**Association between Vitamin D3 and Glutathione levels**

**in COVID 19 individual**

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 **Abstract:**

**Background:** COVID-19 is an infectious disease associated with high rate of infected and death specially for older male when they have low levels of glutathione (GSH) and vitamin D (vit D). The GSH status positively associated with bioavailability of vit D. The GSH deficiency correlated by increased oxidative stress and inflammatory markers which implicate in increase the severity of disease. **Objective**: To verify the vitamin D - GSH levels interaction among healthy and COVID- 19 patient. **Method** :Control healthy group (166) individual and (171) COVID 19 patient were involved in this study. Oxidative stress and antioxidant parameters, Vit D, and inflammatory marker were estimated in both group. **Results**: The COVID-19 patient show significant higher level for malondialdehyde (MDA),protein carbonyl group(PC), inturlukin-6 (IL6),Tumor necrosis factor alpha (TNFα) and C-reactive protein (CRP) and significant low level for GSH and vit D compare to healthy control group, the aged and male COVID-19 group display significant higher level for MDA,PC and significant low level for GSH compare with younger and women group. **Conclusion**: The COVID-19 patient correlated with higher oxidative stress , inflammatory marker and low level of antioxidant GSH and Vit D which develops by age advancing and especially within male .

**Key words:** COVID-19 ,Oxidative stress ,Reduced glutathione(GSH) and Vitamin D3

 **Introduction**

The (COVID-19) still to spread throughout different country, influence many people. The disease severity of people varied from mild, moderate to severe critical illness to asymptomatic, (1).The virus enters to body of human through employed its spike protein and attach to human cell receptor (ACE2) ,later begin to replication within lung contributing to difficult of breathing al so death may be occur(2). Rapid growth of COVID-19 resulting in invasive immune system and overcome mechanism of defense and ‘cytokine storm’ will develops (3).

 The infected older age people with COVID-19 has higher rate of harmful illness ,this

 suggesting the implication of age which make the human more sensitive to the environmental stress factors, such as the infection with the corona virus SARSCoV-2 (4).different studies show that the COVID-19 severity was associated with the gender. The women are less risky ,worsen and death comparable to men. And this due to higher GSH in female than in males (5).

The oxidative stress(OxS) is excess reactive- oxygen species and its correlated with different disorder as COVID-19 disease,(6),Under normal physiological state, OxS is balanced by antioxidants system .The reduced glutathione (GSH) consider the major endogenous antioxidant (7,8,9). It has different function as elimination the free radicals and an antiviral property. The level of cellular GSH keeps varying with sex and age (10).

The vit D play a role in regulate many cell pathways which has magic role in antioxidant system (11). Low level of vit D may involve with increments damage of cell caused by the reactive- oxygen species (ROS) (12).Several studies indicated that vit D deficiency associate with increased COVID-19 severity and notice that vit D positively correlate with level of GSH(13,14).

**COVID 19 and Glutathione**

Thecellular deficiency of GSH may results from decrement biosynthesis or increased depletion of GSH lead to development the OxS, immunity dysfunction and viral invasion (15,16,17).Different data confirm that, GSH deficiency become the most acceptable clarification of increase rate of COVID-19 infection by aging. The old age human are more susceptible for damage cause by oxidative stress due to viral infection as a result for decrement in GSH level and this phenomena shown in COVID-19 patients (18,19).So the inflammation process in lung will be exacerbation resulting in increment disease severity (20).

**COVID 19 and Vitamin D**

Vit. D is steroidal hormone (21). Play significant role in increasing cell immunity achieve by prevention cytokine storm through influencing on TNF- α and interferon- γ [(22) and regulating the immunity (23), an important role in inhibiting the replication respiratory viruses (24). Studies suggest that this deficiency can stimulate the Renin- Angiotensin- system , resulted in cardiovascular disease with make lung function to be insufficient. People has like this comorbidities are possess a higher risk of severe COVID-19 (25).

**Glutathione and Vitamin D**

Many recent observation state , higher reduced (GSH) is parallel with excellent level of active vit D (26).Low level of L.cysteine which is consider precursor for reduced GSH was correlate with low vit D level and vit D. binding protein (27). Thus administration the one-cysteine will improve rGSH and consequence up regulation to VDBP expression , 25-hydroxylase, and vitamin D receptor , So VD value will increase and biomarkers of inflammation will be lower (28).

GSH deficiency is association with OxS increments causing alters regulatory genes of Vitamin D lead to the gene expression suppress consequence decreases in Vit D biosynthetic, and the net results leading to secondary vit D deficiency. finally we conclude that ,GSH is vital for control the endogenously vit D biosynthesis and may use as treatment for vit D deficiency (29).

**Method**

**Study design and patient collection**

A total of 337 individual were share in this study ,they were collected from Hospital of Al-Hakeem,166 of them were consider as healthy control group, and the reminder 171 marked as case study group and confirmed with COVID-19 infection.

Once again the case study group(171 patient) was subdivided in to two subgroup ,the first sub group (129 patient) designed considering the age and the second sub group (171 patient) considering gender and the aim to show the influence of both factors on OxS product MDA,PC and antioxidant stress including reduce glutathione (GSH) , Vit D, and inflammatory marker include IL6 , CRP and TNFα were measurement for all participant

**Biostatistical analysis:**

Our data were express as mean ± stander devotion, student's T- test was tested to verify differences among healthy and COVID-19 patient and among the subgroup of COVID-19 individual depend on age and sex for all estimated parameters .Significant differences was accept when P-value less than 0.05.

**Result :-**

After analysis the date the results from table two display significant higher level for MDA (4.51± 1.6,p=0.001) , PC (1.81±0.92 ,p= 0.003)and significant low level for GSH(2.89±0.42,p=0.001) in patient group compare with healthy group, while the data in table three exhibit significant higher level for fasting glucose (141.1±11.32,p=0.001), IL6(8.94±1.21,p=0.001) ,TNF(5.32±1.03 ,p=0.001) and CRP(10.11±2.01,p=0.001) and significant low level of D3(19.71±8.72,p=0.001) in patient in compare to healthy group .In table four the data demonstrate the age influencing on disease , it show significant higher level for MDA(4.02 ± 1.12,p=0.004),PC(2.05±0.02,p=0.028) and low level for vit D (18.01±6.89,p=0.004)and GSH(2.01 ±0.33,p=0.001) in aging group(67±12 year) compare with other group having age of (62±14year).In other hand the gender factor display significant higher level for MDA(4.34± 1.81,p=0.03) ,PC(2.15±0.11,p=0.002) and significant low level for GSH(2.36 ±0.53 ,p=0.003) in male compare to female and failure to show significant differences for D3(21.91±6.96,p=0.51) among two subgroup as in table five.

 **Table 1 :. The demographic properties for study**

|  |  |  |
| --- | --- | --- |
| **COVID-19 N = 171****Case group))** | **Controls N = 166****(Healthy group)**  | **Parameters**  |
| 64 ±14 | 62 ±12 |  Mean age (year) |
|   (%50.88) 87 (%49.12)84  | 87(52.41%)(%47.59)79  |  Gender  Males  Females  |
|  72 (42.11%) 59 (34.5%)5940 (23.39%) |  There is no |  Symptoms  Mild case  moderate case  Severe case  |
|  167 (97.66%)1 (0.59%)3 (1.75%) |  There is no  |  Comorbidities None  Hypertension  Gall stone  |

 **Table 2:- Oxidative stress and Antioxidant value among Healthy and COVID 19 patient**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Healthy****(Control group)****NO =166** | **COVID 19** **(Case group)****NO = 171** | **P-value** |
| BMI (kg/m2) | 23. 8±1.8  | 24.1±2.2  | 0 .17 |
| MDA (mmol/L) |  1.41 ± 0.09 | 4.51± 1.6 | 0.001 |
| PC( nmol /mg protein |  0.722 ± 0.3 | 1.81±0. 92 | 0.003 |
| GSH (mg/gHb)) |  4.01±0.61  |  2.89±0.42 |  0.001 |

 **Table 3:- Biochemical and inflammatory marker for Healthy and patient**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** |  **Healthy****(Control group)**  **NO=166** |  **COVID 19 (Case group)**  **NO= 171** | **P-value** |
| Glucose ( mg/dl) |  96.4±8.31 |  141.1±11.32 | 0.001 |
| 25(OH)Vit D (ng/ml) |  33.42±10.13 |  19.71±8.72 | 0.001 |
| IL6 (pg/ml) |  3.15±0.11 | 8.94±1.21 | 0.001 |
| CRP (mg/L) | 2.97±1.1  | 10.11±2.01 | 0.001 |
| TNFα (pg/ml) |  1.31±0.93  | 5.32±1.03  | 0.001 |

 **Table 4:- Oxidative stress , Antioxidant and Vit D3 value among COVID 19 patient depend on Age**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **COVID 19 patient NO = 64** | **COVID 19 patient NO =65**  | **P-value** |
| Age years  |  62±14 |  67±12 |  0.03  |
| BMI kg/m2 |  24.21±1.5  |  24.62±1.22  | 0.091 |
| MDA (mmol/L) |  3.52 ± 0.81 |  4.02± 1.12 | 0.004 |
| PC. nmol /mg protein |  1.91 ± 0.51 | 2.05±0.02 |  0.028 |
| GSH (mg/gHb) |  2.77±0.54 |  2.01 ±0.33 | 0.001 |
| 25(OH)Vit D (ng/ml) |  21.61±7.11 | 18.01±6.89 | 0.004 |

 **Table 5:- Oxidative stress , Antioxidant and Vit D3 value among COVID 19 patient**

 **depend on gender**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **COVID 19 patient Female = NO = 84** | **COVID 19 patient Male = NO =87**  | **P-value** |
| Age years  | 65±10 |  66±13 |  0.57 |
| BMI kg/m2 |  24.32±1.6  |  23.92±1.17  |  0.06 |
| MDA (mmol/L) |  3.88 ± 0.81 |  4.34± 1.81 |  0.03 |
| PC. nmol /mg protein |  1.91 ± 0.71 | 2.15±0.11 |  0.002 |
| GSH (mg/gHb) |  2.61±0.54 |  2.36 ±0.53  |  0.003 |
| 25(OH)Vit D (ng/ml) |  22.68±8.33 | 21.91±6.96 |  0.51  |

 **Discussion**

This pandemic is highly spread, appear new generation as alpha, sigma lack of knowledge, absent the information and absent efficient treatment (30).Therefore, it is required to identify certain factors that interact with mechanisms of pathogenicity of this virus to lowering time of hospitalization and mortality rate. The oxidative stress is correlated with disease severity of the specially when there is a decrement in antioxidant level such as GSH, Ascorbic acid, vit D and other. Oxidative stress association with different disease and certain infections as COVID-19 (31).

Different evidence reported that the GSH dearth is most caustic agent for harmful demonstration in addition to the death due to this virus (32). The GSH has antiviral property through inhibit viral replication and this activity lead to prevent increased the massive inflammatory markers liberation into the lung (33). Al so GSH decrement the activity of ACE, reduction ROS synthesis so, GSH keep cytokine storm will be under control (34).

From other hand different studies show prevention role of vit D the against SARS-CoV2- infection(35,36,37).Meltzer et al. during her study, He concluded that there is duplicated

In rate of infective with COVID.19 in human with vit D deficiency (38).So analyze role of vit D in keeping the redox- status of cell become essential, the vit D demonstrate significant rate of reduction in infected cells number lower levels of proinflammatory markers (39,40,41).

The interaction of GSH and Vit D deficiency and overproduction the ROS with pathogenicity of this virus make us to measure intracellular GSH concentrations ,oxidative stress parameter and Vit D in those individuals (42).

Our data in table one exhibit a significant higher level for MDA , PC and significant low level for GSH in case group in compare to healthy group it's not surprising that ,the depletion of GSH able to increasing OxS and more carbonylation of proteins resulting PC (43,44,45,46) and the abnormal production for free radicals will cause lipid cell membranes destroys with MDA formation. It has been show the possible explanation for severity and complication of COVID-19 is GSH deficit (47) ,Different studies were compatible with our output data (48),Karkhanei et al.(49)Muhammad et al.(50). The researchers found elevated levels of oxidative stress and reduced antioxidant status GSH in the patient group (51,52).

The data in table three demonstrate significant higher level for TNF-α, IL-6, , CRP and in patient group compare to healthy group . In COVID‐19 patients the depletion GSH increase OS resulting in increased (TNF-α) and (IL-6) (53,54).

Al so the result show significant higher level for D3 associated with low level of inflammatory marker and oxidative stress in control group in compares to case group, and this explain the role of vit D in down regulate the synthesis these markers and it has role in reducing oxidative stress. (55,56,57). Studies show that the GSH stimulate regulation of vit D gene and elevated concentrations of vit D with in the cell.From other hand vit D influence on the biosynthesis of GSH through increase cellular glutathione formation.(58,59).So there is positive inter action among D3 and GSH in lowering the severity of COVID 19(60).

**COVID-19 and Age**

The age, comorbidities, smoking, and dietary are considers risk agent for COVID-19 infection(61).The age implicated in making such aged-subject highly exposure to stressing factors of environment as infectious virus such SARS-CoV-2.In addition, aging involve in normal immune responses dysfunction and induced dysregulation pathways of inflammation (62).The deterioration of redox homeostasis and oxs come out to be critical biological processes that could account for enhancing human susceptibility to disease in elderly patient (63).The possible causes is depletion of GSH which associated with age advancing.

Our observation are interesting because they exhibit significant GSH deficiency in aged group compare with other group of patient .Our study was agreement with different studies which have reported in (64,65,66).From this point the Age, may considers a factor involve in pathogenesis COVID-19 (67).

Know we discuss the level of Vit D in aged patient and its association with disease severity

The data exhibits significant low level for vit Din aging group (≥67 years compare with other group. Many studies indicated that the aged pupulation are at higher risk towards COVID-19 infection (68,69).Our data agreement with other studies which decided ,the COVID-19 incidence is significantly greater in older patient Specially with vit D deficiency (70,71,72)

The less exposure to sunlight, absence of appetite ,reduction absorption of vit D and other are commonest in old age patient and resulting in deficit of it. Our data indicate significant low level of vit D , GSH in association with significant higher level for oxidative stress(MDA and PC) in aging group. Oxidative stress are neutralize with (GSH) is the most intracellular antioxidant .Older age individual with COVID-19, is high risk of elevated OxS combined with GSH deficiency and vit D deficiency (73,74).

 Recently vit D plays significant role in lowering the oxs by activated of many antioxidant cascade and the block certain pathways which make ROS-activating .So there is sincere interrelationship betwixt oxidative stress , vit D and GSH level specially in old age(75).

 **COVID-19 and gender**

The sex associated COVID-19 infection will disuse in our study as one of the commonest caustic agent responsible for disease .It notice that, the male is more significantly to suffer from COVID-19 infection compare to women (76). In addition, our data show significant lower plasma levels of (GSH) which associated with lower but non-significant vit D level in men in relation to women.(77).So men more susceptible to oxs and inflammation (78 )which observed through significant elevation the MDA (4.34± 1.81,p=0.03) and, PC (2.15±0.11,p= 0.002 )level in man compare to data MDA( 3.88 ± 0.81,p=0.03),( PC 1.91 ± 0.71,p=0.002 )in women.

Several studies was exhibit lower value for GSH in man due to rapid utilization of it compare to woman and the reasons may be due to implicate testosterone hormone in male in exacerbation free radicals and stimulation peroxidation of lipid (79). From other hand it was found the Estrogens female hormone could inhibition synthesis ROS so women has less probability to depilation of GSH (80).

From above data and ample evidence we hypothesis that vit D supplementation and glutathione or its precursor advise to use decrement COVID-19 severity (81).

 **Limitation**

 Small sample size ,the data obtain from only one hospital ,the recommendation for future study should consider these limitation by increase the sample size and measure other antioxidant  **Conclusion:**

The COVID 19 patient display low vitamin D level associated with low level of GSH resulting from increment oxidative stress that implicated in lung injury and reflected the severity of disease .

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