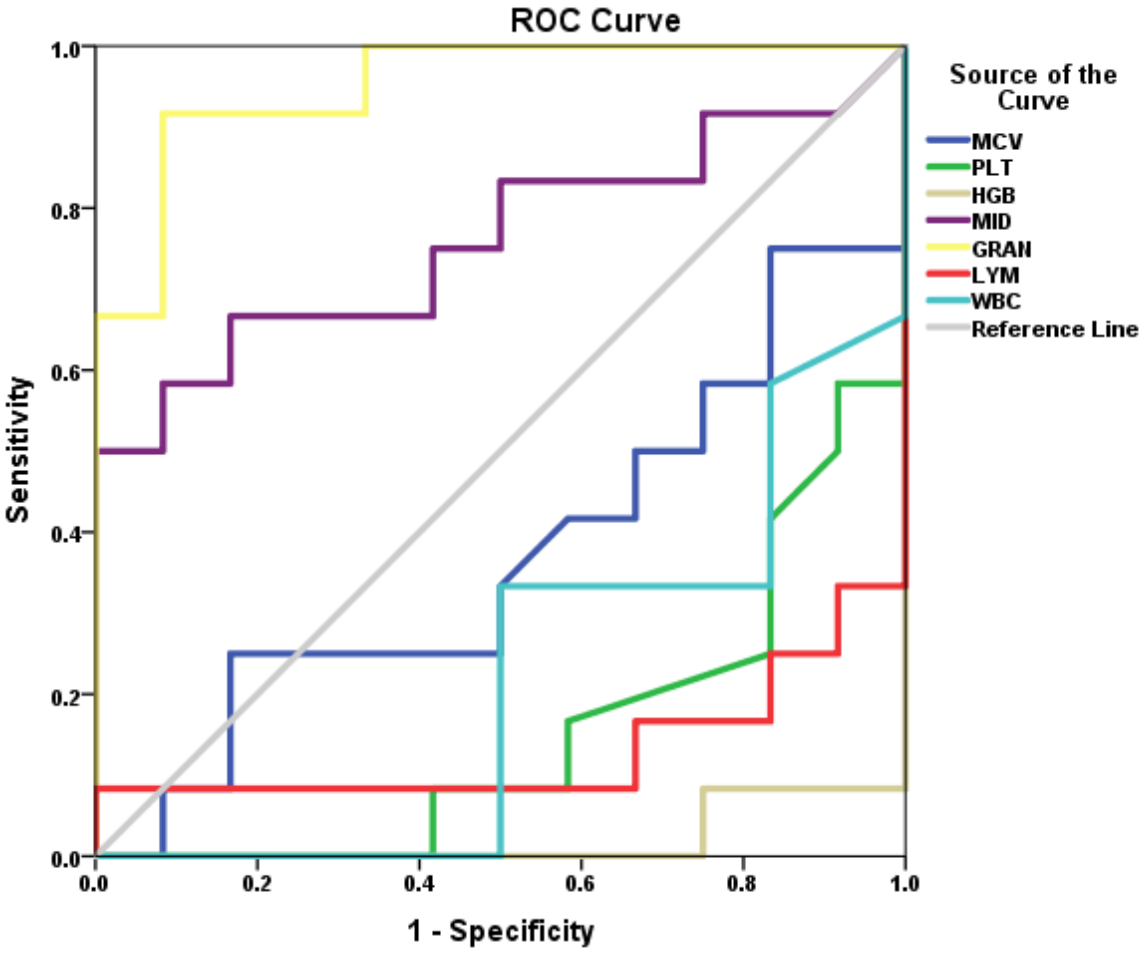

Figure 2; ROC curve, sensitivity and specificity of variables

Discussion

The present study show no significant differences (p>0.05) COVID-19 (Gimati et al., 2020). Based on developing proof suggesting a intercourse-primarily based distinction in COVID-19 effects, assessment of the interest of sex-particular hormones, specifically estrogen, within the pathogenesis of COVID-19 is warranted. Estrogens are regarded to modulate the danger of cardiovascular disease (CVD) and feature set up a position for estrogen in regulating the expression and interest of the renin-angiotensin system (RAS). (Ahmad et al., 2018).

Boccia et al., (2020) showed high levels of WBC in patients with covid-19 than healthy and these

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results contrast to with present study. Inflammation has Crosstalk among COVID-19 patients (Boccia et al., 2020). Changes in peripheral blood cell counts have been well studied in COVID-19. In affected patients, white blood cellular and neutrophil counts increase, at the same time as lymphocyte and platelet counts decrease (Henry et al., 2020). It has been hypothesized that several capacity mechanisms can be responsible, consisting of the effect of viral cytopathic action on lymphocytes, the suppressive effect of cytokine storm, lactic acidosis, and atrophy of lymphoid organs (Terpos et al., 2020).

Terpos et al., *et al*., (2020) presented low levels of WBC in patients than healthy, and these results similar to present study. This decreased in WBC count due to decrease in cytotoxic T lymphocyte cell which mainly fight viral infected cell. Other study in Egypt reported that COVID-19 patients have normal leukocyte count (Terpos et al., 2020).

Henry et al., (2020) presented low levels of lymphocytes in covid 19 patients than healthy and these results similar to present study. Many factors may contribute to lymphopenia that associated with COVID-19. The lymphocytes have been exposed to fast the ACE2 receptor on their surface (Terpos et al., 2020). As a result, SARS-CoV-2 may straight contaminate those cells, eventually leading to their lysis. Furthermore, the cytokine storm is distinguished by significantly elevated levels of interleukins and tumor necrosis factor TNFα, which may promote lymphocyte apoptosis. Significant cytokine start may also be related with lymphoid organ atrophy, counting the spleen, and further damages lymphocyte turnover (Chan *et al*., 2020).

Lymphocytes cell has a main role in adaptive immunity of immune system, such as in antigen recognition and formed memory cell, that causelymphocyte depletion associated with disease progression. So lymphocyte cell count linked with COVID-19 progression and severity and could be an effective indicator to predict COVID-19 severity (Meo *et al.*,2020) . Other study suggested that the COVID-19 inflammation may lead to down-regulation of ACE2 and Antibody-Dependent Enhancement (ADE) as a response to infection which leads to patients progressing to critical case (Jin *et al*., 2020).

The mechanism of decline in the lymphocytes count may be due to;

A. Autopsy and pathological biopsy results hemorrhage and necrosis in spleen and lymph nodes lead to decrease in CD4+ and CD8+ T cells, COVID-19 also can directly attack immune organs, incessantly proliferate, and infect more lymphocytes.
B. The Natural killer (NK) cells have cytotoxic effect and dependent only on antigen recognized soit can kill the target cells earlier than T lymphocytes. In COVID-19 cases, NK cells consumed early and the

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virus invades immune system with failure of the compensation of the cells, lead to lower countsand asymptomatic infections.

c. levated in inhibitory cytokines as IL-10 lead to immune inactivation which associated with T cell depletion (Xiong *et al*., 2020).

Németh *et al*., (2020) showed neutrophil is associated with severity of covid19 diseases, and these results matched with present study. Neutrophils, as part of the chief line of innate immune defenses, have been supposed to play defensive parts throughout bacterial and fungal infections via phagocytosis and neutrophil extracellular traps (NET) formation (Németh *et al*., 2020).

Fortunately, there is no altered phagocytic capability of neutrophils from peripheral blood for mild, moderate and severe subgroups. Neutrophils function the primary line of cellular protection in opposition to microbes, including bacteria, getting rid of them via fusing with their cytoplasmic granules containing proteases, defensins, and antimicrobial peptides or reactive oxygen species (ROS). Also it has extracellular traps (NETs) (Schulte-Schrepping *et al*., 2020). Neutrophil is main cell has a role in clearance of secondary bacterial infection and viral infection, Increasing cells in the G1 to get rid of virus and bacteria remnants.

From research in which hematological parameters related to severe COVID-19 patients were tested, adjustments in the unique coagulation index, i.E., elevated D-dimer degree, extended prothrombin time and decreased wide variety of PLTs, emerged (Mitra et al., 2020). These modifications replicate the hypercoagulable kingdom discovered in intense COVID-19 sufferers, that could promote the development of microthrombosis inside the lungs and different organs. Thus, blockade of hyperactivation and aggregation of PLTs can lessen infection and severity of acute respiration syndrome (Hottz et al., 2018).

As for Covid-19 patients, clots had been diagnosed within the pulmonary veins, bronchial veins, and small lung veins in SARS lung autopsies, indicating that this virus additionally has a prothrombotic impact. Likewise, thrombocytopenia with fewer PLTs became also related to MERS in approximately 37% of sufferers in addition to the presence of microthrombi inside the pulmonary vasculature. However, otherwise from SARS and MERS, preliminary post-mortem research of COVID-19 patients showed the presence of blood clots no longer simplest inside the lungs however additionally in the portal vein and in different blood vessels (Hwang et al., 2019).

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The present look at confirmed lower MCV tiers in patients as compared to wholesome controls and those consequences have been similar to those of Jalil et al. (2022).

Severe COVID-19 sickness primarily based on SARS-CoV-2 has been proven to affect the RBC machine and RBC rheological parameters specially. This may be related to morphological adjustments and can affect oxygen delivery and deliver. The current look at pursuits to address the query of whether slight progression of COVID-19 sickness is related to prolonged modifications in RBC morphology and rheological parameters (Renoux et al., 2021).

The statistics were separated by intercourse due to the fact the results verify recent findings that there are clean differences inside the hematological profile among men and women in standard which have been related to better testosterone ranges in adult males or cyclic menstrual blood loss in girls. Clear variations were also located between males and females in rheological parameters showing higher deformation in wholesome girls in comparison to healthful adult males, which may be defined by means of normal menstruation and for that reason the possibility of a more youthful RBC populace that has been described as having a higher deformation in comparison to older RBCs. . (Graw et al., 2018).

Analysis of the consequences of mild COVID-19 cases investigated here revealed lower hemoglobin concentrations and mean cell hemoglobin in COVID-19 patients, confirming previous findings and additionally suggesting that even patients with mild sickness who triumph over the infection enjoy a huge impact of the virus. On circulating blood cells. The extent of RBC extensions and elongated membrane diagnosed turned into lower in comparison to significantly unwell sufferers. The facts presented right here imply that approximately 2.5% of RBCs in male COVID-19 patients confirmed membrane defects, a higher percent than observed in women with about 1% RBC defects. This approach that among 2 hundred and 500 billion circulating cells show off membrane defects due to the truth that approximately 20 trillion RBCs circulate thru the human frame. Because the blood sampling defined right here turned into scheduled approximately 60 days after infection to rule out the intense infection segment, it appears possible that the amount of RBC defects became probably better at some point of the intense contamination nation. It appears viable that these structural changes limit RBC deformability, which is one of the causes of macrophage erythropoiesis, which may also suggest an acceleration of RBC turnover, which may explain anemia in the course of COVID-19 contamination. In fact, the deformability of RBCs turned into extensively decrease in both COVID-19 corporations, confirming previous findings (Dhont et al., 2020).

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