



A Pilot Study on COVID-19 Positive Subjects: An Excerpt of Post-Infection-Pro-Diabetic Disposition & Related Consequences in Correlation to Hepato-Pancreatic Bio-Markers, Pro-Inflammatory Cytokines and Other Risk Factors

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Received: 28 February 2022 / Accepted: 18 May 2022

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Abstract COVID-19, a global pandemic that led to increased morbidity and mortality worldwide since its outcome at the end of the year 2019. A newly discovered variant of severe acute respiratory distress syndrome coronavirus-2 (SARS-CoV-2) was the arbitrator for spreading the syndrome by droplet transmission causing multi-organ failure in many occasions. A post-infection-pro-diabetic disposition was found evident in this study with the persistence of hepato-pancreatic aberrations in respect of reference range of tissue specific bio-markers in hospital admitted COVID-19 cases. The results of this study show that hyperglycemia is a risk factor in precipitating disease oriented complications to the patients with COVID-19 disease. A post-infection follow-up on glycemic-index and related complexities is a vital need to the COVID-19 infected convalescent subjects. Implementation of guidelines on social measure and awareness of anti-viral interventions may be the only way to prevent COVID-19 transmission.

Keywords SARS-COV-2 · COVID-19 · Hyperglycemia · Insulin · Glucagon · HOMA-IR

Introduction

Coronavirus disease 2019 (COVID-19) has become a global pandemic disease caused by a new variant of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is a member of the genus *Betacoronavirus* like the two other coronaviruses viz. SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (Middle East respiratory syndrome coronavirus) [1–4]. COVID-19 was first reported in Wuhan, China, in late December 2019 [1–3]. This virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. Following the encounter of this disease, the world has experienced an unprecedented effect not only on public health, but also in social and economic ventures. The virus oriented disease severity and its tissue specific affinity, besides its involvement in respiratory disorder, are yet not unclouded. In addition to respiratory symptoms, SARS-CoV-2 infection can also trigger a cytokine storm. Overproduction of pro-inflammatory cytokines assaults the system with multi-organ damage or failure [5]. Diabetes or glycemic imbalance has been reported in infected subjects with a higher risk of severity and fatality of COVID-19 [6, 7]. There are reports describing patients with COVID-19 suffering from acute hepatitis and pancreatitis without a clear aetiology [8–16]. The Fig. 1 represents SARS-COV-2 Structure (Panel-A) and its life cycle (Panel-B) with consequential co-morbidities after infection.

In this pilot study, subjects were very carefully screened with no major bio-medical abnormality and/or any chronic

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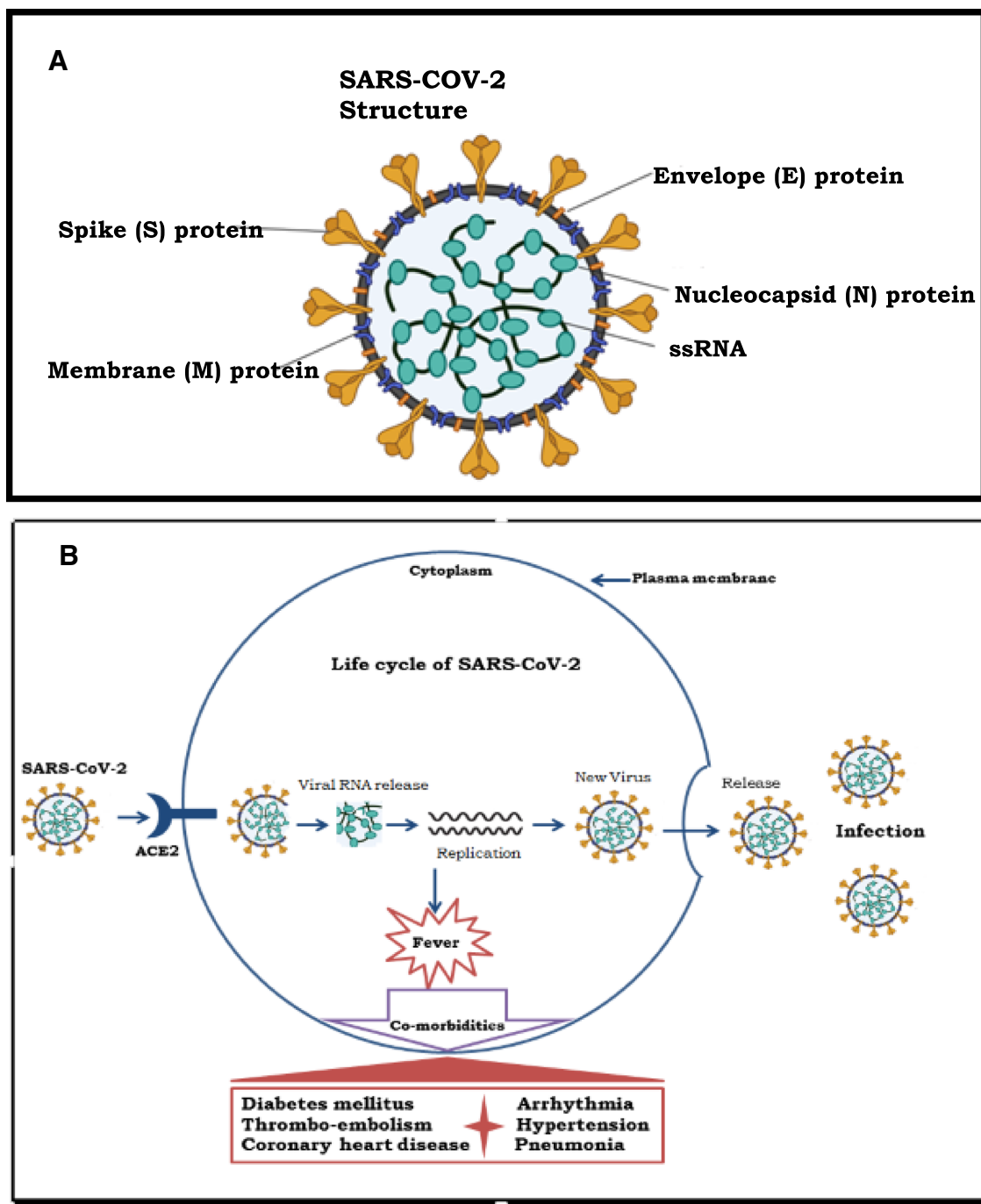


Fig. 1 A: Enveloped spherical SARS-COV-2 virus particle with four structural proteins (S, E, M, N) and a positive-sense single-stranded RNA (ssRNA) genome (30 kb in length). B: Life cycle of SARS-COV-2 and affected co-morbidities after infection. ACE2 (Angiotensin-converting enzyme2) acts as a receptor of S1 subunit of Spike

(S) protein whereas S2 subunit mediates fusion between the membranes of the virus and the host cell. This interaction helps the virus to enter into the host cell and replicate to more new viruses, which can be released to make new infection

drug habit before getting infected with SARS-CoV-2. Scattered studies on COVID-19 positive subjects [17–19] since its outburst have reported increased morbidity and mortality of diabetic subjects as compared to non-diabetics. Hence, in this study, more impetus is given to explore

whether SARS-CoV-2 infection itself is pro-glycemic by choosing only those infected subjects having no pre-diabetic history in order to watch during infection period, if the subjects are getting inclined more to the diabetic stance after the infection. In this context the profile of pancreatic

tissue is also coming into account because blood level of glucose is maintained by insulin whose origin is in pancreas. Since liver is the central regulator of most of the metabolic activities; trace is given in present study to watch bio-chemical changes in hepato-pancreatic lineage along with glycemic status of the subject after SARS-CoV-2 infection. The gross severity of infected subjects has been judged with the proficiency of pro-inflammatory risk factors in circulation. A post-infection-pro-diabetic disposition with existence of high level pro-inflammatory risk factors is the outcome of this study that draws special attention for an intense follow up of the subjects for a while even in post-COVID recovery period.

Materials and Methods

All the experiments in this study were kit-based. Test-kits for glucose, SGPT, SGOT, ALP, LDH and CRP were purchased from Randox Laboratories Ltd., United Kingdom, and Beckman Coulter auto-analyser AU680 model (made in Beckman Coulter Inc. Japan) was used for their estimation in human blood plasma or serum samples following company directed procedure(s). Kits for IL-6, ferritin and insulin were obtained from Siemens Healthcare Diagnostics Inc, USA, and SIEMENS ADVIA Centaur XPT immune assay model system was used for their estimation using kit enclosed protocol(s). D-dimer (a fibrin degradation product) estimation was done in stago compact STA max fully automated machine (assembled in France) by immune-turbidometry method using latex agglutination and the assay reagents, as per company referred assay protocol, were procured from stago proprietary commercially prepared reagent supplier only. Neutrophil:Lymphocyte (N/L) ratio was determined from complete blood count (CBC) apparatus (CBC-Sysmax XN-1000 fully automated 6 parts, erba transasia, made in Japan) by using company directed fluorescence flow cytometry procedure and its reagents were purchased from Sysmax proprietary commercially prepared reagent source only. Homeostatic model assessment for insulin resistance (HOMA-IR) score was computed with the formula: *fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5* (*Diabetologia* 28: 412–419, 1985). Elikine™ Human IL-1β ELISA kit (KET6013) from Abbkine, Inc., China, was used for IL-1β estimation. ELISA (Enzyme linked immunosorbent assay) kits for glucagon (Cat. No. E1023Hu) and glucokinase (Cat. No. E0773Hu) estimation were procured from Bioassay Technology Laboratory (BT LAB), China. Glucagon was measured in human plasma and glucokinase in human serum by using EON ELISA Reader (EON BioTek Instruments, Inc., USA) equipped

with EON™ high performance microplate spectrophotometer.

This study was conducted by following all the Institute's ethical guidelines and taking consents from the associates of the SARS-Cov-2 infected patients who were admitted for therapeutic intervention at our institute. COVID-guidelines [20], in order to avoid transmissible contamination from infected subjects [21–25], confined this study to approach only least number of patients and hence the survey was restricted to a pilot study model on thirty subjects only. Very carefully we screened these thirty patients who did not suffer from any major patho-physiology before this new SARS-CoV-2 variant infected COVID-19 disease and especially they didn't have any pre-diabetic history.

As COVID-19 pandemic made the patients too vulnerable due to mental stress, physical discomfort, death scare and social isolation in hospital environment [26, 27]; frequent sample collection was strategically discouraged. So, the study was programmed by limiting the sample collection twice only—one blood sample immediate after admission and the other one on the 10th day of 1st sample obtained, because recuperated COVID-negative subjects were released mostly by 14th day [28] as the load of incoming infected patients was very high. Only 10 ml whole blood was collected in each time and segmented as per need of serum and plasma for testing elements [29]. In COVID-pandemic time normal healthy people (consider as control group) were unwilling to volunteer in this hospital based study worrying from in-hospital infection. This limitation compelled this survey to desist from case-control template. Hence, the data of our results were judged either against literature based reference range or from company referred test-kit quoted reference values.

Results

Table 1 shows the blood plasma and serum specific profile of glycemic (glucose level) and hepato-pancreatic biomarkers (single tissue specific and common to both tissues) in SARS-CoV-2 infected subjects remained under therapeutic observation in our institutes COVID-care unit. The outcome of the result was evaluated within the window of three category of reference range — normal, moderate and high. Though the data showed an overall healing of patho-physiology based on percentage of subjects showing their existence within the reference range of set-out biochemical parameters defined to evaluate distinct pathogenesis; a considerable percentage of people remained between moderate to high reference range of patho-physiology even on 10th day of hospital admission when they were checked non-symptomatic and RT-PCR (Real time polymerase

Table 1 Blood levels of diabetic (Db), hepatic (Hp), pancreatic (Pn) and common hepato-pancreatic (CHP) parameters of COVID-19 infected subjects on admission and 10th day of admission

Db, Hp, Pn, & CHP markers	Reference range in fasting blood sample. (plasma/serum)	On admission			10th day of admission		
		Severity as per reference range			Severity as per reference range		
		Normal (N)	Moderate (M)	High (H)	Normal (N)	Moderate (M)	High (H)
Diabetic marker (Db) (in plasma)	Glucose	94.75	135.54*	182.33**	88.44	129.85**	180.86**
	Unit: mg/dL	±	±	± ##	±	±	±
	N: 65–110	11.88	10.81	52.42	14.05	16.62	11.66
	M: 111–150	n = 8	n = 13	n = 9	n = 16	n = 7	n = 7
	H: > 150	26.66%	43.33%	30.0%	53.33%	23.33%	23.33%
Hepatic markers (Hp) (in serum)	SGPT	16.62	44.83**	130.0**	16.05	46.22*	104.03**
	Unit: IU/L	±	±	± ##	±	±	± ##
	N: 10–30	07.46	09.63	14.86	08.81	08.55	36.29
	M: 31–60	n = 15	n = 10	n = 5	n = 10	n = 10	n = 10
	H: > 60	50.0%	33.33%	16.66%	33.33%	33.33%	33.33%
	SGOT	22.03	38.45**	72.56**	19.06	38.51**	74.34**
	Unit: IU/L	±	±	± ##	±	±	± ##
	N: 0–30	05.53	05.05	10.65	05.92	04.98	10.84
	M: 31–60	n = 12	n = 12	n = 6	n = 12	n = 13	n = 5
	H: > 60	40.0%	40.0%	20.0%	40.0%	43.33%	16.66%
	ALP	62.66	110.0**		72.11	120.86**	
	Unit: IU/L	±	±		±	±	
	N: 30–90	14.55	13.16	NIL	12.84	19.73	NIL
	M: 91–150	n = 21	n = 9		n = 25	n = 5	
	H: > 150	70.0%	30.0%		83.33%	16.66%	
LDH	374.38	590.29**	1152.3**	368.25	627.51*	1256.7**	
Unit: IU/L	±	±	± ##	±	±	± ##	
N: 230–450	26.58	119.82	172.74	52.23	97.98	294.21	
M: 451–800	n = 5	n = 20	n = 5	n = 7	n = 11	n = 12	
H: > 800	16.66%	66.66%	16.66%	23.33%	36.66%	40.0%	
Pancreatic markers (Pn) (in plasma)	Insulin	13.71	38.14*	98.32**	12.75	37.05**	87.39**
	Unit: µU/mL	±	±	± ##	±	±	± ##
	N: 04–25	08.97	07.38	33.12	05.67	07.01	22.37
	M: 26–50	n = 11	n = 11	n = 8	n = 20	n = 7	n = 3
	H: > 50	36.66%	36.66%	26.66%	66.66%	23.33%	10.0%
	Glucagon	150.05	199.08**	247.83**	131.74	206.14**	252.72**
	Unit: ng/L	±	±	± ##	±	±	± ##
N: 72–180	20.48	12.33	08.33	33.87	11.80	10.61	
M: 181–225	n = 10	n = 11	n = 9	n = 13	n = 11	n = 6	
H: > 225	33.33%	36.66%	30.0%	43.33%	36.66%	20.0%	
Common Hepato-pancreatic markers (CHP) (in serum)	Glucokinase	39.46	63.55**	77.35**	37.43	58.47**	76.71**
	Unit: ng/mL	±	±	± ##	±	±	± ##
	N: 20–50	08.15	08.40	05.95	5.34	06.46	05.58
	M: 51–70	n = 16	n = 10	n = 4	n = 16	n = 10	n = 4
H: > 70	53.33%	33.33%	13.33%	53.33%	33.33%	13.33%	

Result: Mean ± SD (no. of subjects (n), % of total subjects). Total number of subjects: 30

SGPT: Serum glutamic-pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase

* $P < 0.05$ vs Normal range (N), ** $P < 0.005$ vs Normal range (N)

$P < 0.05$ vs Moderate range (M), ## $P < 0.005$ vs Moderate range (M)

chain reaction) negative. Especially, there was no change in *glucokinase* activity with percent of people in each category of reference range within 10 days span of time. *Glucokinase* is an intracellular component of hepatic and pancreatic tissues. Presence of *glucokinase* in the blood stream happens with cellular damage of one or both tissues. Since LDH is typically native element of hepatic tissue and glucagon is of pancreatic origin and as their concentrations remained in higher side along with high *glucokinase* activity throughout the time of this study period, a hepato-pancreatic injury had become apparent as the outcome of this study. In addition, almost 50% of the study-population existed in moderate to high glucose level and about 70% of these hyper-glycemic group existed beyond extreme limit of normal insulin concentration in blood plasma. So, a pro-diabetic disposition (as these subjects had no pre-diabetic history) along with the chances of hepato-pancreatic injury was found to be a post- COVID inception in SARS-CoV-2 infected subjects. Thus, a post-recovery follow-up on hepato-pancreatic bio-markers together with blood sugar profile may be the take home judicious message for COVID-19 infected people.

Table 2 represents the percent of subjects with hepato-pancreatic injury, in terms of variation of bio-marker reference range, by COVID-19 infection over a time-gap of 10 days in relation to diabetic severity (plasma glucose concentration) caused by COVID infection. To be noted that the subjects under this study didn't have any diabetic predisposition before the infection. Subjects with normal plasma glucose level at the time of admission (Group-A) showed a mixed scenario on hepato-pancreatic injury as per the stature of tissue specific bio-markers over the span of 10 days duration of this study. Contrary to high values of other bio-markers, only ALP and LDH reached maximally up to moderate levels of their severity range in this time-period. In case of moderate glucose group (Group-B) at the time of admission, the plasma/serum parameters also remained in random reference-range besides ALP which appeared to be 100% normal over the 10 days time period. Again to note that even in Group-B level, a major percentage of population existed only in moderate to high reference range of hepato-pancreatic injury related bio-markers at the end of 10 days of incubation in hospital care setup. In contrast, the high glucose group (Group-C) at the time of admission showed a major difference. Besides ALP, all other hepato-pancreatic injury bio-markers existed primarily at higher reference-range with a major percentage of population of this Group-C category. The summary of the result from Table 2 relays the message that the gravity of glycemic ferocity earned by SARS-Cov-2 infection is the determinant for COVID-19 disease related risk extremity to presage systemic organ damage.

Table 3 shows the alteration of pro-inflammatory cytokine level in 10 days interval when the infected subjects were in the healing process by hospital care. The initial circulatory high level of pro-inflammatory cytokines went downhill over the time as the infected subjects were recuperating from the stress of the disease.

Table 4 delineates the persistence of COVID-19 disease related other risk factors, assigned to assess COVID-19 disease severity in many other prevalent reports, e.g. ferritin, C-reactive protein (CRP), degraded fibrin dimer (D-dimer) and neutrophil:lymphocyte (N/L) ratio over an exorbitantly high level with a wide range limit during the 10 days recuperating phase of the disease. This incidence again points out the chances of forthcoming risk of some associated consequences in post-COVID era.

Table 5 correlates percentage of glycemic status on the day of hospitalization with the blood groups and associated co-morbidities in COVID-19 infected subjects under study. This is to record that these subjects didn't have any diabetic pre-history before COVID-infection and were maintained in normal room air oxygen environment without having any breathing problem. The blood glycemic range reported in this table is an incidence of SARS-Cov-2 infection only. Besides AB type, the subjects from all other blood groups showed characteristic variance in their blood glycemic picture. Rh(-) blood groups had hardly any consequences of co-morbidities. The subjects of AB blood group didn't suffer from severe hyperglycemia (> 200 mg/dL) too and were free from any incidence of death; while an average 6% death rate was recorded with other blood groups (A, B, O). Co-morbidities like hypertension, coronary artery disease, hypothyroid, irritable bowel syndrome and lung cancer were ascertained in subject-specific manner from all of the three blood groups besides AB type. A rating of decreasing order of risk vulnerability in terms of hyperglycemia, associated co-morbidities and death incidence from COVID-19 disease out burst can be outlined from the data presented in Table 5 as following: Gr-B > Gr-A > Gr-O > Gr-AB.

Discussion

Since the beginning of year 2020, human population has been undergoing through the period of stress with profound challenges over the outburst of COVID-19 disease caused by SARS-CoV-2 infection. Though many of the published reports have claimed that in the majority of cases, COVID-19 is a relatively mild condition; the percentage of worst cases leading to severe manifestations of acute respiratory distress syndrome (ARDS), multi-organ failure, hospitalization and death also cannot be ignored as its global magnitude has been recorded considerably high [30–36].

Table 2 Percent of subjects having hepato-pancreatic severity on admission and 10th day of admission of COVID-19 infected subjects in relation to diabetic severity (plasma glucose concentration) at the time of admission

Group-A: Normal glucose level (65–110 mg/dL) at the time of admission. (n = 8)								
Parameters In Plasma/serum	On admission			10th day of admission				
	No. of Subjects (n) – % of (n = 8)			No. of Subjects (n) – % of (n = 8)				
	Normal (n)	Moderate (m)	High (h)	Normal (n)	Moderate (m)	High (h)		
SGPT	n = 5 62.5%	n = 2 25%	n = 1 12.5%	n = 3 37.5%	n = 2 25%	n = 3 37.5%		
SGOT	n = 3 37.5%	n = 2 25%	n = 3 37.5%	n = 5 62.5%	n = 2 25%	n = 1 12.5%		
ALP	n = 5 62.5%	n = 3 37.5%	n = 0 0%	n = 4 50%	n = 4 50%	n = 0 0%		
LDH	n = 2 25%	n = 3 37.5%	n = 3 37.5%	n = 3 37.5%	n = 5 62.5%	n = 0 0%		
Insulin	n = 2 25%	n = 4 50%	n = 2 25%	n = 3 37.5%	n = 4 50%	n = 1 12.5%		
HOMA-IR	n = 4 50%	n = 2 25%	n = 2 25%	n = 2 25%	n = 4 50%	n = 2 25%		
Glucagon	n = 4 50%	n = 3 37.5%	n = 1 12.5%	n = 4 50%	n = 2 25%	n = 2 25%		
Glucokinase	n = 4 50%	n = 3 37.5%	n = 1 12.5%	n = 6 75%	n = 1 12.5%	n = 1 12.5%		
Group-B: Moderately high glucose level (111–150 mg/dL) at the time of admission.(n = 13)								
Parameters In Plasma/serum	On admission			10th day of admission				
	No. of Subjects (n) – % of (n = 13)			No. of Subjects (n) – % of (n = 13)				
	Normal (n)	Moderate (m)	High (h)	Normal (n)	Moderate (m)	High (h)		
SGPT	n = 6 46%	n = 5 38%	n = 2 15%	n = 4 31%	n = 5 38%	n = 4 31%		
SGOT	n = 4 31%	n = 6 46%	n = 3 23%	n = 4 31%	n = 8 62%	n = 1 7%		
ALP	n = 11 85%	n = 2 15%	n = 0 0%	n = 13 100%	n = 0 0%	n = 0 0%		
LDH	n = 2 15%	n = 11 85%	n = 0 0%	n = 2 15%	n = 4 31%	n = 7 54%		
Insulin	n = 6 46%	n = 4 31%	n = 3 23%	n = 11 85%	n = 2 15%	n = 0 0%		
HOMA-IR	n = 3 23%	n = 2 15%	n = 8 62%	n = 4 31%	n = 4 31%	n = 5 38%		
Glucagon	n = 3 23%	n = 5 38%	n = 5 38%	n = 4 31%	n = 6 46%	n = 3 23%		
Glucokinase	n = 9 70%	n = 3 23%	n = 1 7%	n = 6 46%	n = 6 46%	n = 1 7%		
Group-C: High glucose level (> 150 mg/dL) at the time of admission.(n = 9)								
Parameters In Plasma/seru	On admission			10th day of admission				
	No. of Subjects (n) – % of (n = 9)			No. of Subjects (n) – % of (n = 9)				
	Normal (n)	Moderate (m)	High (h)	Normal (n)	Moderate (m)	High (h)		
SGPT	n = 4 44.5%	n = 3 33.3%	n = 2 22.2%	n = 3 33.3%	n = 3 33.3%	n = 3 33.3%		
SGOT	n = 5 55.5%	n = 4 44.5%	n = 0 0%	n = 3 33.3%	n = 3 33.3%	n = 3 33.3%		
ALP	n = 5 55.5%	n = 4 44.5%	n = 0 0%	n = 8 88.8%	n = 1 11.2%	n = 0 0%		
LDH	n = 1 11.2%	n = 6 66.6%	n = 2 22.2%	n = 2 22.2%	n = 2 22.2%	n = 5 55.5%		
Insulin	n = 3 33.3%	n = 3 33.3%	n = 3 33.3%	n = 6 66.6%	n = 1 11.2%	n = 2 22.2%		
HOMA-IR	n = 1 11.2%	n = 0 0%	n = 8 88.8%	n = 1 11.2%	n = 2 22.2%	n = 6 66.6%		
Glucagon	n = 3 33.3%	n = 3 33.3%	n = 3 33.3%	n = 4 44.5%	n = 3 33.3%	n = 2 22.2%		
Glucokinase	n = 3 33.3%	n = 4 44.5%	n = 2 22.2%	n = 4 44.5%	n = 3 33.3%	n = 2 22.2%		
Reference Range	SGPT IU/L	SGOT IU/L	ALP IU/L	LDH IU/L	Insulin μ U/mL	HOMA-IR	Glucagon ng/L	Glucokinase ng/mL
Normal	10–30	0–30	30–90	230–450	04–25	< 2.60	72–180	20–50
Moderate	31–60	31–60	91–150	451–800	26–50	2.60–3.80	181–225	51–70
High	> 60	> 60	> 150	> 800	> 50	> 3.80	> 225	> 70

Total no. of subjects (n = 30) were divided in three groups (A: n = 8, B: n = 13, C: n = 9) as per plasma glucose level at the time of admission
 SGPT: Serum glutamic-pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; HOMA-IR: Homeostatic model assessment (HOMA)-insulin resistance (IR)

Table 3 Pro-inflammatory cytokines response in COVID-19 infected subjects. (n = 30)

Cytokines level in serum (Normal ref. range)	On admission (n = 30) Mean \pm SD	10th day of admission (n = 30) Mean \pm SD
IL-1 β (0.5–12.0 pg/mL)	11.59 \pm 4.1 pg/mL	9.31 \pm 6.1 pg/mL
IL-6 (0.5–5.0 pg/mL)	5.12 \pm 2.1 pg/mL	2.88 \pm 1.1 pg/mL*

IL-1 β : Interleukin-1 β ; IL-6: Interleukin-6

* $P < 0.05$ vs On admission

Hyperglycemia related anomalies, the prime interest of this present study, is a long known risk creator when gets associated with any other clinical symptoms. Many research laboratories around the world are now trying to get a comprehensive answer to the clinico-pathological impact between diabetes and COVID-19 disease, particularly to interpret the severity and mortality of COVID-19 disease evolved by diabetic collusion [7, 18, 37–43].

The present study has projected an insight on the pro-diabetic disposition along with impaired hepato-pancreatic functional vulnerability in post-COVID era of SARS-CoV-2 infected subjects. Although there was a healing trend of the disease by 10 days of hospital care, a hyperglycaemic propensity persisted along with high values of hepato-pancreatic injury bio-markers beyond the normal reference range in subjects who had no diabetic history before COVID-19 disease. This implies the chances of an insult over normal organ specific functional output by indulging organ damage through diabetic malediction. The results of this study has shown a three level comparison of organ function output in terms of the limits of reference range (normal, moderate, high) of their functional integrity against the background of existed glycemic stature of the SARS-CoV-2 infected victims at the time of hospital admission. In spite of a diverse scenario, the prime consensus of the results from this study exhibits predilection of the chances of organ damage from hyperglycaemic severity of COVID-19 disease. In majority of nascent convalescent subjects (on 10th day), high values of organ specific diagnostic bio-markers accompanying with higher HOMA-IR level were found to be existed particularly with subjects having hyperglycaemic disposition at the time of hospital admission.

Soluble cytokines in blood circulation are the signatures of pro-inflammatory response and is a major cause of disease severity and death in several pathogenic occasions including COVID-19 disease process [44–46]. The elevated serum cytokine levels, viz. IL-1 β and IL-6, near to the extreme higher limits of their reference range in the patients of this study at the time of hospitalization supports the earlier findings in previously published reports [44, 47–49]. Therapeutic intervention and COVID-care indenture in 10 days of hospital-stay have marginally diminished the pro-inflammatory serum constituents (IL-1 β and IL-6) from their extreme upper limits to a considerable safe zone within the normal reference range. This has shown that an acute surge of pro-inflammatory response is an accompanied phenomenon of SARS-CoV-2 infection and this could be the reason of morbidity and mortality related fatal outcome in the progression of COVID-19 disease.

The other associated risk factors that have got priority as prognostic determinants as well as COVID-19 related diagnostic evaluator in many prevalent studies on COVID-19 disease are C-reactive protein (CRP) [50–56], ferritin [57–63], degraded fibrin dimer (D-dimer) [64–71], and neutrophil: lymphocyte ratio (N/L) [72–79]. All of these COVID-19 specific diagnostic biomarkers remained at extremely higher levels beyond their normally existed limits in human serum. The span of existed concentrations of these markers at any stage of SARS-CoV-2 infection was found too random to justify their margins and thus, beyond the scope to score their discrete proportional limits to assess the degree of severity of the disease. In our 10 days follow up, the concentrations of these markers remained randomly anywhere within the range given in table-4, which was too high to only get convinced about the

Table 4 Risk factor range over 10 days period of admission in COVID-19 infected subjects. (n = 30)

Parameters in serum	Reference range (Kit based)	Patient value (range only)
Ferritin	10–300 ng/mL	375.54–513.97 ng/mL
CRP	0–5.0 mg/L	72.34–82.3 mg/L
D-Dimer	< 0.2 μ g/mL	0.85–1.88 μ g/mL
Neutrophil / Lymphocyte ratio	2.0–3.5	5.3–9.5

CRP: C-reactive protein; D-Dimer: Degradative fibrin dimer

ensured morbid episode of new SARS-CoV-2 infected COVID-19 disease.

Lastly, the disposition of hyperglycemic severity in subjects suffered from COVID-19 disease was correlated with the blood group of infected subjects along with other clinical comorbidities. This is to note that all the patients in our study were housed in air oxygen mode in hospital ward only and were not critical for using high flow oxygen delivery system in ICU set up. Although hyperglycemic outburst was common in all groups in 30 participants with varying severity; comorbidities like hypertension, coronary artery disease, hypothyroid, irritable bowel syndrome and lung cancer were found discretely present in A, B and O blood group people. No such side effects were observed in subjects with AB blood group, who were only 6.7% of the total participants (n = 30) in present study. A 6% death was evident in all three blood groups except AB type. Hyperglycemic out-turn was also less in AB type group. The comparison of COVID-19 sensitivity in terms of blood group in this study had given a clue that A and B blood group people were more sensitive to COVID-19 infection. On the other hand, between two other groups the AB type was more resistant to COVID-19 infection over O types within the limits of this pilot study. Top of all, the Rh(-) type of all blood groups in present study remained unaffected from either comorbidities or hyperglycemic indenture. Based on our observation, though we can presume a scale of COVID-19 disease sensitivity as Gr-B > Gr-A > Gr-O > Gr-AB; we cannot solicit this result of our pilot study as universal poster. An elaborate study over more people with large number of each of the four blood groups may only give a universal picture about the blood group sensitivity to COVID-19 infection. Scattered interests on blood group sensitivity to Corona virus infection are also not skimpy [79–85].

The results of this study show that hyperglycemia is an associated risk factor along with disease oriented complications to the patients with COVID-19 disease. A post-infection follow-up on glycemic-index and related complexities is therefore, a vital need to the COVID-19 positive subjects.

Conclusions

A post-infection-pro-diabetic disposition is apparent in COVID-19 infected subjects. Hence, disease oriented acute complications are expected in diabetic subjects when they get COVID-19 infection. Thus, existed hyperglycemia is a risk factor for COVID-infected subjects. Therefore, a post-infection follow-up on glycemic-index is the prime need to prevent post infection complexities in COVID-19 infected convalescent subjects. Implementation of guidelines on

Table 5 Percentage of glycemic status on the day of hospitalization in relation to blood groups and the associated co-morbidities in COVID-19 infected subjects under study. (n = 30)

Blood Gr. (n)	% Availability	% Co-morbidity (out of n = 30)				O ₂ Status	DHBH	Fasting Glucose (% subjects)			DEATH(n)
		HT	CAD	HTh	IBS			HT + CAD	CaLung	< 126 mg/dL	
A Rh(+) n = 8	26.7%	3% n = 1	-	3% n = 1	-	Room Air	NIL	13% n = 4	20% n = 6	3% n = 1	6% n = 2 Rh(+)
Rh(-) n = 3	10%	-	-	-	-	-	-	-	-	-	-
B Rh(+) n = 13	43.3%	10% n = 3	-	-	3% n = 1	-	-	20% n = 6	17% n = 5	7.5% n = 2	6% n = 2 Rh(+)
Rh(-) n = 0	-	-	-	-	-	-	-	-	-	-	-
AB Rh(+) n = 2	6.7%	-	-	-	-	-	-	3% n = 1	3% n = 1	-	-
Rh(-) n = 0	-	-	-	-	-	-	-	-	-	-	-
O Rh(+) n = 3	10%	-	-	-	-	-	-	3% n = 1	7.5% n = 2	3% n = 1	6% n = 1 Rh(+)
Rh(-) n = 1	3.3%	-	-	-	-	3% n = 1	-	-	-	-	Rh(-)
						3% n = 1					

HT: Hypertension; CAD: Coronary artery disease; HTh: Hypothyroid; IBS: Irritable bowel syndrome; Ca-Lung: Lung cancer; DHBH: Diabetic history before hospitalization.

social measure and awareness of anti-viral interventions may be the only way to prevent COVID-19 transmission.

Acknowledgements The authors are thankful to all COVID-19 infected subjects, who volunteered by donating their blood samples to perform this study.

Funding AIIMS PATNA-COVID-19-Research Intramural Institute Grant (Ref. No. I – 18/543).

Declarations

Conflict of interest The authors declare that there is no conflict of interests.

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