Exposure to Mycobacteria influences disease progression in COVID-19 patients

Ajay Gupta (drajaygupta@hotmail.com)
Maulana Azad Medical College, New Delhi https://orcid.org/0000-0001-7128-2795
Sumit Sural
Maulana Azad Medical College, New Delhi
Ayush Gupta
Swami Rama Himalayan University, Dehradun
Shashank Rousa
Maulana Azad Medical College, New Delhi
B.C.Koner
Maulana Azad Medical College, New Delhi
Anju Bhalotra
Maulana Azad Medical College, New Delhi
Rohit Chawla
Maulana Azad Medical College, New Delhi

Research Article

Keywords: Quantiferon, innate, B.C.G., typhoid,

Posted Date: August 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-56141/v1

Abstract

Background: COVID-19–related deaths are significantly higher in countries with higher quality of life. A strong negative correlation is reported between the BCG index and COVID- 19 mortality. The present study explored if a high Th1immunity due to frequent exposure to strong Th1 antigens like Mycobacteria or Salmonella could be the cause for lesser COVID-19–related deaths in Indian population.

Methods: This prospective comparative study was conducted with 3 groups of twenty patients each of mildly symptomatic (A), severely ill (S) Covid patients and healthy volunteers with a Covid Negative report (H).

Results: All severely ill patients showed increased leucocyte counts, lymphopenia and raised D-dimer. A gross reversible unresponsiveness of T cells was seen among all patients in S group with absolutely no response even to the mitogen stimulus. Quantiferon TB test value and distribution of test positivity was significantly lower in group S. Three out of 6 survived patients in S group had positive Quantiferon TB test while 2 patients turned positive on repeat test and the sixth patient showed high TH titre on widal test.

Conclusion: Altered Th1 immunity associated with frequent community exposure of tuberculosis and typhoid antigen in Indian population might be responsible for its relatively lesser prevalence and mortality following Covid-19.

Background

Inflammation is the double-edged sword to contain and eliminate any infection in the body. Pathogens could evoke reduced or even excessive inflammatory reaction depending upon the type and magnitude of infection and the relative availability of different immune reactions. An excessive inflammatory reaction called cytokine storm was seen in patients of SARS influenza virus, avian H5N1, H7N9 and Covid-19 infection[1,2] which helps managing the infection but damages host's own tissues thus leading to acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS).

Breathlessness along with decreasing blood O_2 saturation level, lymphopoenia, high blood D-dimer and very high serum concentration of several unrelated cytokines are important clinical features seen in Covid-19 patients.[2] Covid-19 patients exhibit a Th1 type response where SARS-CoV-2 specific CD4+ T cells express significantly higher frequency of polyfunctional CD4 T cells producing IFN-*γ*, TNF-*α*, and IL2 and CD8 T cells producing IFN-*γ*, TNF-*α*, and CD107a(degranulation).[3] A strong Th1 response associated with increased neutralizing antibodies was seen, leading to recovery in Covid-19 patients, while a Th2 response was seen to be associated with fatal disease.[4] A healthy individual with good immunity, should be able to sufficiently suppress or eliminate Covid-19 virus like other Corona and influenza viruses through a robust locally available innate immunity in the respiratory passage and the sequential cellular immunity with Th1 preponderance.

Innate immune responses, in addition to providing a first line of defence, plays an instructive role in T cell activation and the Th1/Th2 polarization also.[5] Body evokes Th1 response aiming to kill and contain virus. However, in patients with Th2 bias immunity, virus upon reaching to lung macrophages or epithelial cells, polarizes them to M2 state which produce inhibitory cytokines like TNF-α, IL-10 and type-I IFN causing T cell apoptosis and prevention of their proliferation.[6-8] Autocrine IL-10 signalling in dendritic cell (DC) further inhibits chemokine production preventing their trafficking to lymph nodes for maturation which again preferentially stimulate Th2 responses due to decreased signalling through T-cell receptor and low co-stimulatory signals.[9] Influenza virus in an experimental study on mice has been seen to be lethal if it somehow induces a combined Th1 and Th2 response.[10] Therefore, poor Th1 immunity in combination with pre-existing Th2 bias having high levels of inhibitory cytokines e.g., IL-10 and TNF-α might become fatal in Covid-19 patients.

Human population living in a clean and hygienic environment grossly lack in Th1 immunity and overexpresses Th2 immunity due to the cross regulation between Th1 and Th2 immunity[11] and has shown a higher prevalence of Covid-19.[12-14] Could frequent exposure to strong Th1 antigens like Mycobacteria, malaria or Salmonella that is prevalent in the Indian population be the cause for lesser severity and lower mortality due to Covid-19, was the research question which we aimed to answer through the present study. We, therefore, performed measurements of Salmonella antibodies titre and Interferon gamma release assay (IGRA) against Mycobacteria (Mtb) antigen in a group of healthy volunteers, Covid-19 asymptomatic patients and severely affected Covid patients to analyse if immunity against such strong Th1 inducing pathogens has any role in modifying the course of the Covid-19.

Materials And Methods

The present work was a Prospective comparative study conducted at Lok Nayak Hospital and Maulana Azad Medical College, New Delhi and comprised of 60 subjects in three groups of 20 each. **Group A** consisted of asymptomatic or mildly symptomatic cases admitted in a particular male ward on 3 consecutive days with a Covid-19 positive test report, **Group S** consisted of severely ill Covid positive cases admitted in the intensive care unit of the hospital while **Group H** comprised of 20 healthy HCW volunteers working at LN Hospital with a Covid negative report by reverse transcriptase polymerase chain reaction from the oro-naso-pharyngeal swab.

Patient with Co morbid condition like malignancy, chronic renal failure, cardiomyopathy, Chronic respiratory failure with pre-existing dyspnoea, inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease or patients on long term immuno-suppressants were excluded from the study. Approval of the institutional ethical committee was obtained. After an informed consent of all participants, a routine clinical examination for fever, pulse rate, blood pressure and respiratory rate along with any organomegaly was done and recorded. The socio-economic status, past illness like diabetes mellitus, hypertension, asthma or other allergic disorder, intake of ACE2 inhibitors or steroids etc. were recorded. A note was also made of their religious practices including performing Hawan, Artis and the

dietary habits. Any past history of vaccinations including Bacille Calmette Guerin (BCG), influenza, hepatitis in the preceding 10 years including the BCG scar was also recorded. All subjects were subjected to Haemogram, S. Ferritin, S. triglycerides, D-Dimer, Quantiferon TB Gold and Widal test (anti-TO and anti-TH and AH titre) besides the routine required medical treatment depending upon their individual clinical needs.

T test or one-way ANOVA followed by Tukey HSD post-hoc test was used for comparison of parametric data and Mann Whitney U test or Kruskal Wallis followed by corrected Dunn post-hoc test for non-parametric data. Linear regression analysis was used for correcting confounders' effect (lymphocyte count) on Quantiferon test and mitogen stimulation testA p value less than 0.05 was considered as level of significance.

Results

There was a significant difference between the 3 groups in terms of D-Dimer ($\chi2$ = 36.533, p = <0.001, S. Ferritin ($\chi2$ = 19.811, p = <0.001), TAG ($\chi2$ = 12.308, p = 0.002), TLC (F = 15.737, p = <0.001), neutrophil (F = 36.799, p = <0.001), lymphocyte (F = 32.798, p = <0.001) and monocyte counts (F = 8.196, p = 0.001). S group had the highest level of S. Ferritin, TLC, neutrophil and monocyte counts while TAG and lymphocyte were found to be the lowest (Table 1).

There was a significant difference between the various groups in terms of distribution of positive Mtb induced IGRA- Category ($\chi2$ = 13.838, p = 0.001) or mitogen induced IGRA ($\chi2$ = 36.946, p = <0.001) which were lowest in S Group. The difference between levels of Quantiferon TB Gold test was statistically significant (p<0.01) even after correction for lymphocyte count (confounder) by linear regression analysis. There was no significant difference between the various groups in terms of distribution of Salmonella TO, TH or AH Antigens. Sixty percent of the study population were frequently indulged in religious practices to expose them to strong respiratory irritants while majority of them consumed eatables with good spicy constituents.

A comparison was made between 2 subgroups of the Group S based on their outcome measure of Survived or Died. There was a significant difference between the 2 subgroups in terms of TLC (W = 10.000, p = 0.006), positive Quantiferon TB Gold test ($\chi2$ = 8.235, p = 0.018) and mitogen induced IGRA (W = 70.000, p = 0.023) with all patients with positive Quantiferon TB Gold test and higher mitogen induced IGRA surviving (Fig. 1) . No significant difference could be established between the two subgroups in terms of lymphopenia and other haematological parameters, D-dimer and S. ferritin levels (Table 2).

A gross reversible unresponsiveness of T cells was seen among all patients in S group who showed absolutely no response even to the mitogen stimulus with mean of 1.33 ± 2.77 IU/ml against 8.68 ± 5.50 and 13.90 ± 4.09 in A and H Group respectively. Three out of 6 survived patients had positive Quantiferon TB test. A repeat Quantiferon TB test was done on all survived patients 6 weeks after discharge from the hospital. Two of the remaining 3 survived patients also turned positive on repeat test while the sixth

patient still appeared in unresponsive mode with very low response even to mitogen stimulus (1.38 IU/ml). She tested negative on quantiferon TB test but showed high TH titre on widal test.

Discussion

The present study demonstrates higher age, decrease in Hb%, haematocrit and increase in TLC, polymorph count, monocyte count & serum ferritin level in severely ill patients which reflect acute phase response in viral infection. Lower lymphocyte counts in peripheral blood are a consistent finding in patients with severe Covid-19 [2] as was seen in our study as well. Not only lymphocyte depletion, there was a severity dependant decrease in response to mitogen stimulation of lymphocytes also (Table 1). T-cell exhaustion is a phenomenon of dysfunction or physical elimination of antigen-specific T cells reported in viral infections as well as cancerand is often described with severe covid-19 patients.[15,16] In addition to decrease in count, T cells exhaustion is identified with loss of interleukins in a *hierarchical* manner where (IL)-2 and tumor necrosis factor (TNF) are always decreased significantly followed by interferon (IFN)-γ and beta-chemokine production.[17,18] COVID-19 patients however have shown very high levels of IL- 2, IL-7, IL-10, TNF-α, G-CSF, IP-10, MCP-1, and MIP-1A [2,19] which is in contradiction to the exhausted status of T cells with decreased levels of (IL)-2, (TNF) and (IFN)-γ.[17,18] Moreover, T cell exhaustion is described to effect antigen-specific T cells due to excessive and persistent antigenic exposure.[18] The present study however demonstrated poor response to even mitogen which stimulate a large proportion of normal lymphocytes to respond in a manner similar to their response by specific antigens. It could therefore be concluded that there exists a dysregulated immune response in all critically ill Covid-19 patients. However, whether the mechanism to evoke such compromised immunity is T cell exhaustion or T cell anergy which is failure of T cells to proliferate and to produce cytokines preferentially in Th1 cells[20] remains to be proved. All critically ill Covid-19 patients showed severe unresponsiveness by the mycobacterium specific T cells while they remain high on IL-6 level. These patients are also seen to have increased level of IL-10 which is known to be involved in the induction of anergy in CD4 + T cells by selectively impairing signalling through the TCR/CD3 complex[21] thus favouring later to be more probable.

Fifty percent of our study population of healthy volunteers or asymptomatic/mild Covid-19 patients was strongly positive for Mtb IGRA depicting good community exposure to tubercular antigen. The 95% of remaining half of the study group had BCG scar and evidently had Mtb antigenic exposure to their immune system sometime in past. Not only the distribution of Quantiferon TB gold test positivity was lowest (3 out 20 in gr S vs 10 out of 20 in gr A) in group S (table 1), but Quantiferon TB gold test positivity was associated with increased survival also in severe COVID-19 (table2). All these findings indicate that latent TB or recent TB exposure might have some beneficial effect with reference to severity and mortality in COVID-19. Similar observations were made authors who reported strong correlation between the BCG index, an estimation of the degree of universal BCG vaccination deployment in a country, and COVID- 19 mortality in different socially similar European countries ($r2 = 0.88$; $P = 8 \times 10-7$).[12]

COVID-19–related deaths are significantly higher in countries with higher quality of life, contradicting the expectation of lower rates of mortality in countries with improved health care systems.[12] Though India has become the third highest country with maximum number of Covid-19 patients, it continues to shows significantly lower prevalence of the disease, both in terms of disease and deaths per million population in comparison to other top 15-20 countries.[22] Natural course for most of Covid-19 patients in Th1 predominant population may be to remain asymptomatic or minimally symptomatic which is reflected in the epidemiological reports from India and other similar countries like south America etc.[12] Patients with moderate to severe symptoms from such a population, probably have pre-existing low immunity due to malnutrition or an associated co-morbidity. An unforeseen Th2 predominance due to worm infestation, subtle autoimmune or allergic disorder etc. affecting these patients could be another compounding factor and deserves further exploration.

None of the subject from our study group had ever been administered an influenza vaccine showing its nearly negligible prevalence. Consequently, frequent affection to common cold, common habit of eating spicy food, relative unhygienic environment, a common religious practice of performing Hawan and Arti etc. which involves sublimating mixture of wood with odoriferous and medicinal herbs in the fire exposing nasal, respiratory and gut mucosa to many irritants of ancient unproven qualities[23] could possibly be contributing to an enhanced innate immunity among the Indian population. Besides its role in building up of innate immunity, the medicinal smoke from performing Hawan has been found to reduce bacterial counts also by 94% in 60 minutes and was seen to be maintained for 24 hours.[24] Strong Th1 immunity can be attributed to lower worsening or progression of the disease in Covid-19 patients. This however is unlikely to cause lower infectivity of the disease among the population, which could only be obtained through measure like social distancing, tracking and quarantine the patients etc. or some unrecognized biological sentinels in the respiratory passage as pointed above.

D-dimer levels have been reported as a reliable prognostic marker for in-hospital mortality in patients admitted for Covid-19.[25] 75% patients in group A had D-dimer levels much above the cut off value of 2000 ng/ml[25] which qualify them for initiating an anticoagulant therapy. None of such patients were prescribed with any anticoagulants and had no untoward incident for at least 8 weeks after the discharge from the hospital. Interestingly, patients in group S of severe disease with 70% mortality also showed raised D-dimer levels but of lesser magnitude than the Group A. Such observations dictate to hypothesize that increased D-dimer level may be an independent risk factor for producing state of hypercoagulability but is likely to benefit patients through T cell independent immune mechanism. Various Th2 cytokines IL-4, IL- 10 and IL-13 have been shown to deactivate monocytes and macrophages and also to downregulate fibrinogen biosynthesis.[26] Higher fibrinogen degradation products and D-dimer are reported in patients with tuberculosis than community acquired pneumonia.[27] It may therefore be inferred that Th1 immunity predisposes to increased fibrinogen or D-dimer level. D-dimer is reported to stimulate peripheral blood monocytes to secrete IL-6 and TNF-α[28] thus creating an additional pathway for inducing early innate immunity. This is supported by previous studies which suggested cytokine release not to originate from T cells but to trigger from monocytes and macrophages in COVID-19 patients.[3] Such an

altered pattern of D-dimer levels in our population and its pathogenesis deserves to be substantiated with further studies.

The present study is constrained by its smaller sample size and lack of a detailed corroborating cytokine profile of all subjects. BCG vaccine has been suggested to be used as prophylaxis against Covid-19. It has merit theoretically but is difficult to be established due to BCG vaccine's long preparatory period and known poor efficacy in adult population.[12] The present work probably establishes a direct correlation between Mtb induced Th1 immunity and protection against Covid-19. The restoration of Th1/Th2 imbalance may be a more natural strategy to fight against Covid-19. The novel and explicit results should stimulate researchers to explore treatment options with broad and long-term benefits than to develop a highly specific but short-lived prophylaxis.

Declarations

Funding- Cost of few blood test was funded by Systopic Laboratories- a pharma Co.

No competing interest

References

1. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK. Dysregulated type I interferon and inflammatory monocyte- macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 2016;19:181–193.
2. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395:10223;497-506
3. Diao B, Chenhui W, Yingjun T, Xiewan C, et al. Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19). Frontiers in Immunology 2020 :11; 827
4. Li CK, Wu H, Yan H, et al. T cell responses to whole SARS coronavirus in humans. Journal of Immunology 2008. 181(8):5490-5500.
5. Jankovic D, Liu Z, Gause W. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. 2001. 22 (8); 450-457.
6. Makris S, Paulsen M, Johansson C. Type I interferons as regulators of lung inflammation. Front Immunol 2017;8:259.
7. Mehta AK, Croft M. TNF activity and T cells. Cytokine. 2018;101:14–8.
8. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MBA. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 2006;12:1301–9.
9. Schulke S. Induction of Interleukin-10 Producing Dendritic Cells as a Tool to Suppress Allergen-Specific T Helper 2 Responses. Front Immunol 2018; 9: 455.

10. Turianova L, Lachova V, Svetlikova D et al. Comparison of cytokine profiles induced by nonlethal and lethal doses of influenza A virus in mice. Exp Therap Medicine 2019;18: 4397-4405.
11. Okada H, Feillet H, Bach J-F. The “hygiene hypothesis” for autoimmune and allergic diseases: an update. ClinExpImmunol 2010; 160(1):1-9.
12. Escobar LE, Molina-Cruz A, and Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). PNAS. 2020. www.pnas.org/cgi/doi/10.1073/pnas.2008410117
13. Zwerling et al. The BCG world atlas: A database of global vaccination policies and practices. PLos Med 2011; 8.
14. Faust L, Schreiber Y, and Bocking N. A systemic review of BCG vaccination policies among high risk groups in low TB-burden countries. BMC Public Health. 2019 19, 1504.
15. Zheng H-Y, Zhang M, Yang C-X, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cellular & Molecular Immunology 2020. 17:541–543
16. Brooks DG, Teyton L, Oldstone MBA, McGavern DB (2005) Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. J Virol 79:10514–10527.
17. Fuller MJ, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. J Immunol (2004) 172:4204–4214.
18. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol (2003) 77:4911–27.
19. Yang Y., Shen C., Li J., Yuan J., Yang M., Wang F. Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. MedRxiv. 2020; 2020(3)
20. Ebihara M, Hattori M, and Yoshida T. Distinctly Different Sensitivity in the Induction and Reversal of Anergy of Th1 and Th2 Cells. 2007. Biosci Biotechnol Biochem 71 (1), 130–137.
21. Groux H, Bigler M, deVries J, and Roncarolo M-G. Interleukin-10 Induces a Long-Term Antigen-specific Anergic State in Human CD4 + T Cells. 1996. J Exp Med 184:19-29
22. Covid-19 coronavirus pandemic. Available and collected on 27th July 2020 from https://www.worldometers.info/coronavirus
23. Bansal P, Kaur R, Gupta V, Kumar S, Kaur R. Is There Any Scientific Basis of Hawan to be used in Epilepsy-Prevention/Cure? J Epilepsy Res. 2015 Dec 31;5(2):33-45.
24. Nautiyal CS, Chauhan P and Nene YL. Medicinal smoke reduces airborne bacteria. J 2008; 114 (3): 4460451.
25. Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19.J Thromb Haemost. 2020;18(6):1324-1329.
26. Vasse M, Paysant J, Soria J, Mirshahi SS, Vannier JP, Soria C. Down-regulation of fibrinogen biosynthesis by IL-4, IL-10 and IL-13. British Journal of Haematology, 1996, **93**, 955–961

27. Min W, Zi-Feng J, Jian-Lin X, Hao-Hui F. Role of fibrinogen degradation products and D-dimer in the differential diagnoses of pulmonary tuberculosis and community-acquired pneumonia. Clin Lab 2018. 64 (1) 135-140.
28. Jensen T, Kierulf P, Sandset PM et al. Fibrinogen and fibrin induce synthesis of proinflammatory cytokines from isolated peripheral blood mononuclear cells. Thrombosis and Haemostasis 2007. 97(5):822-9

Tables

Table 1: Mean, SD and distribution (%) of different parameters in severely ill (S), asymptomatic or mild symptomatic (A) COVID-19 patients and healthy Subjects (H).

Parameters	Group			p value
	S (n = 20)	A (n = 20)	H (n = 20)	
Age (Years)***	56.65 ± 18.72[Y#]	36.00 ± 10.77	36.30 ± 11.43	<0.001[1]
Gender***				<0.001[2]
Male	9 (45.0%)	20 (100.0%)	20 (100.0%)	
Female	11 (55.0%)	0 (0.0%)	0 (0.0%)	
D-Dimer (ng/ml)***	2527.20 ± 1731.80[Y]	3682.20 ± 1867.32[Y]	319.05 ± 258.93	<0.001[1]
S. Ferritin***	405.82 ± 323.64[Y]	335.94 ± 273.01[Y]	87.72 ± 62.56	<0.001[1]
TAG ***	209.40 ± 85.65[Y#]	138.35 ± 89.30[Y]	247.00 ± 114.40	0.002[1]
Hemoglobin (g/dL)***	11.18 ± 2.16[Y#]	13.19 ± 1.88[Y]	14.49 ± 1.27	<0.001[3]
Hematocrit (%)***	34.59 ± 6.35[Y]	38.64 ± 4.99	39.88 ± 9.27	0.001[1]
TLC (/cu.mm)***	14572.00 ± 7324.18[Y#]	7740.50 ± 3080.92[Y]	7170.50 ± 1240.85	<0.001[3]
Lymphocytes (/cu.mm)***	1927.50 ± 1520.88[Y#]	2014.04 ± 614.58[Y]	2566.65 ± 572.46	0.001[1]
Monocytes (/cu.mm)***	260.75 ± 220.74[Y]	104.11 ± 93.72	102.90 ± 59.56	0.007[1]
Platelet Count (Lac/cu.mm)	2.14 ± 1.75	1.82 ± 0.73	1.76 ± 0.69	0.980[1]
Outcome				1.000[4]
Survived	6 (30.0%)	-	-	
Died	14 (70.0%)	-	-	
Quantiferon TB Gold (iu/ml)***	0.27 ± 0.86[Y#]	1.80 ± 3.01[Y]	4.32 ± 5.65	0.001[1]
Quantiferon TB Gold +ve Category >0.35 iu/ml)***	3 (15.0%)	10 (50.0%)	10 (50.0%)	0.032[4]
Mitogen (iu/ml)***	1.33 ± 2.77[Y#]	8.68 ± 5.50[Y]	13.90 ± 4.09	<0.001[1]
TO Antigen				0.749[2]
<50	16 (80.0%)	18 (90.0%)	16 (80.0%)	
50-100	4 (20.0%)	2 (10.0%)	4 (20.0%)	
TH Antigen				0.111[2]
<50	14 (70.0%)	12 (60.0%)	17 (85.0%)	
50-100	2 (10.0%)	7 (35.0%)	2 (10.0%)	
>100	4 (20.0%)	1 (5.0%)	1 (5.0%)	

Parameters	Group			p value
	S (n = 20)	A (n = 20)	H (n = 20)	
AH Antigen				0.603[2]
<50	17 (85.0%)	19 (95.0%)	19 (95.0%)	
50-100	3 (15.0%)	1 (5.0%)	1 (5.0%)	

****Significant at p<0.05, 1: Kruskal Wallis Test, 2: Fisher's Exact Test, 3: One-Way ANOVA, 4: Chi-Squared Test,* [Y] *p< 0.05 in comparison to control (gr H) and* [#] p< 0.05 in comparison to Group A by post-hoc test.

Table 2: Association between Outcome (Death) and Parameters in Severe COVID-19 cases

Parameters	Outcome		p value
	Survived (n = 6)	Died (n = 14)	
Age (Years)	49.67 ± 22.42	59.64 ± 16.93	0.563[1]
Gender			0.157[2]
Male	1 (16.7%)	8 (57.1%)	
Female	5 (83.3%)	6 (42.9%)	
D-Dimer (ng/ml)	2158.17 ± 1739.71	2685.36 ± 1769.03	0.480[1]
S. Ferritin	359.83 ± 262.29	425.52 ± 353.87	0.967[1]
Hemoglobin (g/dL)	9.97 ± 2.83	11.70 ± 1.66	0.201[1]
TLC (/cu.mm)***	8735.00 ± 3889.63	17073.57 ± 7079.49	0.006[1]
Lymphocytes (/cu.mm)	1783.00 ± 678.63	1989.43 ± 1785.99	0.680[1]
Monocytes (/cu.mm)	216.33 ± 271.33	279.79 ± 203.97	0.248[1]
Platelet Count (Lac/cu.mm)	3.06 ± 2.85	1.75 ± 0.88	0.274[1]
Quantiferon TB Gold (iu/ml)	0.86 ± 1.48	0.02 ± 0.02	0.071[1]
Quantiferon TB Gold (>0.35 iu/ml) (distribution of Positive cases)***	3 (50.0%)	0 (0.0%)	0.018[2]
Mitogen (iu/ml)***	3.56 ± 4.49	0.37 ± 0.43	0.023[1]

****Significant at p<0.05, 1: Wilcoxon-Mann-Whitney U Test, 2: Fisher's Exact Test*

Figures

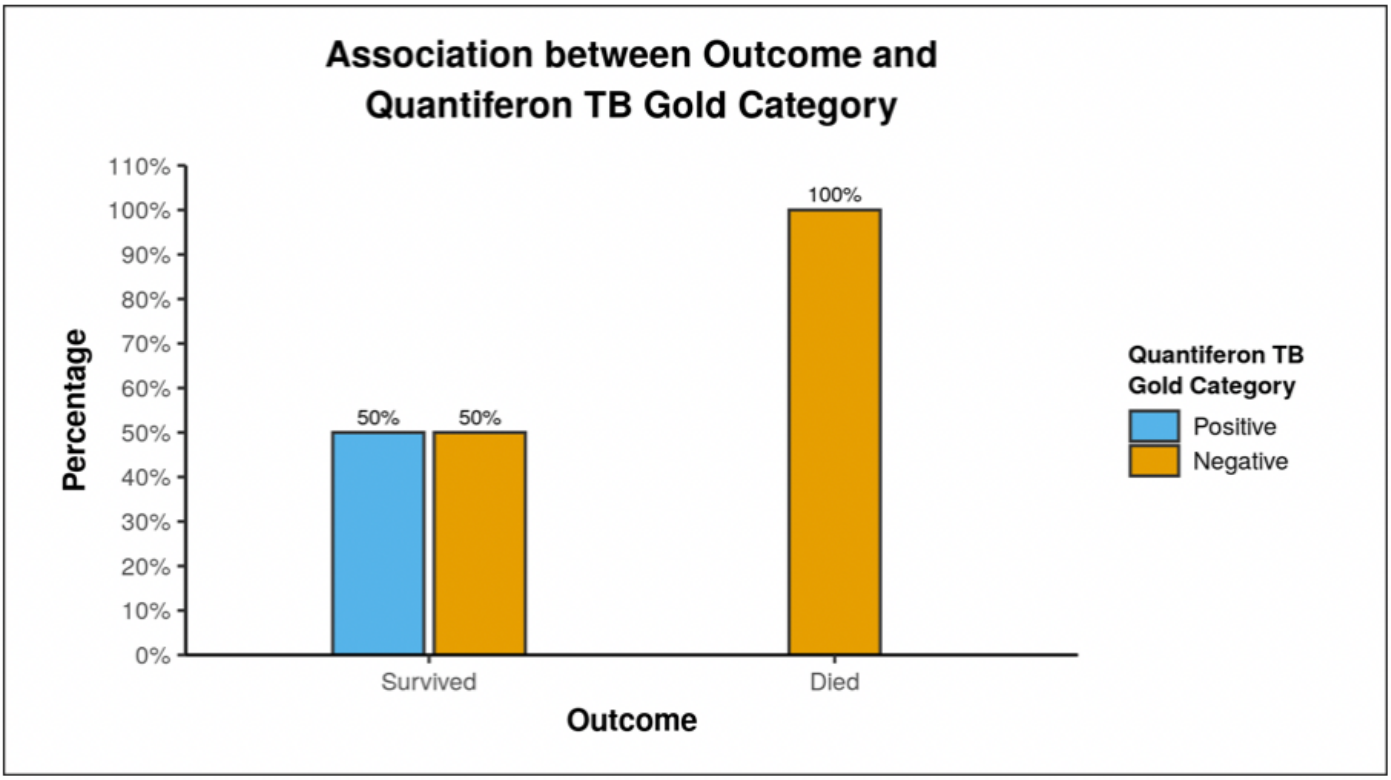

Figure 1

Bar diagram depicting association between Outcome (Death) and Quantiferon TB Gold in severe COVID-19.