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Original Article

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Retrospective Cross-Sectional Study of Hematological and Biochemical Profile in Covid-19 Positive Patients and Their Association with Outcome

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Abstract

Coronavirus disease (COVID-19) infection, can cause common cold symptoms to Acute Respiratory Distress syndrome, acute cardiac injury and multi-organ failure. Use of circulating biomarkers as the prognostic indicator of COVID-19-positive patients, may help in identifying target patients at higher risk. This study evaluated these hematological and biochemical parameters to find their association with outcome. ***Materials and Methods:*** This hospital-based retrospective cross-sectional study included 200 confirmed Covid 19 positive patients admitted at tertiary care hospital. Various hematological and biochemical parameters were studied and comparisons were drawn between initial and final measures in survivors and non-survivors using student t test and chi square test. Data analysis was done by using SPSS (version 22) for windows. ***Results:*** The mean age of non-survivors (69.21 ±10.46 years) was significantly higher compared to survivors (52.76 ±7.55). Risk factors like hypertension with diabetes mellitus was significantly higher in the non-survivor (23%) group compared to survivor group (9%). Total leukocyte count, total bilirubin, Aspartate aminotransferase, Alanine aminotransferase, C-Reactive Protein, D-dimer, Lactate dehydrogenase, ferritin, and Interleukin-6 were found to be significantly lower in survivors compared to non-survivors. ***Conclusion:*** The study envisages that hematological and biochemical parameters can identify patients at higher risk especially in scenarios of overwhelming patient volumes.

Keywords: ***(Biomarkers, COVID-19, Laboratory profile, Risk stratification, Severity)***

Introduction

Coronavirus disease (COVID-19) infection that began in December 2019 in Wuhan, the capital of China's central Hubei province [4,5] is caused by a new subtype type of coronavirus (SARS-CoV2.[6]) that was not previously found in humans. Its genes have more than 85% homology with a SARS-like virus in bats [8]. This enveloped, positive-sense single-stranded RNA virus has a diameter of 60 to 140 nm with characteristic spikes, similar to the Coronaviridae family. The World Health Organization has declared a worldwide health emergency on January 30, 20207 and declared COVID-19 as a pandemic [6].

It has affected more than 216 countries. Currently, more than 43 million cases were diagnosed with 1.1 million deaths resulted from the disease. The case and death in India are also higher affecting 43.1 million with more than 0.5 million death [1].

SARS-CoV-2 gains entry into the hosts' cells by binding the SARS-CoV-2 spike or S protein (S1) to the ACE2 receptors

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abundantly on respiratory epithelium and other organs like the upper esophagus, enterocytes from the ileum, myocardial cells, proximal tubular cells of the kidney, and urothelial cells of the bladder [9].

The spectrum of symptoms vary from asymptomatic to mild illness like fever, cough, sore throat to severe and critical illness including Acute Respiratory Distress syndrome, acute cardiac injury, septic shock and multi-organ failure [1-3]. Hence, COVID-19 can be considered a systemic viral illness. Extrapulmonary manifestations include acute kidney injury (AKI), myocardial ischemia/infarction (MI), and myocarditis, GI symptoms and neurological findings like headache, stroke, impairment of consciousness, seizure disorder, and toxic metabolic encephalopathy. Abnormal blood glucose levels, euglycemic ketosis, and diabetic ketoacidosis have been noted in patients hospitalized with COVID-19 [10]. Acral lesions resembling pseudo chilblains (40.4%) were the most common cutaneous manifestations along with erythematous maculopapular rash (21.3%), vesicular rashes (13%), and urticarial rashes (10.9%) [11].

Early diagnosis is vital considering the rapid onset of acute respiratory distress syndrome after admission to hospital and the high mortality rates in the COVID-19 patients [12]. Laboratory tests are vital to identify the target patients at higher risk. Blood investigations can provide information of the inflammatory process including leucocyte count, neutrophil- or lymphocyte-dominance, neutrophil-lymphocyte ratio (N/L ratio) C-reactive protein (CRP) and collateral organ damage (acute renal failure, acute liver failure). Comparison of hematological parameters between mild and severe cases of COVID-19 showed significant differences in interleukin-6 (IL-6), D-Dimer, glucose (GLU), thrombin time (TT), fibrinogen (FIB) and C-reactive protein (CRP) [13]. Hepatic dysfunction presenting as an acute increase in aspartate transaminase (AST) and alanine transaminase (ALT) occurs more frequently in patients with severe COVID-19 illness [14]. Also, antiviral medications used in COVID-19 may require renal or hepatic dose adjustments [13]. Use of circulating biomarkers as the prognostic indicator of COVID-19-positive patients, may be of great importance. However, its clinical utility in disease diagnosis, monitoring, and risk stratification has not been explored much in detail [12,15]. This study aimed to evaluate hematological and biochemical profile in Covid 19 positive patients and find their association with outcome.

Methods

This hospital-based retrospective cross-sectional study was conducted to evaluate hematological and biochemical parameters in 200 Covid 19 positive patients admitted at tertiary care hospital from 1 January 2021 to 31 December 2021. All admitted COVID-19 patients confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR) of the nasopharyngeal and oropharyngeal swabs were selected Data derived from assessment of hospital records including sociodemographic details such as age, sex, occupation, marital status, religion, education, socio-economic status (according to the standard of living index and BG Prasad's classification), and per capita income were noted. Clinical examination, laboratory findings, chest computed tomographic (CT) scans, and treatment measures (antiviral therapy, Anti-retroviral therapy, anti-malarial therapy, respiratory support), morbidity and outcome were also recorded. Medical laboratory findings, including the counts of white blood cells (WBC), neutrophils and lymphocytes; serum concentrations of, creatinine, lactate dehydrogenase (LDH), albumin, aspartate and alanine transaminases (AST, ALT), hemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), and C-reactive protein (CRP) were collected for each patient. The outcome of interest was in-hospital mortality following COVID-19 infection. The study population was classified into two groups: discharged as cured (survivors) or dead (non-survivors). All data analysis had been done by using SPSS (version 22) for windows. The initial measures of each group were compared with the final measures of the study period and compared between the groups by using student t test and chi square test.

Results

The study was conducted to evaluate the hematological and biochemical parameters in Covid 19 positive patients and find their association with outcome. The outcome of interest was in-hospital mortality and hence, patients were divided into 2 groups: survivors and non-survivors. The mean age of study subjects in survivors (52.76 ±7.55 years) was significantly lower (p=0.002) compared to non survivors (69.21 ±10.46 years). The study showed a slight male preponderance among survivors and non survivors with 60% and 68% respectively with no statistically significant difference. Among survivors 62% subjects had cough, 60% had fever, 42% had dyspnoea, 24% had headache and 45% patients had other symptoms. Among both groups non survivors had more dyspnoea compared to survivors with statistical significance (p=0.03). Patients having hypertension with diabetes mellitus formed 23% of the non-survivor group compared to 9% in survivor group with statistical significance(p=0.02). 48% patients among survivors required oxygen support compared to 65% among non-survivors (p=0.02). 15% patients among survivors were on NIV support compared to 60% among non survivors, found to be statistically significant (P<0.001).

Mean TLC among survivors was less (13.39 ±7.50) compared to non-survivors (18.79 ±5.57) with a statistically significant difference (p<0.001). The mean NLR ratio among survivors was less (9.35 ±3.28) compared to non-survivors (12.69 ±3.89) with statistically significant difference (p=0.01) The mean hemoglobin, lymphocytes and platelets among survivors and non-survivors patients shows no statistically significant difference. (P>0.05). The mean total bilirubin among survivors was less (0.62 ±0.22) compared to non-survivors (1.02 ±1.22) with statistically significant difference (p=0.01). Similarly, the mean SGOT, SGPT, total proteins and albumin among survivors and non-survivors showed statistically significant difference. (p<0.05).

The mean blood urea among survivors was less (18.95 ±33.49) compared to non-survivors (63.78 ±52.99) with statistically significant difference (p=0.003). The mean serum potassium among survivors was less (3.90 ±0.98) compared to non-survivors (4.27 ±0.92) with statistically significant difference (p=0.02). The mean serum creatinine and serum sodium among survivor and non-survivor patients shows no statistically significant difference. (P>0.05). There was 20% survivors who had normal CRP levels as compared to 4% non-survivor with statistically significant difference (p=0.008).

Among survivors 37% patients had D-dimer levels ≤0.5 as compared to only 14% among non-survivor with statistically significant difference (p=0.0001). Among survivors 52% patients had serum LDH levels ≤400 as compared to only 24% among non-survivor with statistically significant difference (p<0.05). Among survivors 31% patients had serum ferritin levels ≤110 as compared to only 11% among non-survivor with statistically significant difference (p<0.05). IL-6 among survivors 57% patients had IL-6 levels <7 as compared to only 14% among non-survivor with statistically significant difference (p<0.05). It was observed that, from among survivors 11% patients had hospital duration of <5 days

compared to 41% among non-survivor with statistically significant difference. (P<0.05)

The survival percentage (89%) is much higher in those who stayed for more than 5 days in hospital as compared to mortality percentage (59%).

Table 1: Distribution of patients according to age

Age	Survivor (%)	Non-survivor (%)	Total (%)
≤20	01	00	01
21-30	08	02	10
31-40	14	04	18
41-50	21	24	45
51-60	25	29	54
>60	31	41	72
Total	100	100	200
Mean Age (years)	52.76 ±7.55	69.21 ±10.46	P=0.002* (S)

Abbreviation: S= Significant

Table 2: Distribution of patients according to sex

Sex	Survivor (%)	Non-survivor (%)	Total (%)	P value
Male	60	68	128	X2=1.48; DF=1:
Female	40	32	72	P=0.32 (NS)
Total	100	100	200	

Abbreviation: NS= Non-Significant

Table 3: Distribution of patients according to symptoms

Symptoms	Survivor (n=100) (%)	Non-survivor (n=100) (%)	P value
Fever	60	68	0.51 (NS)
Cough	62	62	0.98 (NS)
Dyspnea	42	57	0.03 (S)
Headache	24	36	0.06 (NS)
Others	45	48	0.67 (NS)

Abbreviation: S= Significant, NS= Non-Significant

Table 4: Distribution of patients according to co-morbidities

Co-morbidities	Survivor (%)	Non-survivor (%)	P value
Hypertension	06	13	0.14 (NS)
Diabetes mellitus	09	06	0.89 (NS)
HTN + DM	10	23	0.02 (S)*
CKD	02	06	0.09 (NS)
HTN + Others	08	12	0.42 (NS)
DM + Others	05	04	0.99 (NS)

Abbreviation: S= Significant, NS= Non-Significant, HTN= Hypertension, DM= Diabetes Mellitus, CKD= Chronic Kidney Disease

Table 5: Distribution of patients according to respiratory support

Variables	Survivor (%)	Non-survivor (%)	P value
O2 required	48	65	0.02 (S)
NIV support required	15	60	<0.001 (HS)

Abbreviation: S= Significant, HS= Highly-Significant, O2= oxygen, NIV= Non-invasive Ventilation

Table 6: Showing Distribution of patients according to complete blood count

Investigations	Survivor (Mean ±SD)	Non-survivor (Mean ±SD)	P value
Hemoglobin	12.69 ±1.84	11.80 ±2.44	0.75 (NS)
Total Leucocyte Counts	13.39 ±7.50	18.79 ±5.57	<0.001 (S)*
NLR ratio	9.35 ±3.28	12.69 ±3.89	0.01 (S)*
Lymphocytes	2.08 ±1.08	1.98 ±1.54	0.63 (NS)
Platelets	260.6 ±121.4	190.36 ±111.4	0.31 (NS)

Abbreviation: S= Significant, NS= Non-Significant, NLR= Neutrophil Lymphocyte ratio

Table 7: Showing Distribution of patients according to liver function test

Investigations	Survivor (Mean ±SD)	Non-survivor (Mean ±SD)	P value
Total Bilirubin	0.62 ±0.22	1.02 ±1.22	0.01 (S)*
SGOT	37.47 ±18.44	47.60 ±26.40	0.02 (S)*
SGPT	33.09 ±17.80	44.06 ±39.90	0.01 (S)*

Total proteins	6.19 ±0.75	4.21 ±1.23	0.03 (S)
Albumin	2.85 ±0.62	1.29 ±0.78	0.04 (S)

Abbreviation: S= Significant, SGOT= Serum Glutamic Oxalaoacetic Transaminase, SGPT= Serum Glutamic Pyruvic Acid Transaminase, SD= Standard Deviation

Table 8: Showing Distribution of patients according to Kidney function test

Investigations	**Survivor (Mean ±SD)**	**Non-survivor (Mean ±SD)**	**P value**
Blood Urea	18.95 ±33.49	63.78 ±52.99	0.003 (S)*
Sr Creatinine	1.76 ±4.01	1.65 ±1.57	0.29 (NS)
Sr Sodium	134.1 ±17.9	136.6 ±15.3	0.22 (NS)
Sr Potassium	3.90 ±0.98	4.27 ±0.92	0.02 (S)*

Abbreviation: S= Significant, NS= Non-Significant

Table 9: Distribution of patients according to CRP levels

CRP levels	**Survivor (%)**	**Non-survivor (%)**	**P value**
< 5	21	04	(X2=11.73;
5-100	69	63	DF=3: P=0.008
100-200	07	23	(S)
>200	03	10	
Total	100	100	

Abbreviation: S= Significant, CRP= C-Reactive Protein

Table 10: Distribution of patients according to D-dimer levels

D-dimer levels	**Survivor (%)**	**Non-survivor (%)**	**P value**
≤0.5	37	14	(X2=22.44; DF=3:
0.6-5.0	46	29	P=0.0001 (HS)
5.1-10	13	19	
10.1-20	04	38	
Total	100	100	

Abbreviation: HS= Highly Significant, CRP= C-Reactive Protein

Table 11: Distribution of patients according to Serum LDH levels

Serum LDH	**Survivor (%)**	**Non-survivor (%)**	**P value**
<400	52	24	(X2=12.09;
401-1000	40	62	DF=3: P=0.007
1001-1500	06	13	(S)
1501-2000	02	01	
Total	100	100	

Abbreviation: S= Significant, LDH= Lactate Dehydrogenase

Table 12: Distribution of patients according to Serum Ferritin levels

Serum Ferritin	**Survivor (%)**	**Non-survivor (%)**	**P value**
<110	31	11	X2=16.37; DF=3:
110-1000	64	60	P=0.0001 (HS)
1001-2000	03	18	
2000-3000	02	11	
Total	100	100	

Abbreviation: HS= Highly Significant

Table 13: Distribution of patients according to IL-6 levels

IL-6 levels	**Survivor (%)**	**Non-survivor (%)**	**P value**
<7	57	14	X2=6.281;
7.1-500	43	45	DF=1: P=0.004
501-1000	00	24	(S)
1001-1500	00	17	
Total	100	100	

Abbreviation: S= Significant, IL-6= Interleukin-6

Table 14: Distribution of patients according to Hospital duration

Duration (Days)	**Survivor (%)**	**Non-survivor (%)**	**P value**
0-5	11	41	X2=16.4; DF=3:
6-10	39	27	P=0.0001 (HS)

11-15	36	20	
>15	14	12	
Total	100	100	

Abbreviation: HS= Highly Significant

Discussion

The world has been facing an unprecedented coronavirus (Covid-19) outbreak that started in China and rapidly spread internationally since December 2019. Treatment guidelines have been constantly updated in the past few months. Although there is effective treatment and vaccine available for the disease, better disease management and treatment algorithms are needed. It is thereby crucial to determine the factors that affect the mortality and clinical outcome of patients.

The present hospital record based cross sectional study was conducted at tertiary care hospital to study hematological and biochemical parameters in Covid 19 positive patients. The present study was comparative study performed in the tertiary care institute for the period of around 18 months. Total 200 subjects were enrolled in this study into 2 groups, survivors and non survivors based on inclusion and exclusion criteria.

In our study, mean age (Standard deviation) of study subjects in survivors was 52.76 ±7.55 whereas among non survivors 69.21 ±10.46 years, with a minimum of 18 years and maximum of 87 years. The age-wise distribution of study subjects shows that non-survivors were of higher age group compared to survivors with statistically significant difference. (P<0.05). Similar findings were observed in study by Albalawi *et al* [16] and Rajkumar Chinnadurai *et al* [17] where non-survivors were elderly compared to survivors. The demographic analysis indicated that the likelihood of mortality for patients in the older age group (i.e., ≥65 years) was five times higher than those in the younger age group (OR = 5.34, 95% CI 1.71-16.68, p = 0.004) (49) our study is comparable with other studies.

The gender-wise distribution of study subjects show slightly male preponderance among survivors and non survivors with 60% and 68% respectively with no statistically significant difference. (P>0.05) Male preponderance in our study was similar to other Indian studies, though it was lower than western studies.

In our study, 86% patients in the non-survivor group had dyspnoea compared to 42% in the survivor group with a statistically significant difference (p < 0.05). Similar findings were seen in other western studies like Rastad H *et al*, [18] Mesas AE *et al* [19] and Chinnadurai, R *et al* [20] where dyspnoea was significantly more among non-survivors compared to survivors.

COVID-19 patients with comorbidities, have a rapid and severe progression, often leading to death. In our study group patients having hypertension with diabetes mellitus formed 23% of the non-survivor group compared to 9% in survivor group. This shows significantly higher mortality rate among hypertensive with diabetes mellitus population. (p<0.05) Hypertension alone associated with mortality for 13% patients among non survivors as compared to survivors with 6 %. Diabetes with other comorbidities associated equally in survivors and non survivors with 5% and 4% respectively. The Centres for Disease Control and Prevention (CDC) studied a total of 180 patients in 14 different states under COVID-NET, of which 89.3% of the patients had an underlying comorbidity. The most common comorbidities found were, hypertension, and diabetes mellitus [21]. In a retrospective, multicentre cohort study in Wuhan, China, which has been conducted on 191 patients with laboratory-confirmed COVID-19, the risk factors associated with in-hospital death had been explored. The prevalence of DM and CVD was recorded respectively 19% and 8% in hospitalized patients and 31% and 24% in non-survivors [22], indicating a higher prevalence of comorbidities in infected non-survivors in agreement with our findings.

In the present study, 48% patients among survivors were having 02 requirement and 15% patients was on NIV support. This was significantly higher in non survivors where 65% patients were on 02 support and 60% patients were on NIV support (p<0.001).

Gökhan Aksel *et al* [23] in a study on early predictors of mortality for moderate to severely ill patients with Covid-19 observed non-survivors showed significant association with O2 support and mechanical ventilation. Hadith Rastad *et al* [18] observed in non-survivors significantly more likely to present with O2 saturation and receive invasive mechanical ventilation on admission than survivors (all P-values <0.05). These findings were in accordance to present study.

In the present study, mean TLC among survivors was less (13.39 ±7.50) compared to non-survivor (18.79 ±5.57) with statistically significant difference. Higher Mean total bilirubin, SGOT, SGPT, serum urea, and serum potassium among non-survivors were also found significant (p<0.05). Changcheng Shi *et al* [24] in a meta-analysis on mortality in patients with coronavirus disease 2019 observed the values of the following laboratory parameters were significantly higher in the deceased patients than in survivors: white blood cells (WBCs), neutrophils (NEUs), total bilirubin (TBIL), aspartate aminotransferase (AST), creatinine (Cr), blood urea nitrogen (BUN), urea, prothrombin time (PT), D-dimer, C-reactive protein (CRP), procalcitonin (PCT). The lymphocyte (LYM), monocyte (MON), platelet (PLT), albumin (ALB), CD3+, CD4+, and CD8+ cell counts were significantly lower in the non-survivors than in the survivors.

Dramatically reduced LYM levels as well as CD3, CD4, and CD8 cell counts in the deceased patients suggests that SARS-CoV-2 may contribute to destruction of T lymphocytes, decreasing immune function. Hence, patients with poor immune function were more likely to suffer from acute infection and die than those with normal immune function. In the present meta-analysis, a positive correlation between the PCT level or WBC count and mortality was observed, indicating that an increased WBC count (≥10 × 109 /L) may be a useful predictor and that PCT-guided antibiotic therapy might be beneficial in COVID-19 infection [24].

The present study showed that CRP levels, D-dimer levels and serum LDH levels were more among non-survivor patients compared to survivors with statistically significant difference. (<0.05)

Similarly, Gökhan Aksel *et al* [23] observed increased CRP levels, D-dimer levels and serum LDH levels as early predictor of mortality for moderate to severely ill patients with Covid-19 with statistical significance. (P<0.001). Esmaeil Mehraeen *et al* [25] in a systemic review observed CRP, D-dimer and serum LDH as predictors of mortality. Changcheng Shi *et al* [24] in a meta-analysis on predictors of mortality in patients with COVID-19 observed significantly higher values of CRP, D-dimer and serum LDH in the deceased patients than in survivors (P<0.001).

In the present study, among survivors 31% patients had serum ferritin levels ≤110 as compared to only 11% among non-

survivor. This shows that serum ferritin levels were more among non-survivor patients compared to survivors with statistically significant difference. (P<0.05) Similarly, Gökhan Aksel *et al* [23] observed increased serum Ferritin levels as early predictor of mortality for moderate to severely ill patients with Covid-19 with statistical significance. (P<0.001) Changcheng Shi *et al* [24] in a meta-analysis on predictors of mortality in patients with COVID-19 observed significantly higher values of serum ferritin in the deceased patients than in survivors. (P<0.001) Ferritin is a known acute phase reactant secreted by alveolar macrophages in the lungs and also stimulated by various cytokines, including IL-6 in response to inflammation. Various single-centre retrospective studies done in China found higher ferritin levels in patients who succumbed compared to survivors and discovered a decrease in ferritin levels with remission of the disease [26].

In the present study, among survivors 57% patients had IL-6 levels <7 as compared to only 14% among non-survivor. This shows that IL-6 levels were more among non-survivor patients compared to survivors with statistically significant difference. (P<0.05)

Esmaeil Mehraeen *et al* [25] in a systemic review observed IL-6 as predictor of mortality.

Similarly, Gökhan Aksel *et al* [23] observed increased IL-6 levels as early predictor of mortality for moderate to severely ill patients with Covid-19 with statistical significance. (P<0.001)

Changcheng Shi *et al* [24] in a meta-analysis on predictors of mortality in patients with COVID-19 observed the significantly higher values of interleukin-6 (IL-6) in the deceased patients than in survivors. (P<0.001)

Cytokine storm is the excessive and uncontrolled release of proinflammatory cytokines. Huang *et al*. [27] found markedly higher plasma levels of cytokines in COVID-19 patients requiring ICU admission than in those not treated in the ICU, indicating that cytokines are correlated with disease severity. In the present meta-analysis, numerous inflammatory biomarkers (ESR, CRP, PCT, ferritin, and IL-6) were higher in deceased patients than in survivors, providing further evidence for the presence of a cytokine storm that can contribute to the fatal outcome of COVID-19 patients.

It was observed that, from Among survivors 11% patients had hospital duration of <5 days compared to 41% among non-survivor with statistically significant difference. (P<0.05) The survival percentage (89%) is much higher in those who stayed for more than 5 days in hospital as compared to mortality percentage (59%).

Hadith Rastad *et al* [18] observed in survivors, the median time (IQR) of general ward and ICU stay were 5 days (3-7) and 9 days (7-11), respectively and in non-survivors, the median time (IQR) from admission to death was 4 days (2-6).

Conclusion

The present study concludes that older age and male gender conferred a significant increased risk of mortality among patients with COVID-19. The study also concludes that dyspnoea and the presence of comorbid disease like diabetes with hypertension predict mortality among COVID-19 patients.

Laboratory parameters associated with critical COVID-19 disease were lymphopenia, thrombocytopenia, leucocytosis, increased neutrophils, increased C reactive protein and ferritin, increased D dimer, raised ALT and/or AST, decreased albumin, increased cardiac troponin and elevated LDH. The various laboratory markers like elevated CRP levels, D-dimer levels, IL-6 and serum Ferritin demonstrated an independent relationship with increased mortality.

The study envisages that such clinical, hematological and biochemical parameters will go a long way in identifying high risk patients in scenarios of overwhelming patient volumes with limited PCR testing capabilities and healthcare resources.

Declarations

Ethics Approval

The study was approved by the Institutional Ethics Committee of HBT MEDICAL COLLEGE & Dr RN Cooper Hospital, Mumbai, Maharashtra.

Availability of supporting data

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

None

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Financial relationships

All authors have declared that they have no financial relationships at present or within the previous three years or with any organizations that might have an interest in the submitted work.

Author's contributions

Dr. Prakash Ram Relwani, Dr. Sandeep Tekade and Dr. Neelam N. Redkar conceptualised the study and were the principal investigators. Dr. Omkar E. Paradkar, Dr. Abhilasha Srivastava and Dr.Arun Saruk assisted with data interpretation and literature review. Dr. Diksha Samsukha assisted with manuscript writing. Dr. Alhad Mulkalwar assisted in the final review of the manuscript. All authors read and approved the final manuscript.

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