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EFFECT OF RECTAL OZONE THERAPY IN PATIENTS WITH MODERATE AND SEVERE COVID-19 PNEUMONIA ON BLOOD SUGAR LEVELS AND INFLAMMATORY MARKERS - A PRELIMINARY STUDY

Anaesthesiology

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ABSTRACT

Background: A novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the causative agent of coronavirus pandemic 2019. Systemic ozone therapy could be potentially useful in the clinical management as shown in research studies to be highly effective at decreasing organ damage mediated by inflammation and oxidative stress. Improved glycaemic control, normalized levels of organic peroxides, activated superoxide dismutase, improved tissue oxygenation, besides its anti-inflammatory and immunomodulatory properties play an important role in the management of cytokine storm associated with COVID. We used rectal O3 insufflation therapy assuming that it may have a beneficial role in the management of COVID-19 disease. **Objectives:** To evaluate the safety, anti-inflammatory effect, glycemic controlling effect of 100ml rectal Ozone therapy at 35µg/ml concentration given once a day for 7 consecutive days in these patients. **Methods:** A total of 10 patients admitted in ICU with RT-PCR positive for SARS-CoV2 moderate and severe COVID-19 pneumonia at our institute were evaluated in this study. **Results:** In ozone group the mean differences in inflammatory markers and blood sugars levels between day 1 and day 7 were CRP 5.12 & SD of 0.93113 with p-0.000, D-dimer levels were 209.00 & SD of 46.61008 with p-0.001, LDH levels were 84.80 & SD of 23.32809 with p-0.001, RBS levels were 60.80 & SD of 5.44977 with p-0.000 respectively. In non-ozone group the mean differences of the same were CRP 1.76 & SD of 1.16748 with p-0.028, D-dimer 56.80 & SD of 34.52825 with p-0.021, LDH 22.60 & SD of 11.52389 with p=0.012, RBS 24.40 7 SD of 16.28803 with p-0.029 respectively. **Conclusion:** We conclude, reduced inflammatory markers level and better glycemic control in patients with moderate and severe COVID pneumonia receiving rectal ozone therapy is because of its multimodal effects.

KEYWORDS

COVID pneumonia, Rectal ozone therapy, cyto-protective therapy.

INTRODUCTION

Corona virus disease 2019 (COVID 19), a systemic inflammatory disease caused by the novel severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). First isolated in December 2019 in Wuhan, China. It was declared a pandemic on 11 March 2020 by the World Health Organization (WHO). The clinical features of this novel Coronavirus Disease 2019 (nCOVID-19), is very much similar to viral pneumonia[1]. The outbreak of COVID-19 in China and its worldwide spread led to the WHO declaring it as a Public Health Emergency on January 2020[2]. Since then, more than 160 million infected cases have been reported worldwide with a death toll of almost 3.5 million individuals[3].The clinical features of COVID-19 are highly variable, ranging from asymptomatic patients to severe forms of respiratory failure and may change remarkably with time. With better understanding of the pathophysiology of COVID-19 achieved over a short period of time has led to a significant improvement in medical management.

Acute inflammation in the lungs is a complex pathophysiological mechanism involving inflammatory mediators such as cytokines and chemokines which stimulate the macrophages in the alveoli leading to poor regulation of the immune system[4]. In humans, the clinical progression of the novel coronavirus induced disease exist in triphasic form. The clinical features in first phase include fever, dry cough, myalgia and other systemic infections that are likely to be increased by the replication of the virus and cell necrosis. The associated features of second phase, is the onset of IgG immunoglobulin conversion, correlated with increase in viral replication. During this phase uncontrolled viral replication occurs causing severe worsening of symptoms. The exact hypothesis behind this might be the severe damage to alveoli caused by over exuberant immune response of the host, majority of the population recover after second phase but one third of patients progress to third phase of the disease which is characterized by severe lung inflammation leading to ARDS that is acute respiratory distress syndrome[1'4]. The study conducted by Jyoti Upadhyay et al suggested that the role of inflammatory mediators in nCOVID-19 infected patients which is diverse and multifunctional. In majority of the cases the immunopathogenesis of nCOVID-19 infection involves disturbances of inflammatory mediators that do not cause the disease but helps in progression of the disease. Some specific set of cytokines and chemokines may be therapeutically targeted and used as prognostic tools in clinical outcomes[5].

The standard of care for COVID-19 is only supportive and symptomatic. Acute respiratory distress syndrome (ARDS) will cause respiratory failure and finally death in COVID-19 patients if left untreated. Although most patients develop mild symptoms, a small percentage of patients develop hyperinflammation and Interferon gamma related cytokine storm syndrome and ARDS will constitute the leading cause of mortality[6'7]. The treatment of hyperinflammation will decrease the mortality rate therefore they hypothesize the anti-inflammatory drugs such as corticosteroids, monoclonal antibodies and even Ozone could be recommended at this stage[8]. Corticosteroids used in management of moderate to severe COVID pneumonia increase blood sugar levels in both diabetics and non-diabetics. It was very difficult to control blood sugar levels in these patients. Ozone(O3) is capable of modulating pain and inflammation. The recognized bactericidal, fungicidal, virucidal and anti-parasitic properties of Ozone are attributed to the fact that is supported by clinical studies that come from countries where the practice of Ozone therapy is well regulated like Cuba, Italy, Germany, Russia, India and Spain. Many water purification plants worldwide use Ozone because of its germicidal effect and its biological properties. Fernandez-Cuadros ME et al have postulated ozone as an alternative therapy for the management of SARS-CoV-2 pandemic[4]. Ozone's virucidal immunomodulatory and vasodilatory properties favour O2 transport to hypoxemic tissues[9], hence can be used in COVID pneumonia. Many authors postulate Ozone as promising alternative in COVID-19 management.

With recent studies, the therapeutic advantages of Ozone have gained more importance through its strong ability to induce controlled and moderated oxidative stress when administered in accurate therapeutic doses. There are many evidences with scientific trails showing that the

activation of hypoxia inducible factor-1α(HIF-1a), nuclear factor of activated T-cells (NFAT) and activated protein-1(AP-1) pathways are the main molecular mechanisms responsible for the therapeutic effects of ozone therapy[9]. Activation of these molecular pathways will result in up-regulation of endogenous antioxidant systems, stimulation of immune functions and also suppression of inflammatory processes which are important for the reduction of oxidative stress in diabetic patients and thereby reducing diabetes related complications including hyperglycaemia[10'11]. It has been seen in many prospective studies that hyperglycemia is also an important cause of microvascular complications (ex. retinopathy, nephropathy, neuropathy) seen in diabetic patients. There are many evidences showing the key role played by hyperglycemia together with insulin resistance in the pathogenesis of macrovascular damage (atherosclerosis), predominantly in type-2 diabetes mellitus which is characterized by early onset and a high incidence of cardiovascular complications[11'12]. A strong relation has been shown between postprandial glucose levels, glycemic spikes, cardiovascular risk and atherosclerosis. A unifying theory for the pathogenesis of microvascular complications of diabetes and hyperglycemia points at the chronic oxidative stress as the common denominator in most of the known biochemical mechanisms[13,14]. In this background, we studied the effects of rectal Ozone therapy on both inflammatory response and blood sugars levels in patients with moderate and severe COVID pneumonia admitted to ICU in our institution.

AIMS & OBJECTIVES

1)To evaluate the safety and effectiveness of 100ml rectal Ozone therapy at 35µg/ml concentration given once a day for 7 consecutive days in these patients.
2)To evaluate the effects on inflammatory response measured with CRP, D-dimer, LDH levels.
3)To evaluate the effects on blood sugar levels measured with RBS.

MATERIALS AND METHODS

Patients admitted in ICU with moderate and severe COVID-19 pneumonia at Adichunchanagiri Institute of Medical Sciences, ACU, BG Nagara, Karnataka were included in this study. A total of 10 patients with moderate to severe COVID clinical symptoms and RT-PCR positive for SARS-CoV2 were evaluated in the study. Institutional ethical committee approval was obtained. Out of 10 patients,5 patients (Group A) were managed with standard treatment protocol along with rectal O3 therapy once a day for 7 consecutive days, rest 5 patients (Group B) were managed with standard treatment protocol alone. Rectal Ozone therapy was done using
(a) Ozone generator.
(b) 14 French rectal probe.
(c) Two silicon syringes of 50 ml capacity/ozone rectal bag.

After seven sessions of Ozone protocol the final evaluation was made using the clinical and biochemical analysis, daily random blood sugar level analysis and chest radiographs. Adverse effects, if any were also recorded.

Inclusion Criteria

1)Male or female between 18 to 80 years of age.
2)With positive detection of new corona virus nucleic acid (RT-PCR SARS-CoV-2) and with clinical signs and symptoms suggestive of SARS-CoV-2 with lung parenchymal involvement on chest X-ray or HRCT.
3)Diagnosed with moderate-severe pneumonia (SpO_2 <□ 93% or PaO_2/FiO_2 <□300 mmHg).
4)Confirmation of lung lesions with chest X-ray or HRCT thorax (according to Taylor's scale).

Exclusion Criteria

1)Pregnancy or lactation.
2)G-6PD (glucose 6-phosphate dehydrogenase) deficiency.
3)Patients who have participated in other clinical studies.

METHODOLOGY

Informed written consent from every patient or legal representatives of the patients was obtained. During the initial assessment, the objectives of the treatment, procedure, indications and contraindications were explained to the patients and/or to their legal representative. On Day1 initial levels of CRP, LDH, D-DIMER, RBS and serum electrolytes were noted along with other necessary investigations. Group A patients were administered standard treatment protocol along with 100 mL of Ozone per rectally at a concentration of 35µg/mL once a day for 7 consecutive days and Group B patients were managed with standard treatment protocol alone. For administration of rectal O3, patients were placed in the supine or lateral decubitus position with the lower limbs flexed depending on their co-operation, two 50ml syringes were loaded with Ozone at a concentration of 35µg/ml and slowly injected rectally through a 14-French rectal tube after lubrication with medical gel-type solution. The administration rate was 1 ml/s. Daily inflammatory markers and blood sugar levels were monitored with CRP, D-dimer, LDH once a day and RBS thrice daily at 8 hours interval respectively. Inflammatory markers and RBS levels on Day1 and Day7 were compared in both the groups after completion of seven days of Ozone (O3) therapy. The findings in both the groups were recorded in Microsoft excel sheet and the final evaluation was performed using relevant statistical methods and tests in SPSS version 19 software. Any adverse effects during the study were also recorded.

RESULTS

GROUP A

Sl. No	BEFORE O3 THERAPY				AFTER O3 THERAPY			
	CRP	D-DIMER	LDH	RBS	CRP	D-DIMER	LDH	RBS
1	19.1	1050	635	322	13.1	885	586	258
2	18.6	988	530	284	14.8	725	435	223
3	18.2	960	632	301	13.5	758	543	246
4	17.9	876	591	388	12.8	625	479	320
5	20.2	789	572	300	14.2	625	493	244

GROUP B

Sl. No	BEFORE STANDARD THERAPY				AFTER STANDARD THERAPY			
	CRP	D-DIMER	LDH	RBS	CRP	D-DIMER	LDH	RBS
1	18.8	1200	650	396	17.9	1185	640	387
2	20	998	685	298	19.6	970	678	288
3	19	1300	712	334	18.9	1275	709	324
4	17	1285	526	356	16.9	1248	518	344
5	18	1100	622	375	17.8	1075	617	362

Statistical Analysis

T-Test: CRP, D-dimer, LDH, RBS levels in both groups.

Group Statistics					
	Groups	N	Mean	Std. Deviation	Std. Error Mean
Pre CRP	Group A	5	18.8000	.90277	.40373
	Group B	5	26.3600	9.74926	4.36000
Pre. D -dimer	Group A	5	932.6000	101.76836	45.51220
	Group B	5	1176.6000	127.76071	57.13633
Pre LDH	Group A	5	592.0000	43.85772	19.61377
	Group B	5	639.0000	71.80529	32.11230
Pre RBS	Group A	5	319.0000	40.86563	18.27567
	Group B	5	351.8000	37.81799	16.91272
Post CRP	Group A	5	13.6800	.81670	.36524
	Group B	5	24.6000	8.62699	3.85811
Post D-dimer	Group A	5	723.6000	108.02685	48.31108
	Group B	5	1119.8000	142.88352	63.89945
Post LDH	Group A	5	507.2000	58.52521	26.17327
	Group B	5	616.4000	73.63627	32.93114
Post RBS	Group A	5	258.2000	36.77227	16.44506
	Group B	5	327.4000	38.70788	17.31069

Independent Samples Test: CRP, D-dimer, LDH, RBS levels				
	t-test for Equality of Means			
	T	Df	Sig. (2-tailed)	Mean Difference
Pre CRP	-1.727	8	.123	-7.56000
Pre. D -dimer	-3.340	8	.010	-244.00000
Pre LDH	-1.249	8	.247	-47.00000
Pre RBS	-1.317	8	.224	-32.80000
Post CRP	-2.818	8	.023	-10.92000
Post D-dimer	-4.946	8	.001	-396.20000
Post LDH	-2.596	8	.032	-109.20000
Post RBS	-2.898	8	.020	-69.20000

N Par Tests: Group A Only

Wilcoxon Signed Ranks Test: CRP, D-dimer, LDH, RBS levels in group A.

Ranks				
		N	Mean Rank	Sum of Ranks
Post CRP – Pre CRP	Negative Ranks	5[a]	3.00	15.00
	Positive Ranks	0[b]	.00	.00
	Ties	0[c]		
	Total	5		
Post D-dimer – Pre. D -dimer	Negative Ranks	5[d]	3.00	15.00
	Positive Ranks	0[e]	.00	.00
	Ties	0[f]		
	Total	5		
Post LDH – Pre LDH	Negative Ranks	5[g]	3.00	15.00
	Positive Ranks	0[h]	.00	.00
	Ties	0[i]		
	Total	5		
Post RBS – Pre RBS	Negative Ranks	5[j]	3.00	15.00
	Positive Ranks	0[k]	.00	.00
	Ties	0[l]		
	Total	5		
a. Post CRP < Pre CRP				
b. Post CRP > Pre CRP				
c. Post CRP = Pre CRP				
d. Post D-dimer < Pre. D -dimer				
e. Post D-dimer > Pre. D-dimer				
f. Post D-dimer = Pre. D-dimer				
g. Post LDH < Pre LDH				
h. Post LDH > Pre LDH				
i. Post LDH = Pre LDH				
j. Post RBS < Pre RBS				
k. Post RBS > Pre RBS				
l. Post RBS = Pre RBS				

Test Statistics				
	Post CRP – Pre CRP	Post D-dimer – Pre. D -dimer	Post LDH – Pre LDH	Post RBS – Pre RBS
Z	-2.032[b]	-2.023[b]	-2.023[b]	-2.023[b]
Asymp. Sig. (2-tailed)	.042	.043	.043	.043
a. Wilcoxon Signed Ranks Test				
b. Based on positive ranks.				

N Par Tests: group B only

Wilcoxon Signed Ranks Test: CRP, D-dimer, LDH, RBS levels in group B.

Ranks				
		N	Mean Rank	Sum of Ranks
Post CRP – Pre CRP	Negative Ranks	5[a]	3.00	15.00
	Positive Ranks	0[b]	.00	.00
	Ties	0[c]		
	Total	5		
Post D-dimer – Pre. D -dimer	Negative Ranks	5[d]	3.00	15.00
	Positive Ranks	0[e]	.00	.00
	Ties	0[f]		
	Total	5		
Post LDH – Pre LDH	Negative Ranks	5[g]	3.00	15.00
	Positive Ranks	0[h]	.00	.00
	Ties	0[i]		
	Total	5		
Post RBS – Pre RBS	Negative Ranks	5[j]	3.00	15.00
	Positive Ranks	0[k]	.00	.00
	Ties	0[l]		
	Total	5		
a. Post CRP < Pre CRP				
b. Post CRP > Pre CRP				
c. Post CRP = Pre CRP				
d. Post D-dimer < Pre. D -dimer				
e. Post D-dimer > Pre. D-dimer				
f. Post D-dimer = Pre. D-dimer				
g. Post LDH < Pre LDH				
h. Post LDH > pre LDH				
i. Post LDH = Pre LDH				
j. Post RBS < Pre RBS				
k. Post RBS > Pre RBS				
l. Post RBS = Pre RBS				

Test Statistics				
	Post CRP – Pre CRP	Post D-dimer – Pre, D-dimer	Post LDH – pre LDH	Post RBS – Pre RBS
Z	-2.032[b]	-2.023[b]	-2.023[b]	-2.023[b]
Asymp. Sig. (2-tailed)	.042	.043	.043	.043
a. Wilcoxon Signed Ranks Test				
b. Based on positive ranks.				

Mann-Whitney Tests: CRP, D-dimer, LDH, RBS levels both groups.

Ranks				
	Groups	N	Mean Rank	Sum of Ranks
Pre CRP	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Pre, D-dimer	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Pre LDH	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Pre RBS	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Post CRP	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Post D-dimer	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Post LDH	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
Post RBS	Group A	0[a]	.00	.00
	Group B	5	3.00	15.00
	Total	5		
a. Mann-Whitney Test cannot be performed on empty groups.				

T-Test: Group A only. CRP, D-dimer, LDH, RBS levels.

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre CRP	18.8000	5	.90277	.40373
	Post CRP	13.6800	5	.81670	.36524
Pair 2	Pre, D-dimer	932.6000	5	101.76836	45.51220
	Post D-dimer	723.6000	5	108.02685	48.31108
Pair 3	Pre LDH	592.0000	5	43.85772	19.61377
	Post LDH	507.2000	5	58.52521	26.17327
Pair 4	Pre RBS	319.0000	5	40.86563	18.27567
	Post RBS	258.2000	5	36.77227	16.44506

Paired Samples Test						
		Paired Differences		T	Df	Sig. (2-tailed)
		Mean	Std. Deviation			
Pair 1	Pre CRP – Post CRP	5.12000	.93113	12.295	4	.000
Pair 2	Pre. D -dimer – post D-dimer	209.00000	46.61008	10.027	4	.001
Pair 3	Pre LDH – post LDH	84.80000	23.32809	8.128	4	.001
Pair 4	Pre RBS – post RBS	60.80000	5.44977	24.947	4	.000

T-Test: Group B only. CRP, D-dimer, LDH, RBS levels.

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre CRP	26.3600	5	9.74926	4.36000
	Post CRP	24.6000	5	8.62699	3.85811
Pair 2	Pre. D-dimer	1176.6000	5	127.76071	57.13633
	Post D-dimer	1119.8000	5	142.88352	63.89945
Pair 3	Pre LDH	639.0000	5	71.80529	32.11230
	Post LDH	616.4000	5	73.63627	32.93114
Pair 4	Pre RBS	351.8000	5	37.81799	16.91272
	Post RBS	327.4000	5	38.70788	17.31069

Paired Samples Test						
		Paired Differences		T	Df	Sig. (2-tailed)
		Mean	Std. Deviation			
Pair 1	Pre CRP – post CRP	1.76000	1.16748	3.371	4	.028
Pair 2	Pre. D -dimer – post D-dimer	56.80000	34.52825	3.678	4	.021
Pair 3	Pre LDH – post LDH	22.60000	11.52389	4.385	4	.012
Pair 4	Pre RBS – post RBS	24.40000	16.28803	3.350	4	.029

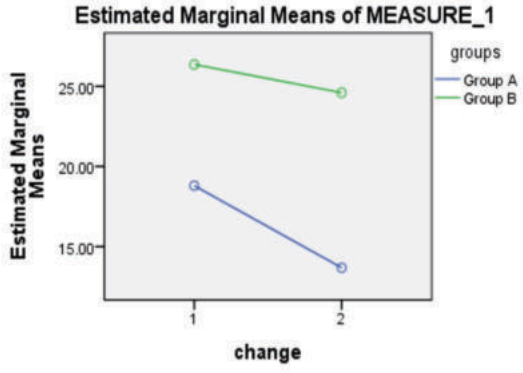
General Linear Model: CRP Levels Both Groups.

Descriptive Statistics				
	Groups	Mean	Std. Deviation	N
Pre CRP	Group A	18.8000	.90277	5
	Group B	26.3600	9.74926	5
	Total	22.5800	7.64734	10
Post CRP	Group A	13.6800	.81670	5
	Group B	24.6000	8.62699	5
	Total	19.1400	8.15464	10

Tests of Within-Subjects Effects					
Measure: MEASURE_1					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Change	59.168	1	59.168	106.131	.000
change * groups	14.112	1	14.112	25.313	.001
Error(change)	4.460	8	.558		

Tests of Between-Subjects Effects					
Measure: MEASURE_1 Transformed Variable: Average					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	8702.792	1	8702.792	102.482	.000
Groups	426.888	1	426.888	5.027	.055
Error	679.360	8	84.920		

Profile Plots: CRP levels.



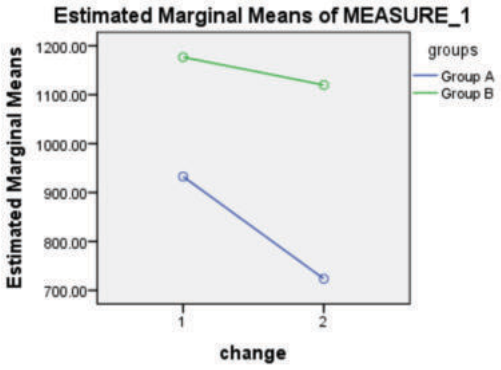
General Linear Model: D-dimer Levels Both Groups.

Descriptive Statistics				
	groups	Mean	Std. Deviation	N
Pre. D -dimer	Group A	932.6000	101.76836	5
	Group B	1176.6000	127.76071	5
	Total	1054.6000	168.50928	10
Post D-dimer	Group A	723.6000	108.02685	5
	Group B	1119.8000	142.88352	5
	Total	921.7000	240.54986	10

Tests of Within-Subjects Effects					
Measure: MEASURE_1					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Change	88312.050	1	88312.050	104.987	.000
change * groups	28956.050	1	28956.050	34.423	.000
Error(change)	6729.400	8	841.175		

Tests of Between-Subjects Effects					
Measure: MEASURE_1 Transformed Variable: Average					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	19528808.450	1	19528808.450	684.228	.000
Groups	512320.050	1	512320.050	17.950	.003
Error	228331.000	8	28541.375		

Profile Plots: D-dimer levels.



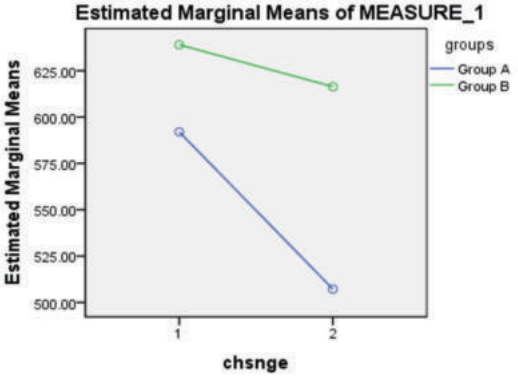
General Linear Model: LDH levels both groups.

Descriptive Statistics				
	Groups	Mean	Std. Deviation	N
Pre LDH	Group A	592.0000	43.85772	5
	Group B	639.0000	71.80529	5
	Total	615.5000	61.31929	10
Post LDH	Group A	507.2000	58.52521	5
	Group B	616.4000	73.63627	5
	Total	561.8000	85.11535	10

Tests of Within-Subjects Effects					
Measure: MEASURE_1					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Change	14418.450	1	14418.450	85.190	.000
change * groups	4836.050	1	4836.050	28.573	.001
Error(change)	1354.000	8	169.250		

Tests of Between-Subjects Effects					
Measure: MEASURE_1 Transformed Variable: Average					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	6930176.450	1	6930176.450	889.140	.000
Groups	30498.050	1	30498.050	3.913	.083
Error	62354.000	8	7794.250		

Profile Plots: LDH levels.



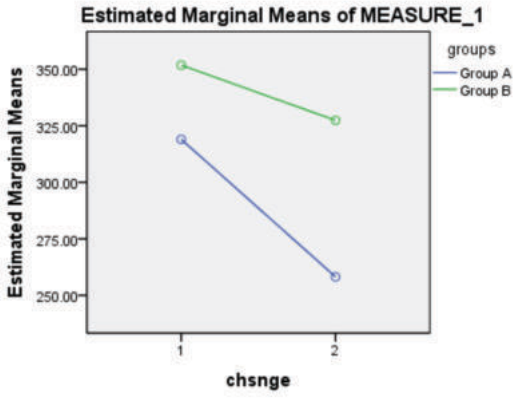
General Linear Model: RBS Levels Both Groups.

Descriptive Statistics				
	Groups	Mean	Std. Deviation	N
Pre RBS	Group A	319.0000	40.86563	5
	Group B	351.8000	37.81799	5
	Total	335.4000	40.94766	10
Post RBS	Group A	258.2000	36.77227	5
	Group B	327.4000	38.70788	5
	Total	292.8000	50.96142	10

Tests of Within-Subjects Effects					
Measure: MEASURE_1					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Change	9073.800	1	9073.800	123.035	.000
change * groups	1656.200	1	1656.200	22.457	.001
Error(change)	590.000	8	73.750		

Tests of Between-Subjects Effects					
Measure: MEASURE_1 Transformed Variable: Average					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	1973176.200	1	1973176.200	680.030	.000
Groups	13005.000	1	13005.000	4.482	.067
Error	23212.800	8	2901.600		

Profile Plots: RBS levels.



DISCUSSION

COVID-19 is a systemic inflammatory viral disease involving multiple organ systems predominantly lungs leading to ARDS[1]. Severe and uncontrolled inflammatory response, ARDS, cytokine storm, thromboembolic phenomena contribute to mortality in these patients[6'7]. Even though mainstay of treatment is symptomatic and supportive, anti-inflammatory drugs, immunotherapy, Ozone are excellent alternate therapies[4'8'10'11]. Ozone being a strong anti-inflammatory, anti-oxidant, cytoprotective agent has additional advantage of reducing blood sugar levels, HbA1C and many other complications associated with hyperglycemia[11'12'14]. Our preliminary study included a total of 10 patients with moderate and severe COVID-19 pneumonia admitted in ICU at our institution, out of which 5 patients (GROUP A) were treated with 100ml of rectal O3 therapy at 35µg/ml concentration once a day for 7 consecutive days along with standard management protocol and rest 5 patients (GROUP B) were treated with standard management protocol alone. We studied the effects of rectal O3 therapy on inflammatory markers and blood sugar levels by evaluating the changes in the levels of CRP, LDH, D-Dimer once a day along with 8th hourly random blood sugar (RBS) monitoring on daily basis for 7 consecutive days in both the groups. On comparing the levels of all the abovementioned inflammatory markers and RBS levels in both the groups we found out that there was clinically significant decline in levels of both inflammatory markers and blood sugars levels in group A patients receiving rectal ozone therapy. This significant decrease in the levels of inflammatory markers and RBS levels were clearly seen in statistical analysis with paired samples T-test also. In group A the mean differences in inflammatory markers and blood sugars levels between day 1 and day 7 were CRP 5.12 & SD of 0.93113 with p-0.000, D-dimer levels were 209.00 & SD of 46.61008 with p-0.001, LDH levels were 84.80 & SD of 23.32809 with p-0.001, RBS levels were 60.80 & SD of 5.44977 with p-0.000 respectively. Whereas in group B the mean differences of the same were CRP 1.76 & SD of 1.16748 with p-0.028, D-dimer 56.80 & SD of 34.52825 with p-0.021, LDH 22.60 & SD of 11.52389 with p=0.012.RBS 24.40 7 SD of 16.28803 with p-0.029 respectively. While considering the mean differences in levels of CRP, D-dimer, LDH, RBS from day 1 to day 7 in both the groups we found that the mean differences of all the parameters measured were significantly higher in group A indicating a reduction in all parameter levels under evaluation to a statistically greater extent compared to group B. Meanwhile in group B the mean differences in the levels of all parameters between day 1 and day 7 were comparatively lower indicating statistically insignificant reduction. From the above analysis it is very evident that the reduction of inflammatory markers and blood sugar levels in group A was statistically more significant when compared to group B which was reflected by lower p-values in group A than group B. The statistical results conclude that the reduction in inflammatory markers and blood sugar levels were significantly higher in group A patients receiving rectal O3 therapy along with standard management protocol compared to group B patients receiving standard management protocol alone. This reduction in levels of inflammatory markers and blood glucose levels can be directly attributed to anti-inflammatory and anti-oxidant effects of rectal ozone therapy[11'12'15'16].

Our findings on anti-inflammatory and anti-oxidant effects of ozone were similar with to those of studies by Brown Lee et al (2001) (2005).Brown Lee et al concluded, an increased oxidative stress and oxidative destructive process resulting in cellular damage cause dysregulated blood glucose levels[9'15'16].A well-established regimen in managing type 2 Diabetes is modulation of glucose levels by low fat diet, life style changes like reduced smoking, regular physical activity, anti-hyperglycemic drugs orally and Insulin. Alternatively, antioxidant therapy can reduce the oxidative stress associated with diabetes and can also lower the incidence of diabetic foot with better glycemic control[10'17]. Anti-oxidant therapy will further improve and prevent occurrence of neuropathy, vasculopathy[14'17]. Antiviral, anti-inflammatory, anti-oxidant, cytoprotective and glycemic controlling properties of ozone should have contributed to reduced inflammatory markers and reduced blood glucose levels in patients receiving rectal ozone[9-12'18-21]. In our study, we observed patients receiving rectal ozone therapy had lesser mean CRP, D-dimer, LDH and RBS levels on Day 7 compared to Day 1. The mean RBS levels in Group A patients receiving ozone therapy had significantly reduced at different time intervals of the day by day 7 as compared to day 1. Also, the mean RBS levels in Ozone Group A were significantly lesser on day 7 as compared to Group B non- ozone Group of patients. Our findings in this study were consistent with the study conducted by Martínez-Sánchez G et al[10]. The anti-inflammatory and anti-oxidant effects of rectal ozone not only reduced inflammatory markers and blood sugar levels but also significantly accelerated overall recovery further reducing the number of days in ICU in this subset of patients. We have followed up all the patients involved in the study for a long time, we could not find any ozone or procedure related complications. Nil adverse effects were observed in any of the patients during our study making it a very safe and reliable modality of treatment in covid-19 pneumonia.

CONCLUSION

With this preliminary study conducted in 10 patients we conclude, reduced inflammatory markers level and better glycemic control in patients with moderate and severe COVID pneumonia receiving rectal ozone therapy is because of its multimodal effects. The use of 100ml rectal ozone therapy at 35µg/ml is very safe, effective, economic and also very much reliable in reducing inflammation and blood sugar levels. It is a simple alternative and adjunctive therapeutic modality in management of moderate and severe COVID-19 pneumonia patients with negligible side effects. Rectal ozone therapy is a method capable of protecting antioxidant systems and also to control blood sugar levels in COVID-19 pneumonia patients thereby attaining better glycemic control. However, further studies and trials have to be conducted to know the advantages and disadvantages of various routes of administration, concentration of ozone therapy and its side effects.

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