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Estimation of inflammatory marker levels associated with Pfizer/BioNTech vaccinated subjects from Baghdad hospital

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Abstract: The most hopeful chance to stop the global COVID-19 pandemic has come from the introduction of COVID-19 vaccinations. The COVID-19 vaccines, however, have been connected to severe side effects and increased blood indicators. This is a prospective study investigating the impact of different pfizer vaccine on inflammation markers (IL-1b and IL-10) along with blood markers among the vaccinated and controls (no vaccination). The study included 70 vaccinated individuals and 25 non-vaccinated healthy controls. Blood glucose levels, Triglycerides, HDL, LDL, WBC count, Lymphocyte count, haemoglobin, platelet count, cholesterol and D-dimers were estimated and measured using the Cobas c 111® clinical chemistry automated system. The main findings where that vaccinated individuals (both doses) enhanced inflammation and platelet activation. We found a strong correlation between vaccination and both IL-1β and IL-10 ($p<0.01$). Spearman rank correlation analysis showed that IL-1β was directly proportional to doses within the plasma ($r = 0.55132$, $P < 0.001$). We also found IL-10 levels to be elevated with vaccination in the serum in all the subjects ($p<0.01$). However there is no significant comparison seen between the male and female subjects ($p<0.01$). In the event that vaccinated experience a more evident and severe COVID-19 clinical course, this may help to ensure the best possible vaccine response.

Key words: COVID19, Vaccination, Interleukins, Blood markers.

Introduction: The World Health Organisation (WHO) classified coronavirus disease 2019 (COVID-19) as a pandemic in March 2020. More than 163 million COVID-19 cases are seen. Worldwide, the sickness has claimed the lives of more than 3.3 million individuals. Inflammation is a prominent aspect of COVID-19, as it is with many other disorders [Wong RSY, 2021]. Overwhelming inflammation can have negative effects or even be fatal. Worldwide teams of researchers are always looking for new treatment strategies to lower or

manage inflammation in COVID-19. In COVID-19, there is growing evidence linking immune response to disease development, and abnormalities in immune cells, inflammatory markers, and the cytokine storm have all been linked to disease severity and prognosis. Additionally, patients with severe disease have been linked to cytokine storm, hyperinflammation, and multi-organ failure [Wong RSY, 2021].

Although most infected people are asymptomatic or only exhibit mild to moderate symptoms, a tiny proportion of them have severe to life-threatening illness. [Wong RSY, 2020]. This is due to the fact that the overactive immune reaction has more negative effects than the infection itself, which causes severe organ damage that cannot be repaired. A cytokine storm has been connected to the excessive immunological response in COVID-19 [Soy M, 2020]. Cytokines are typically tiny proteins that cells, particularly immune cells, release to control the immune system's response to infections or inflammation. A wide variety of chemicals are included in them, such as chemokines (which are involved in chemotactic processes), lymphokines (cytokines released by lymphocytes), interleukins (IL) (cytokines released by white blood cells that affect other white blood cells), and monokines (cytokines released by monocytes). Interferons (IFN) and tumour necrosis factors (TNF) are further types of cytokines. Cytokines can affect cells in close proximity to the site of secretion (autocrine impact), cells nearby (paracrine effect), or cells far from the site of secretion (endocrine effect). According to Tisoncik JR (2021), while others are anti-inflammatory, certain cytokines are pro-inflammatory.

Patients with severe COVID-19 are more likely to have abnormal immune cell numbers and noticeably elevated proinflammatory markers. According to a study by Qin et al. [Qin C, 2020], Fewer B cells, T cells, and natural killer (NK) cells were seen in COVID-19 patients. The severe group tended to have even fewer of these cells than the non-severe group. The latter, however, had a smaller percentage of basophils, eosinophils, and monocytes and a higher neutrophil-lymphocyte ratio (NLR), leukocyte, and neutrophil count.

Both the helper and suppressor T cells in the T cells were impacted. The severity of COVID-19 cases was shown to be associated with increased levels of a few inflammatory and infection-related indicators, such as tumour necrosis factor (TNF), procalcitonin, C-reactive protein (CRP), serum ferritin, interleukin (IL)-2R, IL-6, and IL-8 [Qin C, 2020]. Tan & al. showed that severe COVID-19 patients often had lower levels of B cells, NK cells, and T

cells (both CD4+ and CD8+ cells), as well as more dramatic levels of IL-6, IL-10, and CRP. These findings were consistent with those of Tan et al.'s other study. [Tan M, 2020].

As of August 13, 2021, there are 138 vaccine candidates in development, of which 21 have received global approval for emergency use [Dan JM, 2021]. Their mode of action depends on the immune response to essential components of the virus (DNA, RNA or proteins). The primary antigenic feature with which the viral particle initiates the pathogenesis is the spike proteins (S proteins) present on the surface of SARS-CoV-2 [Dan JM, 2021]. The majority of candidate vaccines have been developed and have been used for immunization since August 13, 2021 and they include whole virus (live attenuated and inactivated), vector viruses (replicating and non-replicating), protein subunits, nucleic acids (DNA and RNA), and virus seed vaccines (VLP). There are at least 21 distinct vaccines that have been authorised for use in emergencies globally through August 13, 2021. The following vaccinations are included in this list: BNT162b2, mRNA-1273, Ad26.COV2.S, ChAdOx1 nCoV-19, Gam-COVID-Vac, BBIBP-CorV, BBV152, Ad5-nCoV, CoronaVac, ZF2001, Sinopharm-Whuan, EpiVacCorona, CoviVac, Abdala/CIGB-66, QazCovid-in, K [Uthaya Kumar A, 2019].

The World Health Organisation (WHO) recommended six immunisations (BNT162b2, mRNA-1273, Ad26.COV2.S, ChAdOx1 nCoV-19, CoronaVac, and BBIBP-CorV) for SARS-CoV-2 but the CDC and FDA only acknowledged three vaccines (BNT162b2, mRNA-1273, and Ad26.COV2.S) for emergency use. The COVID-19 vaccine race has come a long way, and on August 23, 2021, the FDA fully approved the BNT162b2 vaccine [Tanriover MD, 2021]. In patients with COVID-19 disease, IL-10 has recently been identified as an important biomarker of disease severity and mortality (Neumann J, 2020). Early expression of IL-10 may have immunosuppressive or anti-inflammatory effects, reducing the over-inflammatory properties of SARS-CoV-2 infection. However, it has been documented to attenuate T-cell-mediated immune responses and even attenuate them in peripheral blood when released by regulatory T cells in patients with COVID-19. -19 is severe (Han H, 2020). Thus, in the present situation of immunized individuals with a previous infection and elevated basal IL-10 levels, this cytokine may have the potential to decrease the cell-mediated immune response. T cells to vaccination while polarizing it to a significant B-cell-mediated response.

The overproduction of pro-inflammatory cytokines as a result of excessive immune cell activation (also known as "cytokine storm") is a prevalent symptom and the likely cause of

death in patients with severe cases of coronavirus disease 2019 (COVID-19; caused by the SARS-CoV-2 virus). (Tang Y, 2020). Multiple studies suggest that high levels of the anti-inflammatory cytokine IL-10 predict poor outcomes in COVID-19 patients and that this cytokine may be a distinctive marker of hyperinflammation following severe SARS-CoV-2 infection (Chan JF, 2013).

The rapid increase in IL-10 could be viewed as an effort to control tissue damage and hyperinflammation given its well-established roles as an anti-inflammatory and immunosuppressive cytokine. But given that IL-10 levels are rising at the same time as several pro-inflammatory cytokines and that there is a correlation between high IL-10 levels and disease severity. It is also possible that IL-10 is either failing to suppress inflammation as it should as has been seen in other inflammatory conditions (Moore KW, 2001) or acting in a manner that is inconsistent with its traditional function as an anti-inflammatory molecule. In fact, IL-10's capacity to serve as a pro-inflammatory and immunostimulatory molecule under specific circumstances is one reason for the seemingly paradoxical discovery of concurrently high IL-10 and pro-inflammatory cytokine levels (Neidhart M, 2005).

Multisystem inflammatory syndrome, which is quite frequent, is brought on by a variety of environmental, nutritional, and lifestyle variables. Due to the induction following infection, inflammatory marker research has recently gained significant interest with the introduction of COVID-19. Numerous reports also mentioned severe inflammation following infection and after receiving the SARS-CoV-2 vaccine [Xia S, 2021]. In this study, we intend to examine how the COVID vaccine affects the expression of inflammatory markers. We hypothesized vaccination aid in the enhancement of inflammatory markers like IL-1 and IL-10 [H_0: $\mu_1 = \mu_0$ ($p<0.05$)].

Methods

Study population: About 95 individuals (n=95) were included in the study. Patients who received double vaccination and booster dose (subjects) were studied from December 2022 to April 2023 and study was conducted in, the medical City - Baghdad hospital /Iraq. Demographics were collected from the subjects and with informed consent. Evaluation of inflammatory biomarkers like Il-1 and Il-10 were estimated from the subjects post

vaccination. Subjects who were recorded with no vaccination were considered as control group (n=25).

Inclusion criteria: Patients who received double and booster dose were selected randomly for the study, and history was collected through a questionaire and with informed consent.

Exclusion criteria: Patients who received only one dose of vaccination were not included in the study. During our study, we didn't encounter any such subject.

Sample collection: The blood sample was taken from the anterior axillary vein on an empty stomach. Biochemical tests and blood routine tests were detected by an automatic biochemical analyzer in a fasting condition. Two hours after the blood sample was collected. Test methods and procedures are unified laboratory standard protocols and guidelines. The collected blood sample was made into 2 aliquotes. First aliquot was used to prepare serum and used for the blood markers estimation. A fraction of the serum was stored in the freezer and used for IL-1b and IL-10 assays.

Biochemical assays: Blood glucose level (Normal Range: less than 100mg/dL) was estimated using Folin Wu method. Tiglycerides (Normal Range: less than 150mg/dL), HDL (Normal Range: 35-80mg/dl), LDL (Normal Range: less than 100mg/dL), WBC count (normal range: 4,500 to 11,000/ml), Lymphocyte count (normal range: 1000-4800/ml), hemoglobin (Normal range: 12 to 18g/dl), platelet count (150,000 to 450,000 /ml) was estimated and measured using the Cobas c 111® clinical chemistry automated system. Samples were processed as per the manufacturers instructions.Cholesterol levels were quantified in the blood serum with UV/Vis spectrophotometer (1800 Shimadzu). (Normal range: 130 - 250 mg/dL).

Estimation of Il-1β: IL-1β (Cat No: SEA563Hu, Cloud-Clone Corp, Iraq) levels in serum was estimated with ELISA test kit. Protocol was followed as mentioned in the instructions manual. Concentration of test sample was estimated using standard graph drawn with Standard IL-1β ($y=1.0892x2 + 1.4232x$; $R^2= 0.9972$). In brief, plates were impregnated with 50µl aliquots of 100 fold diluted serum to the respective wells and incubated at room temperature (20–25^{0}C) for 2hr. the plates were then incubated after adding 50µl aliquots of IL-1β standard (5ng/ml to 100ng/ml) in their labelled wells. Following washing, 50µl of biotin-conjugated anti IL-1β was added to each well and kept on shaker for 1hr. About 50µl of the HRP-conjugated avidin solution was then added and again incubated at room temperature for 30min. Following washing, 50µl of chromogenic substrate was added and

incubated on shaker for 20 min till the development of color. Absorbance was recorded at 450nm using a SpectraFluor-Plus (Tecan, USA) plate reader (Mardi A, 2021).

Estimation of IL-10:

IL-10 (cat No: SEA056Hu, Cloud-Clone Corp, Iraq) levels in serum was estimated with ELISA test kit. Protocol was followed as mentioned in the instructions manual. Concentration of test sample was estimated using standard graph drawn with Standard IL-10 ($y=1.1785X^2$ + 1.3713x; R^2= 0.9912).

In brief, plates were impregnated with 50µl aliquots of 100 fold diluted serum to the respective wells and incubated at room temperature (20–25^{0}C) for 2hr. the plates were then incubated after adding 50µl aliquots of IL-10 standard (5ng/ml to 100ng/ml) in their labelled wells. Following washing, 50µl of biotin-conjugated anti IL-10 was added to each well and kept on shaker for 1hr. About 50µl of the HRP-conjugated avidin solution was then added and again incubated at room temperature for 30min. Following washing, 50µl of chromogenic substrate was added and incubated on shaker for 20 min till the development of color. Absorbance was recorded at 450nm using a SpectraFluor-Plus (Tecan, USA) plate reader (Makoto Kinoshita, 2004).

Statistics: Interquartile ranges (IQR) are used to depict demographic, biomarker, and aggregate data. Individuals who had received their second and third doses of vaccination as well as unvaccinated controls were compared using median values and interquartile range. For all analyses and graphics, the faculty version of IBM SPSS statistics was utilised. P-values of less than 0.05 and P-values of less than 0.01 when necessary were deemed significant.

Results:

The median age of the subjects was found to be 27, 44 and 25.5 years for 2nd, 3rd and control group respectively. The median weight of the subjects was found to be 77, 82 and 79kg for 2nd, 3rd and control group respectively. The median length of the subjects was found to be 167, 172 and 170.5cm for 2nd, 3rd and control group respectively.

Table 1: Table showing the Media (Inter quartle range; IQR) of the demographics of the subjects collected through questionnaire (n=95).

	age	Weight	length
2nd Dose	27 (24-41)	77 (66-87)	167 (162-174.25)
3rd Dose	44 (29.5-53.75)	82 (78.5-92)	172 (167.5-179)
Control	25.5 (19.75-37)	79 (69.75-83)	170.5 (164.5-176.25)

Table 2: Table showing the mean of the demographics parameters of the subjects (n=95).

	Spo2	**Heartrate**	**Weight**	**length**
2 dose	97.07 ± 1.751	90.32±11.799	78.61±19.371	164.95±17.450
3 dose	96.83± 1.465	96.17±13.27	86.67±13.248	170.83±13.338
control	97.60±1.500	86.10±9.552	78.40±11.193	169.50±8.223

Blood glucose levels: From the data we could find an elevation in the subjects who received the vaccination (2 and 3rd dose) than the control ($p<0.01$). The elevation was found to be more in case of 3rd dose than the 2nd dose ($P<0.01$). The blood glucose level was found to be 135.500 ± 70.74 and 114.702 ±72.926 after 3rd and 2nd dose respectively. Control level was found to be 94.700 ± 14.3494 which is less than the normal range (70-100mg/dl**).** Though the levels seems to be within the normal range, but still alarm rise was seen after the vaccination.

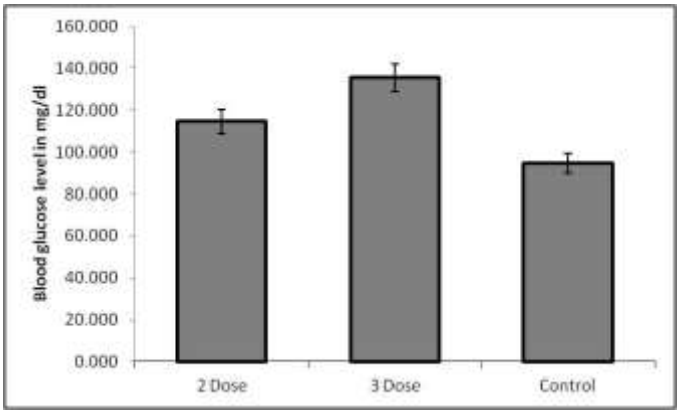

Figure 1: Graph showing the Blood glucose levels in mg/dl of the subjects who are administered with second, third and no dose Covid vaccination. All the values are average of triplicates and expressed as value ± SD.

Cholesterol estimation: From the data we could find an elevation in the subjects who received the vaccination (2 and 3rd dose) than the control ($p<0.01$). The elevation was found to be 209.21 ± 42.82 and 190 ± 67.60 for second and third dose respectively ($p<0.01$). However there was a slight reduction in the level after 3rd dose. Control level was found to be 146 ± 38.24 which is less than the normal range (130 - 250 mg/dl**).** Though the levels seems to be within the normal range, but still alarm rise was seen after the vaccination.

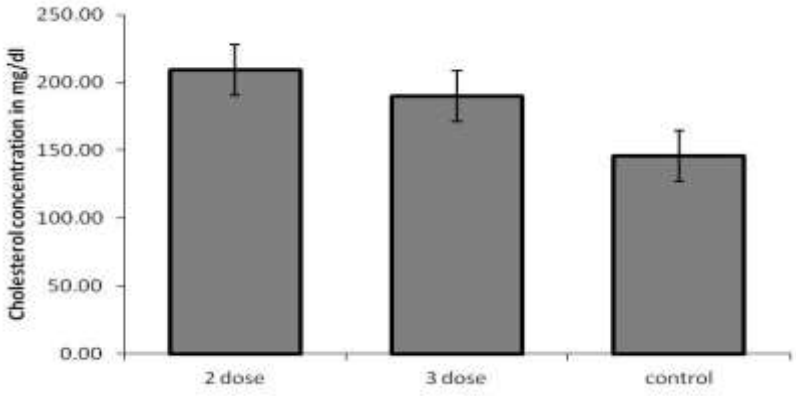

Figure 2: Graph showing the concentration of cholesterol in mg/dl of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

Triglycerides estimation: The mean concentration of the TG of the control was found to be 153.7 ± 71.248 which is slightly more than the normal range (less than 150mg/dl). TG levels of the subjects were found to be 184.3157 ± 59.302 and 192.333 ± 70.368 for 2nd and 3rd dose subjects respectively ($P<0.01$) [Figure 3]. In both the cases the levels seem to be elevated when compared to the control ($p<0.01$).

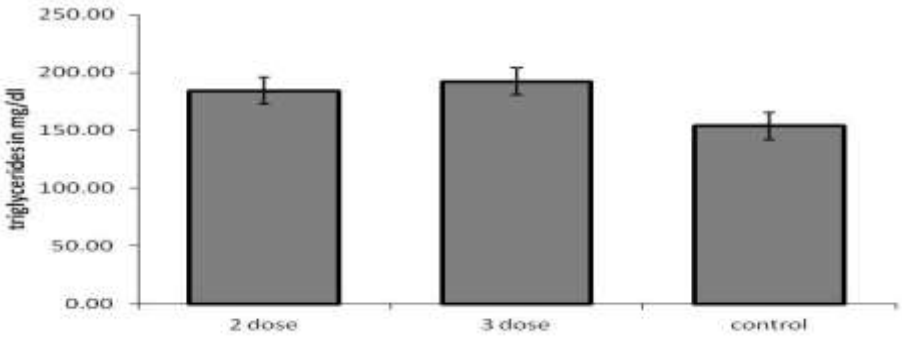

Figure 3: Graph showing the concentration of triglycerides (TG) in mg/dl of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

HDL and LDL levels: Mean concentration of HDL of the subjects after 2nd and 3rd dose was found to be 41.561± 11.8140 and 34.388 ± 8.513 respectively. The level was found to be within the normal range (Normal Range: 35-80mg/dl). On the other hand control value was found to be 38.65 ± 8.1645. However there is no significant comparison seen between the male and female subjects ($p<0.01$). Mean concentration of LDL of the subjects after 2nd and 3rd dose was found to be 129.263 ± 36.380 and 120.5 ± 57.1182 respectively. The level was found to be within the normal range (Normal Range: less than 100mg/dL). On the other hand control value was found to be 78.45 ± 32.652. The LDL levels seem to be elevated post vaccination in both the cases (2nd and 3rd dose) when compared to the control. However there is no significant comparison seen between the male and female subjects ($p<0.01$).

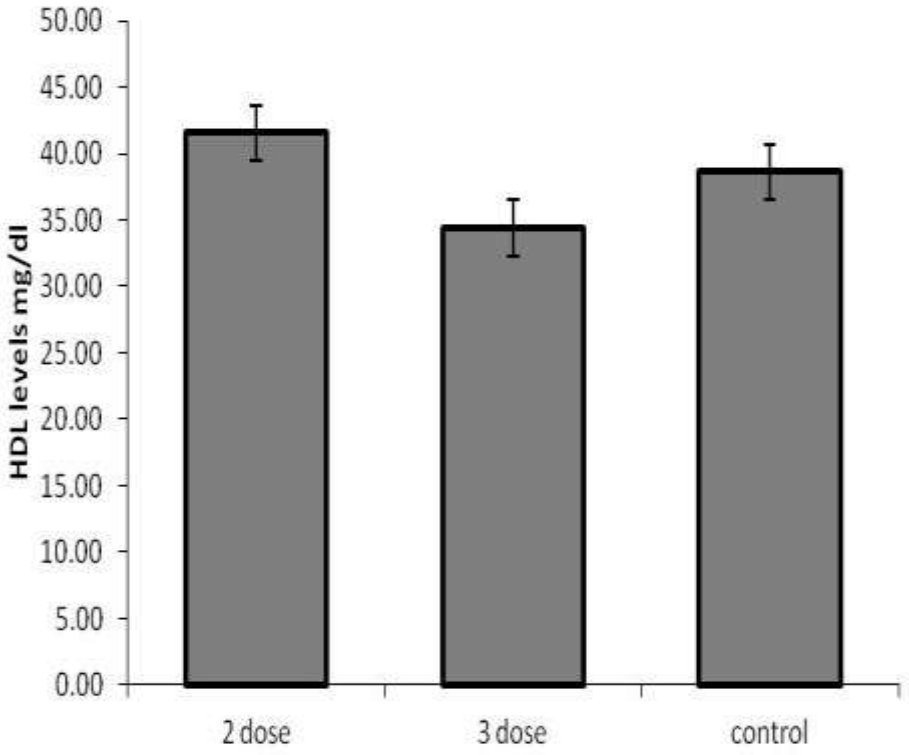

Figure 4: Graph showing the concentration of HDL in mg/dl of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

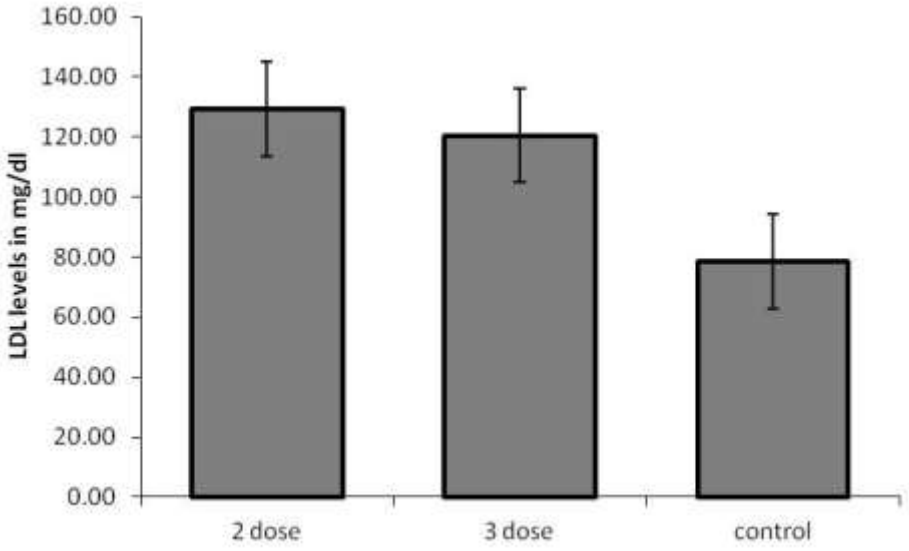

Figure 5: Graph showing the concentration of LDL in mg/dl of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

Estmation of Il-1B from serum samples: Mean concentration of IL-1b of the subjects was found to be 263.40 ± 10.205 which was significantly higher than the control (74.28±2.147). The level seems to abnormally rise after third dose to 626.51±48.628ng/ml which is very high when compared to control ($p<0.01$). However there is no significant comparison seen between the male and female subjects ($p<0.01$). **[**Normal range: 0.5 to 12pg/mL].

Figure 10: Graph showing the standard curve of Il-1b. All the values are average of triplicates. Values are expressed as value ± SD. (R^2 =0.9859).

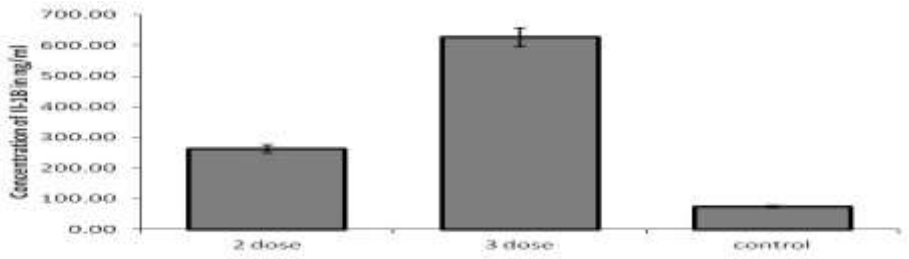

Figure 6: Graph showing the inflammatory marker IL-1b concentration of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

Estimation of Il-10 from serum: Il-1b and IL-10 showed significant difference among the subjects who were administered and not administered with vaccine. IL-10 was abnormally lowered between groups post-vaccination. On the other hand, Il-1B was abnormally elevated in both groups when compared to control ($P<0.01$). On the other hand, mean concentration of Il-10 of the subjects was found to be 211.56 ± 14.365ng/ml which was less than the control (560.79 ± 24.877). Even the value got significantly reduced to 77.97 ± 5.01728ng/ml after receiving 3rd dose of vaccination ($p<0.01$). [Normal range: 0 - 2.8pg/ml].

Figure 7: Graph showing the standard curve of Il-10. All the values are average of triplicates. Values are expressed as value ± SD. ($R^2 = 0.9932$)

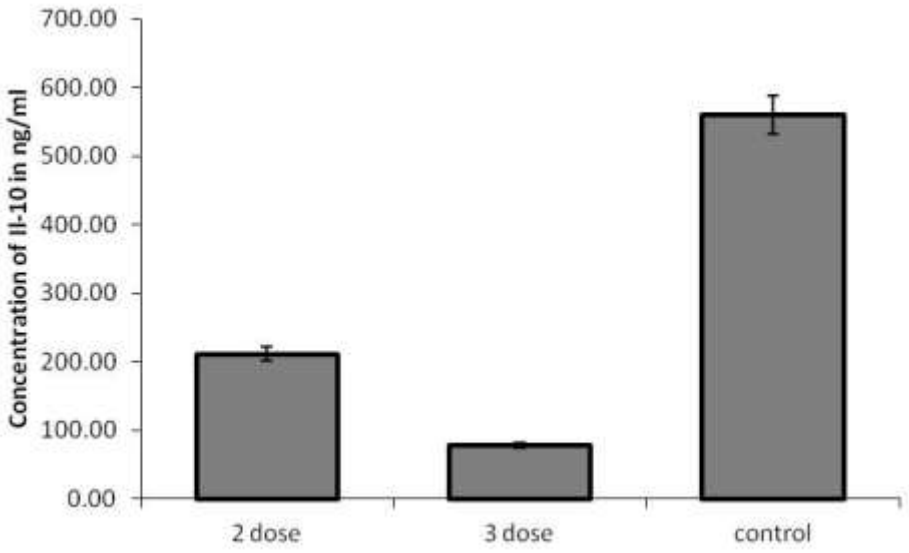

Figure 8: Graph showing the inflammatory marker IL-10 concentration of the subjects who are administered with second, third and no dose covid vaccination. All the values are average of triplicates and expressed as value ± SD.

Estimation of D-Dimer from serum:

Mean concentration of D-dimer of the subjects was found to be elevated significantly after both the doses. The concentration of the dimer was found to be 290.614 ± 358.559 and 300.7 ± 213.495 for 2nd and 3rd dose subjects respectively when compared to control (187.35 ± 76.766) ($p<0.01$) [Table 3]. However there is no significant comparison seen between the male and female subjects ($p<0.01$). **[**Normal range: <500ng/ml].

Platelets: Platelets levels remained higher in the 2rd dose subjects followed by 3rd dose subjects ($p<0.01$). Though the difference between the control and 3rd dose was lower, but there was a significant level rise when compared to control ($p<0.05$). Platelet levels are found to be 274 x 10^3 ± 69 and 254 x 10^3 ± 64 after 2nd and 3rd dose respectively. Level of the control subjects was found to be 258 x 10^3 ± 51 [Table 3]. The normal range of the count was expected to be 150 – 450 x 10^3. Though the levels were found to be in the normal range, but still a flat rise was seen among the subjects.

Lymphocytes: Lymphocytes levels remained a higher in the 3rd dose subjects followed by 2nd dose subjects ($p<0.01$). Though the difference between the control and 3rd dose was lower, but there was a significant level rise when compared to control ($p<0.05$). Lymphocyte count was found to be 30 x 10^2 and 31 x 10^2 after 2nd and 3rd dose respectively. Level of the control subjects was found to be 28 x 10^2. The normal range of the count was expected to be 10 - 48 x 10^2 [Table 3]. Though the levels were found to be in the normal range, but still a flat rise was seen among the subjects.

Table 3: Table showing the levels of D-dimers, lymphocyte and platelet count of the subjects who are not administered with any dose of covid vaccination (control). All the values are average of triplicates and expressed as value ± SD.

	Lymphocytes	**Platelets**	**D-dimer**
2 Dose	30 ± 9	274 x 10^3 ± 69	290.614 ± 358.559
3 Dose	31 ± 7	254 x 10^3 ± 64	300.7 ± 213.495
Control	28 ± 6	258 x 10^3 ± 51	187.35 ± 76.766

Table 4: Table showing the median Interquartile range of the inflammatory markers (IL-1β and IL-10). Comparison between the control and vaccinated individuals are done by Mann-Whitney U test and Z2 values are represented. P-values <0.01 are considered significant.

Group	Unit	2 Dose Mean IQR	3 Dose Mean IQR	Control Mean IQR	2D vs C (Z2 P <0.01)	3D vs C (Z2 P <0.01)
Inflammatory markers						
IL-1β	ng/ml	277.4 (228.5 - 288.4)	600.7 (580.32 - 628.85)	73.55 (69.9 - 77.25)	6.61583 (0.0001)	-5.247

IL-10	ng/ml	201.5 (188.3-239.5)	77.95 (69.675-86.87)	574.9 (517.42-601.65)	-6.6158	5.24773

Correlation between vaccination and Inflammatory markers: IL-1β remained higher in the 3rd dose subjects followed by 2nd dose subjects ($p<0.01$). IL-10 levels remained lower than the control groups. 2nd dose subjects showed more values than 3rd dose. Post-vaccination, differences between the subjects were observed in both IL-1β and IL-10 levels. This clearly states the status of inflammation post vaccination. We found there is a strong correlation between vaccination and both IL-1β and IL-10 ($p<0.01$). Spearman rank correlation analysis showed that IL-1β was directly proportional to doses within the plasma ($r = 0.55132$, $P < 0.001$). We also found IL-10 levels to be elevated with vaccination in the serum in all the subjects ($p<0.01$) [Table 4]. However there is no significant comparison seen between the male and female subjects ($p<0.01$). The ROC analysis results showed that there is a strong correlation between the control and the subjects for both IL-1β and IL-10. For Il-1 (cut-off = 1.512), the sensitivity was 100.0% (AUC=1.00) and for Il-10 (cut-off = 1.007), the sensitivity was 100.0% (AUC=1.00) [Figure 9].

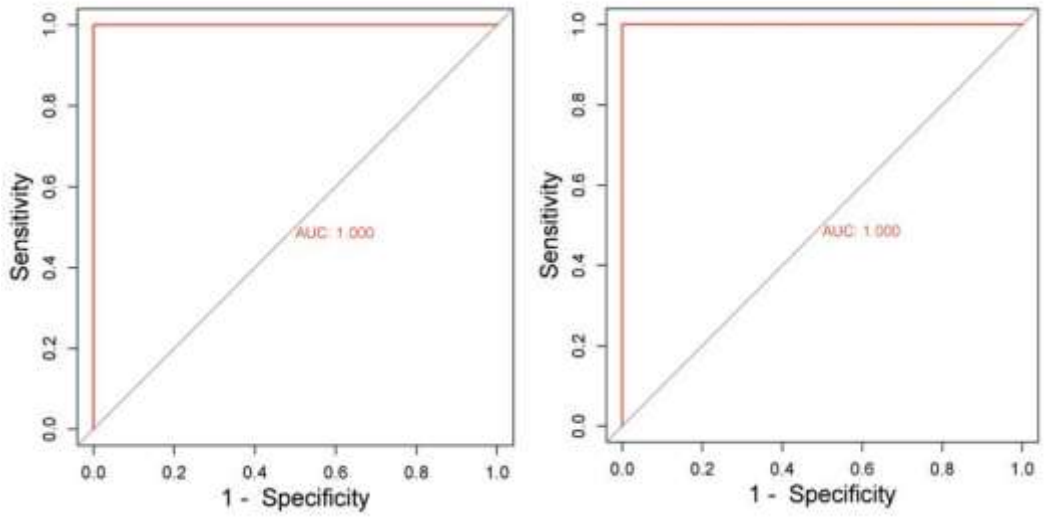

Figure 9: ROC analysis depicting the diagnostic value of the IL-1β and IL-10 to discriminate COVID vaccinated from controls (no vaccination).

Discussion: This study was conducted to find the post effect of the COVID vaccine on the expression of inflammatory markers like IL-1β and IL-10. Alongside blood markers like Glucose, Cholesterol, triglycerides (HDL & LDL), D - dimer and haemoglobin concentration were also studied post vaccination. In addition, lymphocyte, platelet count was also determined to see the after math effects on the immune regulation. The key finding was that post vaccination, interleukin levels were found to be elevated when compared to control group (without vaccination dose).

In this study, about 95 subjects were included. Among them 57 received 2nd dose, 18 of them received 3rd dose and rest 20 of them serves as control. Control groups did not receive any dosage of vaccination (Reason not listed). As of 6 March 2022, including booster doses, more than 10 billion doses of the COVID-19 vaccines had been administered globally. They are believed to be both safe and effective with only minor to moderate side effects (fever, fatigue, headache, muscle ache, chills, diarrhoea, and pain, redness, and swelling at the injection site, among others) (WHO, 2021). Nevertheless, a small number of cases of post-vaccination allergic reactions and other severe adverse events have been documented, including vaccine-induced immune thrombotic thrombocytopenia (VITT) with concurrent mortality, Guillain-Barré Syndrome (GBS), myocarditis, and pericarditis (Varghese E, 2021). The mRNA-based Pfizer-BioNTech COVID-19 vaccine (INN: tozinameran), which is marketed as Comirnaty, was created by the German biotechnology company BioNTech. (Thomas K, 2020).

Maintaining blood glucose levels has been a constant problem for diabetic patients and the doctors who care for them due to the rise in diabetic cases and associated comorbidities. In light of the COVID-19 epidemic, blood glucose control has taken on increased importance. The severity of the condition, the requirement for ICU admissions, and the mortality rates of diabetic COVID-19 patients were all higher than those of nondiabetic COVID-19 patients, according to a number of previously published data [Samuel S.M, 2021]. There have been reports linking SARS-CoV-2/COVID-19 infection in diabetic and non-diabetic patients to acute pancreatitis and subsequent hyperglycemia or new onset of diabetes because of the direct or indirect cellular damage brought on by the viral binding, accumulation, and replication in ACE2 receptor-expressing islets of the pancreas. [Khunti, 2021].

In our study, we investigated the levels of glucose post vaccination to trace the "vaccine-induced" hyperglycemia (ViHG) among the subjects. We found an elevation of the blood glucose in the subjects who received the vaccination (both 2^{nd} and 3^{rd} dose) than the control ($p<0.01$). Control level was found to be 94.700 ± 14.3494 which is less than the normal range (70-100mg/dl**).** Though the levels seems to be within the normal range, but still alarm rise was seen after the vaccination.

There have been many reports of COVID-19 vaccination-induced hyperglycemia (ViHG) and its side effects [Edwards A.E, 2021]. One to six days after getting their first dose of the Covishield (AstraZeneca) vaccine, three diabetic individuals developed postvaccination hyperglycemia [Mishra A, 2021]. It's interesting to note that prior to receiving the COVID-19 immunisation, the documented episode of hospitalisation, and/or ICU treatment, every reported case had a stable glycemic control. Most patients had nocturia, polyuria, and polydipsia, which are normal osmotic symptoms of hyperglycemia; nevertheless, some also had considerable weight loss, confusion, and dizziness (Abu-Rumaileh M.A., 2021). Our results are consistent with these important discoveries, which show that post-vaccination hyperglycemia occurs.

Interleukins, which are cytokines, have a significant impact in balancing the pro-inflammatory response in a variety of viral disorders. Interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-13, IL-19, and IL-35 are important anti-inflammatory interleukins (Cuneo, A. A., 2009). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the newly discovered coronavirus disease 2019 (COVID-19), which is a global public health concern and poses a threat to the lives of millions of people. Older males, especially those with underlying illnesses, appear to be most susceptible to infection by the virus. The primary cause of severe pneumonia that results in acute lung injury, systemic inflammatory response syndrome, acute respiratory distress syndrome, and ultimately multiple organ dysfunction syndromes, as well as death in many cases, is likely the cytokine storm that follows hyperactivated immune responses brought on by SARS-CoV-2 infection. Interleukin (IL)-1 levels were found to be increased after COVID-19 infection, according to several investigations. As a result of unchecked immunological responses brought on by COVID-19 infection, the IL-1 cytokine family also plays a critical part in the production of cytokine storm (Mardi A, 2021).

In addition to lymphocytopenia, patients with severe disease manifestations and poor prognosis have significantly higher levels of several inflammatory biomarkers, such as C-reactive protein (CRP), numerous cytokines (IL-1, IL-4, IL-6, IL-8, IL-17A), monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor (TNF), and interferon gamma (IFN- γ) (Potere N, 2020). Numerous studies have shown that the interaction of the SARS-CoV-2 with the ACE2 receptor causes the NLRP3 inflammasome to be activated, releasing IL-1 β and other inflammatory cytokines that cause broad organ damage, hyperinflammation, and inflammatory cell death **(**Kucia M, 2021).

We found similar results wherein the mean concentration of IL-1β was significantly elevated in both the doses when compared to the control (74.28±2.147). But strikingly, after 3rd dose, the level increased to 626.51±48.628ng/ml which is very high when compared to control ($p<0.01$). This was uniform across the sex of the subjects. Our studies are in accordance to Schultheiß C et al (2022) where they confirmed higher plasma levels of IL-1β, IL-6, and TNF, rather than autoantibodies, are what PASC is linked with in a validation cohort with 333 more subjects and a longer duration since infection of 10 months.

Due to the cytokine storm that might develop during COVID-19, a number of possible therapeutic strategies may include either more focused cytokine inhibitors or non-selective cytokine production inhibition through the use of corticosteroids. Clinicians have lately become interested in IL-1 blocking medicines among targeted cytokine inhibitors because of IL-1's crucial role in coordinating the innate immune system's response to viral infections and tissue damage. A common treatment for a variety of aberrant hyperinflammatory immune response syndromes, such as Still's disease, macrophage activation syndrome, and cytokine release syndrome, is the use of IL-1 blockers. In patients with sepsis and macrophage activation syndrome, IL-1 receptor blockers have also increased survival rates (Curtis JR, 2021).

Since blockers are of great importance, the abnormal rise in the level of IL-1β might pose a serious threat to the tissues and organs post infection. This hyperinflammation might induce excessive tissue damage leading to multiple organ failure. IL-10 was abnormally lowered between groups post-vaccination. We found the mean concentration of Il-10 among the vaccinated subjects to be lowered to as low as 211.56 ± 14.365ng/ml which was very less than the control (560.79 ± 24.877). This level strikingly reduced to 77.97 ± 5.01728ng/ml after receiving 3rd dose of vaccination ($p<0.01$).

The elevated rise of interleukin 10 (IL-10) during the cytokine storm in coronavirus disease 2019 (COVID-19) is a distinctive characteristic. This was believed to be a form of negative feedback to reduce inflammation. However, a number of lines of clinical data imply that a pathogenic function for severe early proinflammatory IL10 rise in the severity of COVID-19. (Ligong Lu, 2021).

In patients with COVID-19 illness, IL-10 has recently been identified as a critical indicator of severity and death (Zhao Y, 2020). Early IL-10 expression may have an immunosuppressive or anti-inflammatory impact, reducing the hyper-inflammation that is characteristic of SARS-CoV-2 infection. However, it has been shown that it might lessen the immunological response mediated by T lymphocytes and even their depletion in peripheral blood when released by regulatory T cells in individuals with severe COVID-19 illness (Diao B, 2019).

Similar to our findings, Ayaat A. *et al* (2023) confirmed the reduction of IL-10 post vaccination with Pfizer-BioNTech Vaccine. They worked with 90 subjects from Al-Iraqia University who received Pfizer-BioNTech vaccine (2^{nd} dose). They reported a very low expression of IL-10 among the subjects post vaccination.

This result might support the claim what Pfizer shouts aloud. They claim their vaccines to be anti-inflammatory and reduces the tissue damage post vaccination, which is not satisfactory with other vaccines. In certain investigations, COVID-19 severe infected people showed a remarkable early elevation of Interleukin-10 along with a cytokine storm, which can be explained as a method of negative feedback to suppress inflammation (Wang F, 2020). Contrarily, a large body of clinical evidence from human investigations suggested that the early, substantial rise in IL-10 following SARS-CoV-2 infection may instead have a negative pathogenic effect as a pro-inflammatory in the severity of COVID-19 (Lu L, 2020). Many other reports also were in support of this low expression of IL-10 post vaccination of Pfizer based vaccine (Baden L R, 2021).

Conclusion: As a result, our study offers significant new knowledge about the immunomodulatory effects of interleukins in COVID-19 on a variety of immune cells (T and NK cells), inflammatory cytokines, chemokines, and growth factors. This knowledge can be used to further assess in vivo the immunomodulatory effects of interleukins on inflammatory diseases like COVID-19. Moreover, these studies, will provide clear insights of how much

safe and effective the vaccines are, when administered on a bulk scale and in emergency. This study not only throws light on the effectiveness of the vaccine, but also on the adverse effects if any, so as to handle the concern with future vaccine strategies. Whether these findings translate to clinical influences for COVID-19 therapy remains to be evaluated by randomized, controlled, clinical trials with a large sample size. Our study showed that IL-10 serum levels significantly dropped down post vaccination. And contrastingly, IL-1β was found to be abnormally elevated post vaccination. Importantly, our findings are in accordance with many recent epidemiologic studies, reporting that COVID 19 vaccination is associated with an increase in inflammatory markers including other blood markers. This study offers significant new insights into the immune-modulating effects of IL-10 and IL-1β in COVID-19 and may offer useful knowledge for future in vivo studies.

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