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Comparison of Biochemical Parameters in COVID-19 and non-COVID-19 Individuals in Gandaki Province, Nepal

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Abstract

Introduction: The corona virus disease causes various organ dysfunctions which brings about changes in pathophysiology including biochemical shifts. Therefore, this study aimed to analyze different change in biochemical parameters in COVID-19 patients.

Material and Methods: It is a case control study. Venous blood (5 ml) was collected from a total of 157 samples; 57 of COVID-19 patients and 100 non-COVID-19 individuals as control, allowed to clot at room temperature, and centrifuged at 1500 rpm for 15 minutes. Serum was separated and AST, ALT, CK-MB, γ-GT, LDH and Albumin were analyzed using semi-automated analyzer, TFT was analyzed using CLIA, D-dimer by immunofluorescence and PT/INR by coagulation method. SPSS version 20.00 was used for statistical analysis.

Results: Clinical spectrum among the COVID-19 patients varied from being asymptomatic to having symptoms like cough (60%), fever (68%), headache (29.8%), dyspnea (24.5%) with comorbidities, (19%) diabetes mellitus, liver disease and (5%) thyroid disorders. The level of SGOT (p=0.0001), SGPT (p=0.0001), γ-GT (p=0.05), CK-MB (p=0.01), D-dimer (p=0.01), PT/INR (p=0.0001) levels were significantly higher in COVID-19 patients. There were significantly higher mean values of SGOT, SGPT, LDH, PT/INR and D-dimer (72.59±44.55, 71.71±41.71, 520.88±142.75, 1.27±0.413 and 1219.3±919.18) in severe COVID-19 patients.

Conclusion: In this study, we concluded that the level of D-dimer, SGOT, SGPT, γ-GT, CK-MB and LDH can be used to assess the severity of COVID-19 cases. In COVID-19 patients, we recommend close monitoring n D-dimer, LDH, SGOT and SGPT as a clinical indicator for potential progression to critical illness.

Keywords: Biochemical parameters, COVID-19, D-dimer, Nepal, SARS-CoV- 2

Abbreviation

COVID-19: Coronavirus Disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; CK-MB: Creatine Kinase-Muscle Brain; CK-B: Creatine Kinase- Brain; CK-M: Creatine Kinase-Muscle; GGT: Gamma glutamyl transferase; PT-INR: Prothrombin Time

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International Normalized Ratio; TFT: Thyroid Function Tests; T:3 Triiodothyronine; T:4 Thyroxine; TSH: Thyroid Stimulating hormone; DVT: Deep vein thrombosis

Background

The novel coronavirus disease 2019 (COVID-19) is an infection caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), it was originated in Wuhan, China, spreaded rapidly and was declared as a global pandemic by World Health Organization (WHO) [1]. The primary organ targeted in severe acute respiratory syndrome (SARS) is the lung; hence it is also named as 'severe acute respiratory syndrome' and 'SARS atypical pneumonia.' However, other organ dysfunctions, including gastrointestinal symptoms, abnormal liver functions, lymphadenopathy and splenic atrophy, have also been observed in patients [2]. Multiple studies have observed the association of elevated liver enzymes in patients with COVID-19 infection that causes liver damage either via direct hepatotoxic injury with viral infection, or drug toxicity, or immune mediated response [3]

Clinical laboratories play an important role in the detection of coronavirus as well as helps in monitoring their evolution and management of COVID-19 [4]. There are several biochemical markers linked with predicting the severity of coronavirus disease. Liver biochemical parameters could be used to reflect the extent of liver damage in clinical practice [5].

There is limited research done regarding the biochemical parameters in COVID-19 patients as well as safety of drugs used to treat COVID-19 patients with liver injury are still missing. Thus, this study aimed to compare biochemical parameter like Albumin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Creatine Kinase-Muscle Brain (CK-MB), Gamma Glutamyl Transferase (γ-GT), Lactate Dehydrogenase (LDH), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), D-dimer, Prothrombin Time International Normalized Ratio (PT/INR), Thyroid Function Tests (TFT) like Free triiodothyronine (FT3), Free Thyroxine (FT4), Thyroid Stimulating Hormone (TSH) in both COVID-19 and non-COVID-19 patients in order to provide baseline data, suggesting the more advanced research to be done on this scenario. This study might help to formulate guideline and improve the overall health condition of COVID-19 patients in future.

Methods and Materials

Materials

All material used were of analytical grade which was provided by biochemistry lab of Fishtail Hospital and Research Center Pvt. Ltd. such as Syringe, Spirit, Tourniquet, Cotton Roll , Gloves, Mask, Test Tubes, Test Tube rack, Gel tube, Water bath, Pipette tips 22, Tips holder, Measuring Cylinder, Conical flask, Refrigerator, Centrifuge, Semi-automated analyzer (BeneSphere), Sodium Citrate tube, CLIA (Adiva Centaur CP) and reagent used were SGPT reagent, SGOT reagent, LDH reagent, PT reagent, GGT reagent CK reagent, D-dimer reagent, Albumin reagent, Thyroid reagent, Distilled water, Buffer.

Methods

Patient selection

This is a case control study conducted among in and out patients of the study center (Fishtail Hospital and Research Center Pvt. Ltd) from May 4, 2021 to June 18, 2021. For this study, ethical consideration was approved by Pokhara University Research Center (PURC) with reference number 57/077/078. The genetic detection of SARS-CoV-2 was done by using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) from nasopharyngeal or throat swab.

Sample size

Total sample size was 157, out of which 57 were of COVID-19 individuals where 100 were of non COVID-19 individuals.

Inclusion criteria

As case

1. Patients tested positive for COVID-19 by RT-PCR having underlying disease.
2. Both in and out patients visiting Fishtail Hospital

As Control

1. People tested negative for COVID-19 with or without underlying disease.

As case and control

1. Both sexes included
2. Only those people who are willing to participate in our study
3. Cases inside Gandaki Province

Exclusion criteria

As case

1. Pregnancy
2. HIV patients, patients with hematological malignancy
3. Chronic liver disease
4. Surgery or trauma within 30 days
5. Patients with deep vein thrombosis (DVT)

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As Case and Control

1. Inadequately collected and improperly labeled samples
2. Eligible samples of which the consent cannot be taken
3. Cases outside Gandaki Province

Sample collection and laboratory analysis

Before starting of the study, informed consent and fulfillment of questionnaire were taken from the patients or from their guardians. The patients' medical records were reviewed to collect baseline data (age, sex, address, history and comorbidities) and details of initial symptoms (fever, cough sore throat, shortness of breath, runny nose, general weakness, headache, diarrhea, irritability, muscle pain and others). Then, 5 ml of venous blood was collected from the selected patients in a gel tube and trisodium citrate tube, allowed to clot at room temperature and then centrifuged at 1500 rpm for 15 minutes. The collected sample was stored at 2-8ºC or frozen at -20ºC at the Biochemistry laboratory, Fishtail Hospital and Research Center Pvt. Ltd. AST, ALT, CK-MB, γ-GT, LDH and Albumin were analyzed using semi-automated analyzer. TFT was analyzed using Chemiluminescent Immunoassay (CLIA). D-dimer was analyzed using Immunofluorescence and PT/INR was analyzed by coagulation method. The final report was verified by the head of department of biochemistry.

Statistical analysis

The data was expressed in mean ± standard deviation (SD) and data analysis was performed using Statistical Package for The Social Sciences (SPSS) version 20.0.

Results

Demographic and clinical condition on COVID-19 patients

Our study comprises of 57 COVID-19 patients as cases, out of which 63% were male and 37% were female. Most infected patients were from Kaski district (56%) followed by Tanahun (26%), Syangja (10%) and Baglung (8%). 82% were married. Cough (60%), fever (68%) and headache (29.8%) were the most common clinical symptoms observed. Demographic and clinical condition in COVID-19 patients in our study is shown in Table 1.

Baseline characteristics of COVID-19 and non-COVID-19 individuals

The study population included 57 patients with COVID-19 and 100 individuals with non- COVID-19. For COVID-19 patients, the median age was 52 years and 63% were male. For non COVID-19 patients, the median age was 47 years and 57% were male. Both of the COVID-19 patients and non-COVID-19 individuals had one or more co-existing medical conditions; and compared with COVID-19 patients, non-COVID-19 individuals were more likely to have co-existing medical conditions including cardiovascular diseases which is shown in Table 2.

Table 1: Demographic and clinical condition on COVID-19 patients

S. N.	Variables		Total (n %)
1	Gender	Male	36(63%)
		Female	21(37%)
2	District	Kaski	32(56%)
		Tanahun	15(26%)
		Syangja	6(10%)
		Baglung	4(8%)
3	Marital Status	Married	47(82%)
		Unmarried	10(18%)
4	Clinical Symptoms	Fever	39(68%)
		Headache	30(29.8%)
		Cough	34(60%)
		Myalgia	13(22.8%)
		Fatigue	20(35%)
		Anosmia	8(14%)
		Dyspnea	14(24.5%)
		Diarrhea	5 (10%)

Table 2: Baseline characteristics of COVID-19 and non-COVID-19 individuals

S. N.	Variables	COVID-19 (n=57)	Non-COVID-19 (n=100)	P-value
1	Age group	52	47	0.032
2	Male	36 (63%)	57 (57%)	0.141
3	Female	21 (37%)	43 (43%)	0.211
4	Cardiovascular disease including hypertension	4 (7%)	2 (2%)	0.205
5	Diabetes	11 (19%)	14 (14%)	0.76
6	Renal disease	4 (7%)	12 (12%)	0.058
7	Liver disease	5 (9%)	6 (6%)	0.295
8	Thyroid disorders	3 (5%)	6 (6%)	0.205
9	Pulmonary disease	1 (2%)	3 (3%)	0.295

Comparison and evaluation of laboratory results of different biochemical parameters in COVID-19 and non-COVID-19 individuals.

There were significantly lower mean values of Albumin, FT3, TSH respectively among COVID-19 patients than non-COVID-19 patients ($p>0.05$). Additionally, there were significantly higher mean values of D-dimer, PT/INR, SGOT, SGPT, LDH, CK-MB, γ-GT, FT4 respectively in Covid-19 patients than non-COVID-19 patients ($p<0.05$) as shown in Table 3.

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Table 3: Comparison of laboratory results of different biochemical parameters in COVID-19 and non-COVID-19 individuals

S. N.	Variables	COVID-19 (mean±SD) (n=57)	Non-COVID-19 (mean±SD) (n=100)	P- value
1	Albumin (g/dl)	3.51±0.47	4.13±3.32	0.5
2	SGOT (U/L)	54.02±33.11	31.35±11.05	0.0001
3	SGPT (U/L)	49.05±31.84	33.65±12.46	0.0001
4	LDH (U/L)	446.44±140.89	221.13±44.63	0.0001
5	CK-MB (U/L)	22.85±8.15	16.34±5.24	0.01
6	γ-GT (U/L)	37.95±14.28	20.74±6.54	0.0001
7	PT/INR	1.10±0.25	1.02±0.04	0.0001
8	D-dimer (ng/ml)	573.89±678.11	44.82±23.72	0.01
9	FT3 (pg /dl)	2.55±0.74	3.18±0.32	0.0001
10	FT4 (ng/dl)	1.21±0.37	1.15±0.13	0.0013
11	TSH (uIU/ml)	2.13±1.55	2.66±1.69	0.22

Comparison of results of different biochemical parameters on the basis of the severity in COVID-19 patients

There were significantly higher mean values of SGOT, SGPT, LDH, PT/INR and D-dimer respectively in severe COVID-19 compared to asymptomatic, mild and moderate. There were lower mean values of Albumin (g/dl) (3.35±0.48) in severe COVID-19 compare to asymptomatic, mild and moderate cases. As regards, the analysis of the abnormalities of the measured biochemical parameters as categorical data, there were no significant difference ($p>0.05$) between covid-19 severity categories as regards the frequency percentage of Albumin, CK-MB, γ-GT, FT3, FT4 and TSH which is shown in Table 4.

Table 4: Comparison of different laboratory parameters on the basis of severity in COVID-19 patients

S. N.	Variables	Asymptomatic (n=16)	Mild (n=13)	Moderate (n=11)	Severe (n=17)	P-value
1	Albumin	3.53±0.55	3.68±0.39	3.52±0.36	3.35±0.48	0.298
2	SGOT	34.25±9.37	42.15±15.70	68.09±30.38	72.59±44.55	0.0001
3	SGPT	30.56±12.21	44.00±22.61	46.91±24.46	71.71±41.71	0.0001
4	LDH	364.75±139.63	449.77±110.98	446.27±120.13	520.88±142.75	0.014
5	CK-MB	19.58±7.22	24.04±7.68	24.49±9.92	24.00±7.95	0.312
6	γ-GT	32.31±11.32	38.69±10.64	37.27±11.22	43.12±19.20	0.189
7	PT/INR	1.00±0.001	1.07±0.86	1.05±0.93	1.27±0.413	0.012
8	D-dimer	98.52±86.24	488.53±141.7	368.90±250.2	1219.30±919.8	0.0001
9	FT3	2.73±0.95	2.80±0.54	2.27±0.63	2.38±0.68	0.194
10	FT4	1.25±0.61	1.10±0.96	1.14±0.14	1.29±0.30	0.473
11	TSH	2.41±1.601	2.23±1.26	2.32±1.75	1.68±1.63	0.559

Discussion

The COVID-19 pandemic has caused considerable consequences globally with the rapid spread of virus, since it's outbreak in China [6]. The virüs affect multiple organs causing a series of physiological damage [3]. However, we still have to know about the effect of COVID-19 on different biochemical profiles. Therefore, we summarized a case control study between COVID-19 and non COVID-19 individuals. Our study findings gave good support to the accuracy of depending on biochemical parameters for the diagnosis of patients with COVID-19 infection. We reported a case-control study on total 57 individuals with laboratory confirmed COVID-19 infection and 100 individuals without COVID-19 infection. The primary symptoms in COVID-19 patients were fever and cough, which is accordance with the research result of Zhao D et al., (2019) [20]. Other symptoms included headache, myalgia, fatigue, and dyspnea. Only 10% of respondents rarely developed intestinal signs and symptoms (i.e. diarrhea) [7-8]. Most of the infected individuals in Gandaki Province were from Kaski (56%) followed by Tanahun (26%), Syangja (10%) and Baglung (8%). Male respondents were more affected (63%) than

female in our study which was consistence with the results from other studies [9]. Among the co-morbidities associated with COVID-19 in our study, diabetes mellitus, liver disease, cardiovascular disease including hypertension were most common. These findings were in accordance with a retrospective study which reported that diabetes mellitus, hypertension and cardiovascular disease were the most prevalent underlying disease among hospitalized COVID-19 individuals [10]. Our study showed that diabetes mellitus was seen most in severe COVID-19 patients. In line with our findings, Singh et al., 2020 provided evidence of increased incidence and severity of COVID-19 in diabetic patients [11].

Liver injury in COVID-19 patients might be due to viral infection of liver cells or due to the other causes like drug hepatotoxicity and immune-mediated inflammation [12]. Liver dysfunction could be more in severe cases of COVID-19. Severe liver injury can lead to various complications such as liver damage, liver failure or even death. Our study showed that the levels of liver associate markers (SGOT, SGPT and γ-GT) were significantly higher in COVID-19 when compared with non COVID-19 patients. A study done by Kaushik A et al., (2020) reported that 59.04% of admitted COVID-19 patients had abnormal LFT with elevated SGOT in 45.71% and elevated SGPT in 25.71% [13]. Another similar study from Pakistan by Asghar MS et al., (2020) showed ratio of elevation of γ-GT (77.84± 84.18) was highest followed by SGOT (73.71± 172.20) being second while SGPT (66.49± 157.00) [14]. But our study showed significant higher elevation of SGOT followed by SGPT and γ-GT in COVID-19 patients. Wan X et al., (2020) reported that there was no significant difference in SGPT of COVID-19 patients based on the severity of the disease [15]. However, our study showed significant difference in SGPT in COVID-19 on the basis of severity. Zhang Y et al., demonstrated that the levels of AST, ALT and LDH in COVID-19 patients were no significantly different than those of non COVID-19 which indicates that liver is not the main target organ of SARS-CoV-2 infection which varies from our study [16]. During critical illness, inflammatory mediators decrease albumin synthesis in order to prioritize synthesis of other acute phase reactants [17]. The mechanism for hypoalbuminemia in COVID-19 have not been thoroughly studied or explained. Our study observed that hypoalbuminemia was common in COVID-19 with severe disease compared with mild/moderate COVID-19 cases. In addition, the continuous decreased in serum albumin level was observed in those progressed with critical illness. This is in accordance to the study done by Zang Y et al., (2020), which showed that hypoalbuminemia was seen predominantly in severe COVID-19 cases compared with mild/moderate cases [16].

LDH levels have been associated with worse outcome in SARS [18]. COVID-19 may lead to inadequate tissue perfusion and multiple organ failure due to various mechanisms, including thrombosis which leads to LDH elevation [19]. Several studies suggested that the serum LDH was elevated in severe COVID-19 patients. Zhao D et al., (2019) observed that higher LDH levels (31.58%) have been found in COVID-19 patients than in patients with SARS-CoV-2 negative confirmed pneumonia [20]. The present study showed that the level of CK-MB significantly elevated in adverse prognosis groups, and this finding was according to similar report published by Mishra et al. [21]. Abnormal coagulation parameters (D-dimer and PT-INR) could be considered as important indicators of severe COVID-19 associated with mortality. D-dimer is relatively small protein fragment formed by degradation of fibrin [22]. We observed that COVID-19 patients have significantly higher D-dimer levels which highlights the possibility of more obvious activation of the coagulation system. The anomalous rise in COVID-19 patients can be an indication of active anticoagulation therapy. Khatri P et al., (2021) reported that 87.36% had elevated D-dimer levels out of total admitted patients. Increased D-dimer was observed in 100% of death during hospital stay [23]. PT/INR is a coagulation test widely used for routine pre-operative screening for bleeding disorders or coagulation status [24]. Our study also showed that the level of PT/INR in COVID-19 patients was slightly higher than non-COVID-19 patients. This is accordance to the study done by Wang L et al., (2020) which showed that the prothrombin time is one of the predictive factors for clinical outcomes of COVID-19 patients [25].

During COVID-19 infection, there is excess cytokine release in the body which might affect the thyroid functions and may result in thyroid disorder [26]. We found that the levels of FT3 and TSH were lower in COVID-19 than non COVID-19 individuals. However, FT4 was found to be in normal range and was not found to be correlated with the gravity of disease. The results showed that the thyroid function abnormalities are common in COVID-19 patients, especially in severe cases. This is in line with a meta analysis study which shows that the presence of thyroid disease was associated with the severity of COVID-19 [27]. However, Khoo B et al., 2021 observed that patients with COVID-19 had lower TSH and FT4 compared to those in non COVID-19 [28].

Limitations

1. A sample size of 57 cases is smaller as compared to other studies that have been conducted but is still relevant to show a distinct derangement in liver enzyme levels.
2. Sample size remained small due to limited budget.
3. It's a single centred study.
4. The qualitative analysis (positive or negative) of SARS-CoV-2 RNA is used to guide the diagnosis and treatment of COVID-19 patients. Although CT (cycle- threshold)

– value for viral load can support in the better interpretation of clinical decisions, in this study, the quantification of SARS-Cov-2 viral load is not available.

Recommendation

1. Similar studies involving larger sample size and area are needed.
2. We need to concentrate more on studies which help to understand the good clinical laboratory and bio safety practices prevail and that the patient be the center of attention.

Conclusion

The prognosis of COVID-19 infection is dependent on various parameters, all of which promote to a person's illness and severity of illness. A number of biomarkers that include SGOT, SGPT, LDH, CK-MB, γ-GT, D-dimer and PT/INR increased in COVID-19 patients and biomarkers such as Albumin, FT3 and TSH decreased in comparison to non-COVID-19 patients. FT4 was seen to be normal (within range) in this study. Hence, it can be concluded that the level of D-dimer, SGOT, SGPT, LDH, γ-GT and CK-MB can be used to assess the severity and prognosis of COVID-19.

Declarations

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Conflict of Interest:

None

Informed consent/ Ethical approval

This is a case control study conducted among the in patients and out patients of the study area from May 4, 2021 to June 18, 2021. Before starting the study, informed consent and fulfillment of questionnaire were taken from the patients or their guardians. For this study, ethical consideration was approved by Pokhara University Research Center (PURC) with reference number 57/077/078.

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