



Role Of Blood Glutathione (GSH) In Post-COVID-19 Health Consequences.

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Abstract:

From the time when the onset of the global pandemic in early 2020, coronavirus disease in the year 2019 (COVID-19) has posed a mass challenge to health care systems all over the world. In order to fight from these challenges and to develop appropriate therapeutic strategies, it becomes of principal importance to explain the pathophysiology of this illness. Many researchers have concluded one key pathologic component associated with infection severity and course, termed as an immunothrombosis. This appeared as a dominant pathological process impairing morbidity and mortality in acute- and long-COVID-19 infections after 2 years of Covid-19 exposure. The hypercoagulable state is due in part to immune system disturb regulation, inflammation and cellular damage, as well as a reduction in defence systems. One defence mechanism in particular is glutathione (GSH), a ubiquitously found antioxidant. GSH is a vital antioxidant that naturally produce in our body and works to limit the levels of dangerous reactive oxygen species (ROS). The group of adverse effects caused by virus as a result of GSH depletion in states like COVID-19 suggest that GSH reduction is a dominant mechanism of immunothrombosis. It is quite difficult to compare these health issues as side effects of vaccination or it is due to Covid-19 infection, because large proportion of vaccinated individuals with double dose and booster dose are facing different health issues after two years of Covid-19 exposure as per our publication (Sarfaraz Hanfi, *et al.*, 2024). The group of adverse health effects as a result of GSH depletion in conditions like COVID-19 suggest that GSH reduction is a dominant mechanism of immunothrombosis and other related health issues. We aim to observe and conclude the fluctuation in GSH level of post COVID-19 survivors with compare to Non Covid-19 individual's and the level of GSH towards

Covid-19 vaccinated and Non-vaccinated subjects. GSH is most important antioxidant that naturally produce in our body it works to limit the levels of dangerous reactive oxygen species (ROS). Decreased levels of GSH and elevated levels of ROS have been associated with COVID-19 pathogenesis which shows major health issues in elderly aged as well as young individuals. (Silvagno F, *et al.*, 2020). Proper diet high in antioxidants, physical exercise and routine health check-up are required to ease the risk of many diseases (including Heart disease, Arthritis and some cancers). Dietary antioxidants help to prevent and reduce the damage caused by oxidative stress.

Keywords: GSH (Blood Glutathione), Immunothrombrosis, ROS (Reactive Oxygen Species),

Introduction:

The COVID-19 pandemic has claimed nearby 6.7 million lives as of January 2023 per the World Health Organization data. SARS-CoV-2 is well known as an enveloped positive-sense single-stranded RNA virus spreads by direct, indirect, or close any contact with infected hosts by respiratory droplets and fluids. The main entry route of the virus in human cells was initiate to be via the SARS-CoV-2 spike (S) protein binding to the cell surface receptor angiotensin-converting-enzyme-2 (ACE2) expressed by type II pneumocytes (Bonaventura A, *et al.*, 2021). Binding and entry leads to a cascade of steps resulting in inflammation and ultimately systemic disease (Kumar A, *et al.*, 2021). This interaction of virus with cellular system increases the oxidative stress leads to cellular damage, one of the naturally occurring antioxidant in our body is Glutathione (GSH) is an important antioxidant that naturally works to limit the levels of dangerous reactive oxygen species (ROS). Glutathione (GSH) found in most of the tissues, especially in high concentrations in the liver, and plays a particularly important role in defending the hepatocytes, erythrocytes, and other cells against toxic injury. It is involved in many enzymatic and nonenzymatic reactions. (P.K. Gupta, 2016). Glutathione produced in the cells. It's consist largely by three amino acids: glutamine, glycine, and cysteine. Glutathione levels in the body may be reduced by a number of factors, including poor nutrition, environmental toxins, and stress. Its levels is age dependent it means with increasing age GSH level decreases. Any major inflammation caused by Viruses and other infection may leads to chronic condition and it can increase oxidative stress. These diseases include Rheumatoid arthritis, Celiac disease, and Lupus. Glutathione helps to reduce oxidative stress by either stimulating or reducing the body's immunological response in the right

manner. Autoimmune diseases attack the mitochondria in cells. Glutathione works to protect cell mitochondria by removing free radicals. Glutathione has been demonstrated to have diverse effects on the immune system, either stimulating or inhibiting the immunological response in order to control inflammation caused by Viruses or any other means. The study of interactions between glutathione and the immune system has attracted many investigators since years (Carlo Perricone, *et al.*, 2009).

In present study, A total of 980 volunteer participated, 500 volunteer are Non Covid-19 (Healthy) & 480 volunteer are Covid-19 survivors with a response rate of 89.5%. Age of the population was divided into two age groups 18 to 40 years & 41 to 80 years. After taking approval from IEC, JNCH & RC, Bhopal (98.4/JNCH/RES/26.07.2021). Blood was taken to analyse the level of Blood Glutathione of different study groups. The significance of these findings identify the basic and an additional pathologic component of COVID-19 and also serve to identify novel adjunctive therapies to overcome with long Covid-19 health issues. After confirming the role GSH in the pathophysiology of COVID-19 and immunothrombosis can serve as a scaffold for future therapeutic research.

Material & Methods:

A total of 980 volunteer participated in the study, 500 volunteer are Non Covid-19 & 480 volunteer are Covid-19 survivors with a response rate of 89.5%. Age of the population was divided into two age groups 18 to 40 years & 41 to 80 years. After taking approval from IEC, JNCH & RC, Bhopal (98.4/JNCH/RES/26.07.2021). IEC approved health history questioner was taken with informed consent form was signed by the volunteer. Peripheral blood was taken in heparinised sample tubes to conduct laboratory parameters like Blood Glutathione (GSH). And for Micronucleus assay.

Blood Glutathione Test (GSH):

To estimate the Blood glutathione level of all test groups following procedures are followed; 0.1 ml of the heparinized blood in 5ml (Borosilicate) glass test tubes, 0.4 ml of PBS (pH7.4) (Himedia), 0.2 ml Sodium Azide (Himedia), 0.2 ml EDTA (Qualigens, Thermo Fisher Scientifics), 0.2 ml of Hydrogen peroxide, H₂O₂ (RFCL. Ltd) and 0.2 ml of reduced glutathione (CDH Pvt. Ltd) was added and volume was made upto 2 ml with distilled water. The tubes were incubated at 37°C for 10 minutes in pre warmed water bath (Scintech, i-therm, AI-7782D) and 1 ml of trichloroacetic acid was added to terminate the reaction. The reaction mixture was centrifuged at 3000 rpm for 10 min, only 1 ml of supernatant was taken and 2 ml of disodium hydrogen

phosphate and 1 ml of trichloroacetic acid (Himedia) was added and 1 ml of DTNB (Himedia) was added just prior to the estimation. The absorbance was taken at 410- 420 nm by using (BioTek 800 ELISA reader) against a blank (without blood sample) by using 24 wells ELISA plates (Himedia). The activity was expressed as mg/dl with normal range is 20- 40 mg/dl. (Test code GSH by Portions ©2024 Mayo Foundation for Medical Education and Research).

Observation:

Table No. 01

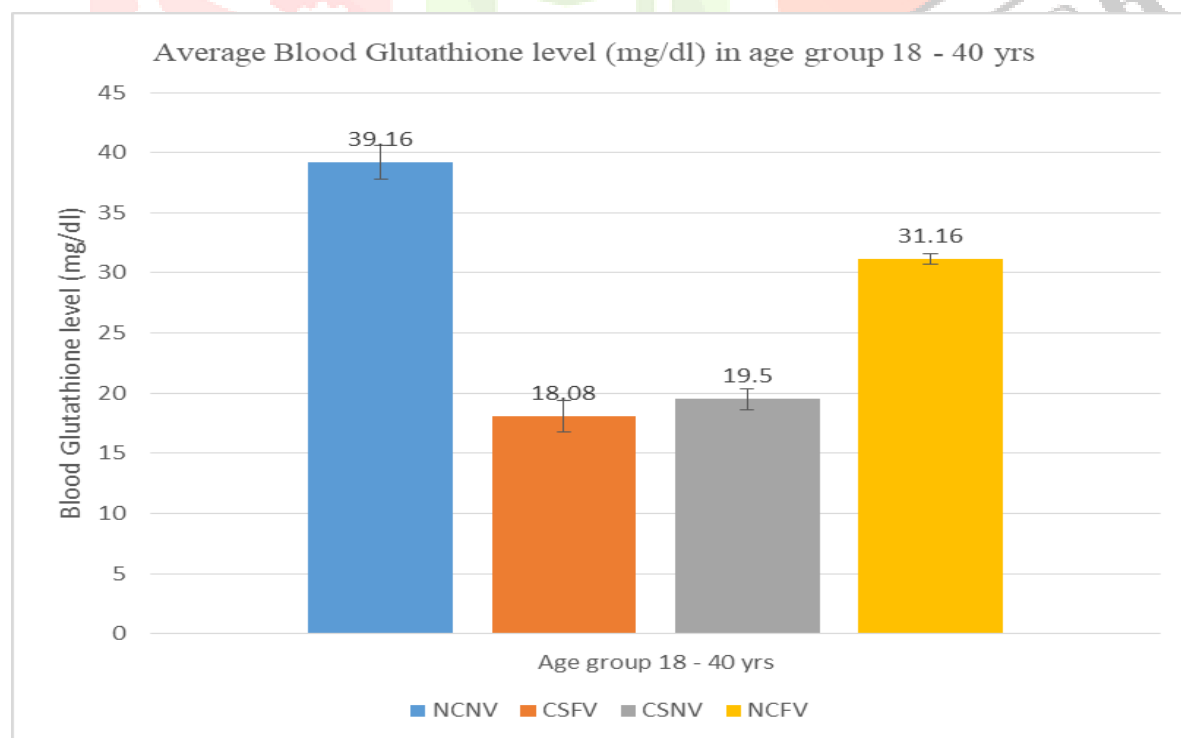
Blood Glutathione average data of registered individuals of different test groups of 18-40 years of age.

STUDY GROUPS	Mean± SE	SD	TOTAL SUBJECTS
No Covid-19/ No Vaccination	39.16 ± 1.39	3.1	250
Covid-19 Survivor/ Vaccinated- I, II & *III dose	18.08 ± 1.34	3.01	85
Covid-19 Survivor/ Non- Vaccinated	19.5 ± 0.86	1.92	73
No Covid-19/ Vaccinated- I, II, *III dose	31.16 ± 0.40	0.89	95

* Only 15% subjects have taken 'III-Booster dose'

Table No.01: Showing Different test groups of (Age: 18-40 Years) with Mean ± SE values, its standard deviation and sample size of 503 registered subjects in the study age group.

Note: Significance is usually denoted by a p-value, or probability value. Statistical significance of above data is P<0.0001.



Graph. No: 01, Showing Blood Glutathione level of different study groups; **NCNV**-No Covid-19 No Vaccination, **CSFV**-Covid-19 survivor fully vaccinated, **CSNV**-Covid-19 survivor Non vaccinated and **NCFV**-Non Covid-19 fully vaccinated. Belongs to 18-40 years age group.

Table No.02

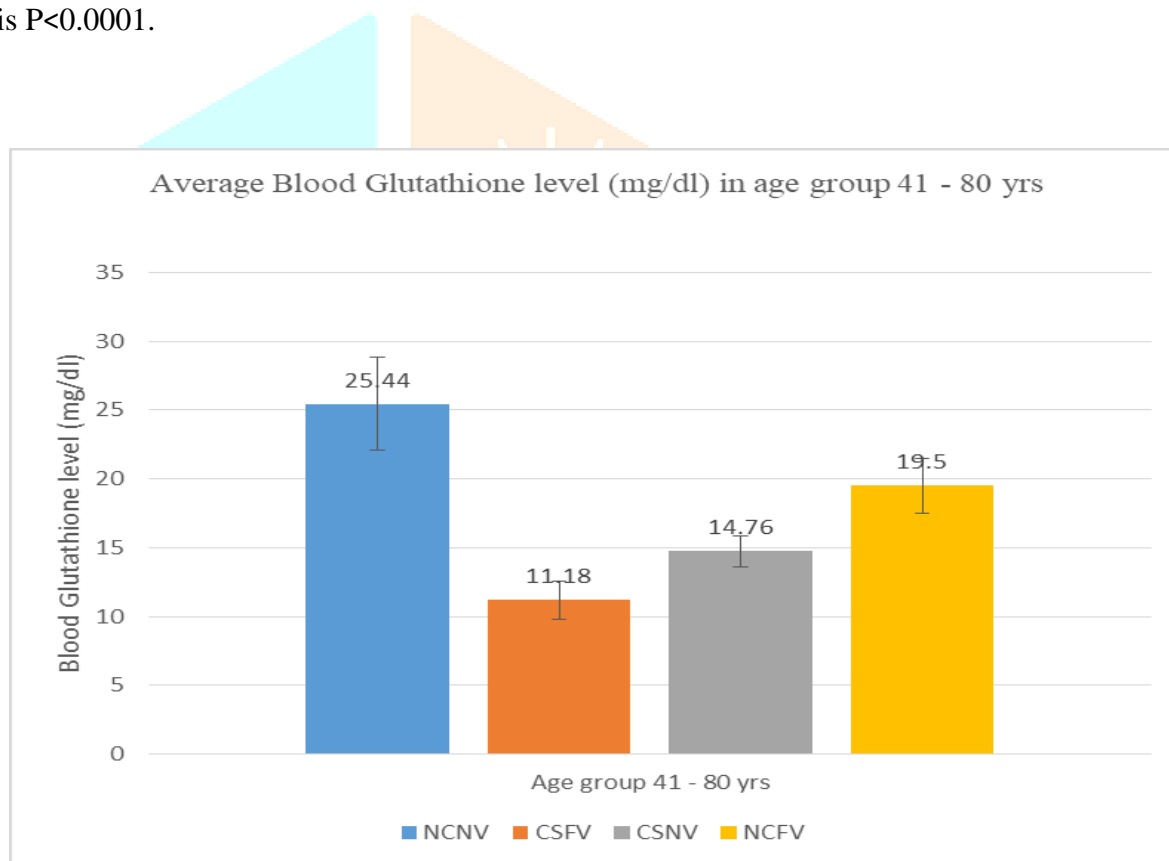
Blood Glutathione average data of registered individuals of different test groups of 41-80 years of age.

STUDY GROUPS	Mean± SE	SD	TOTAL SUBJECTS
No Covid-19/ No Vaccination	25.44 ± 3.39	7.59	250
Covid-19 Survivor/ Vaccinated- I, II, *III-Doses	11.18 ± 1.40	3.14	65
Covid-19 Survivor/ Non- Vaccinated	14.76 ± 1.13	2.54	82
No Covid-19/ Vaccinated- I, II, *III- Doses	19.5 ± 2	4.47	80

*Only 20.4% subjects have taken 'III-Booster dose'

Table No.02: Showing Different test groups of (Age: 41- 80 Years) with Mean ± SE values, its standard deviation and sample size of 477 registered subjects in the study age group.

Note: Significance is usually denoted by a p-value, or probability value. Statistical significance of above data is $P < 0.0001$.



Graph. No: 02, Showing Blood Glutathione level of different study groups; **NCNV**-No Covid-19 No Vaccination, **CSFV**-Covid-19 survivor fully vaccinated, **CSNV**-Covid-19 survivor Non vaccinated and **NCFV**-Non Covid-19 fully vaccinated. Belongs to 1- 80 years age group.

Results:

A total of **980** individual enrolled in the study, **500** are Non Covid-19 and **480** are Covid-19 survivors with (42.3% Females & 57.7% Male with 'p' value of < 0.001 two tailed) out of these **480** individuals **335** are Non-hospitalized (House Isolated) patients (69.8%), and **145** are hospitalized (30.2%) under medical supervision

were included. 64.58% of Covid-19 survivors belongs to age group (18-40 years) and 35.41% belongs to (41-80 years). Age group (41-80 Years) have shown maximum health issues after Covid-19 infection.

Age Group: 41- 80 years:

As per Blood Glutathione level (GSH) the major fall was recorded in Covid-19 survivor with fully vaccinated group (Dose-I, II, III) with $(11.18 \pm 1.40 \text{ mg/dl})$ Mean \pm SE (Graph.No: 02) and same age group belongs to Covid-19 survivor with No-vaccination shows notable downfall $14.76 \pm 1.13 \text{ mg/dl}$ Mean \pm SE of GSH level with compare to No Covid-19 & Non vaccinated (Healthy), this group shows the average GSH level within the normal range $25.44 \pm 3.39 \text{ mg/dl}$ Mean \pm SE (Table No:02).

Age Group: 18- 40 years:

As per Blood Glutathione level (GSH) the major fall was recorded in Covid-19 survivor with fully vaccinated group (Dose-I, II, III) with $(18.08 \pm 1.34 \text{ mg/dl})$ Mean \pm SE (Graph.No: 01) and same age group belongs to Covid-19 survivor with No-vaccination shows notable downfall $19.5 \pm 0.86 \text{ mg/dl}$ (Mean \pm SE) of in GSH level with compare to No Covid-19 & Non vaccinated (Healthy), this group shows the average GSH level within the normal range $39.16 \pm 1.39 \text{ mg/dl}$ (Mean \pm SE) (Table No:01).

As per health consequences of Covid-19 survivors after more than two years the Blood Glutathione level plays a crucial role in the present health status, GSH level is age dependent but the young age group (18-40 years) shows makeable downfall after two years of Covid-19 exposure, Due to low GSH level subjects belongs to this group facing different health issues especially autoimmune problems, Diabetes and other issues published by (Sarfaraz Hanfi, *et al.*, 2024). The study outcome point to that there is an crucial need to explore the pathogenesis of long COVID and develop effective interventions to reduce the risk of long COVID in all age group to overcome form the health consequences facing by Covid-19 survivors .

Conclusion:

In modest terms, an unevenness in free radical production are pointers to increased oxidative stress in the body of Covid-19 survivors due to virus and human cell interaction. Oxidative stress have closely linked with our immune system's our ability to fight from free radicals and deactivate the damage caused by this oxidative stress. Due to Covid-19 exposure the high levels of oxidative stress created due to viral load plays a vital role to become a precursor to several chronic and fatal diseases now a days Covid-19 survivors are facing. These

conditions typically include autoimmune diseases, Hypertension, Cancer, and Diabetes. Antioxidants produced in our body help in neutralize stress and damage, among all Glutathione is more potent than most. Also known as the key antioxidant naturally produced in our body, it brings the ability to warfare and neutralize the full range of free radicals and control the damage caused by viral attack. As per the health concern there is a need routine health check-up, natural antioxidant rich diet management, physical workout and therapeutic agents to combat post-COVID-19 syndromes. Now a majority of researchers also suggest a close link between glutathione deficiency and the progression of different health issues after Covid-19 exposure.

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None.

Conflict of Interest:

None.

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