Possible Expression of Viral seromarkers and inflammatory responses in patients with decreased plasma Superoxide dismutase

Abstract

Study Background: Triggers of immune responses and oxidative stress include viral infections. Expression of viral seromarkers indicate viral infection or immunity while plasma cytokines and Superoxide Dismutase(SOD) are biomarkers of pro/anti-inflammatory responses and anti-oxidative bioactivities respectively. **Aim and Objective:** This work was designed to determine possible expression of viral seromarkers and inflammatory responses in patients with decreased plasma Superoxide dismutase **Materials and methods:** Sixty three patients with decreased SOD, normal blood glucose who were negative to AFB test and Giemsa thick blood film technique for *Plasmodium* were studied as test subjects (n-63 ; Male-33; Female-30; Age- 27 – 71 years). while

individuals with normal SOD, normal blood glucose who were negative to AFB test, Giemsa thick blood film technique for *Plasmodium* and did not express seromarkers of HIV, HBV and HCV were studied as control subjects (n-70 ; Male-35; Female-35; Age- 27 – 71 years). Identification of Plasmodium was carried out by Giemsa thick blood film technique, AFB by Ziehl Neelsen staining technique, blood glucose and SOD by spectrophotometry while viral seromarkers, plasma TNFα and IL-10 were determined in the subjects by ELISA **Results:** The results obtained in this work in patients with decreased plasma Superoxide dismutase showed a frequency of viral seromarkers of 11.1%(7) Anti-HCV; 20.6%(13) Anti-HBe ; 20.6%(13) HBeAg ; 20.6%(13) HBsAg ; 3.2% (2) HIVag(p24)/Ab ; 0%(0) Anti-HCV + HIVAg/Ab ; 3.2% (2) HBsAg + HBeAg + HIVAg/Ab and 20.6%(13) HBeAg + HBsAg + anti-HBe. Seromarker of hepatitis B virus was found to be more prevalent while 3.2% (2) of the patients expressed HBsAg + HBeAg + HIVAg/Ab. The frequency of viral seromarkers of hepatitis B virus were more than the seromarkers of hepatitis C virus and Human Immunodeficiency Virus while seromarkers of both HBV and HCV were more than those of HIV. Only coinfection of HIV and HBV (3.2% (2)) was found in the patients expressed as anti-HBe, HBsAg + HBeAg + HIVAg/Ab. There was also a significant increase in plasma TNFα and a significant decrease in IL-10 in patients who expressed seromarkers of HBV, HCV and HIV which was more pronounced in patients who expressed HIV seromarkers and also in those who co-expressed HIV and HBV seromarkers($p<0.05$). There was a significant decrease in the plasma SOD in patients who expressed Anti-HCV, anti-HBe , HBeAg , HBsAg, HIVAg-Ab, HBsAg+HBeAg+HIV and HBeAg+HBsAg+ anti-HBe compred with the results obtained in the control($p<0.05$).**Conclusion:** Patients with decreased SOD expressed viral seromarkers including significant inflammatory response indicated by an increase in TNFα and decreased IL-10 which generally signify evidence of immune or inflammatory responses as well as evidence of active viral infection.

Keywords : Viral seromarkers, TNFα, IL-10 , Superoxide dismutase

Introduction

Superoxide dismutase is an important antioxidant that protects cell exposed to reactive oxygen from cellular damage. It is an enzyme involved in the dismutation/ partitioning of superoxide ($O2^-$) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2). Superoxide is produced from the metabolism of oxygen and, if not controlled can cause cell damage. Hydrogen peroxide which can also cause cellular damage. It can be degraded by other enzymes such as catalase (McCord and Fridovich, 1988 ; Hayyan *et al.,* 2016).

Superoxide dismutase acts as antioxidant to protect cell damage by converting the reactive oxygen ($O2^-$) species to less damaging species. In other words Superoxide dismutase dismutes

superoxide anion radical (O2−) to O2 and hydrogen peroxide (H2O2) to prevent cell damage(McCord and Fridovich, 1988 ; Hayyan *et al.,* 2016).

Cytokines are substances that play important roles in pro and anti-inflammatory immune responses. They are involved in humoral and cell mediated immune responses (Swardfager *et al.,* 2010). An example of cytokines is Tumor Necrosis factor - alpha (TNF-α) which is a pro-inflammatory cytokine that regulates immune cells (Cannon , 2000.; Locksley *et al.,* 2001).

. This cytokine is an endogenous pyrogen that can bring about fever, apoptotic cell death, cachexia and inflammation. It can also inhibit tumorigenesis and viral replication. Tumor Necrosis factor - alpha (TNF-α) can act on macrophages to stimulate phagocytosis and liver to generate acute phase response(Cannon , 2000.; Locksley *et al.,* 2001).

Another example of cytokines is Interleukin 10 (IL-10) or human cytokine synthesis inhibitory factor (CSIF) (Brennan *et al.,* 2008; Mosser and Zhang, 2008).

.Interleukin 10 (IL-10) is an anti-inflammatory cytokine that can inhibit the synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF. The cytokine (IL-10) regulates the enzyme responsible for the conversion of TNF-α(Brennan *et al.,* 2008; Mosser and Zhang, 2008).

Viral Seromarkers are expressed or characterized in the serum or plasma as a result of viral infection. Viral infection stimulates immune response leading to the production of antibody specific to the invading virus(antigen) [8][9][10] . This immune product can which are always in the serum are detected or characterized as viral seromarkers to indicate evidence of viral infection. They are expressed in the serum or plasma after seroconversion(Prabina *et al.,* 2019; Lee *et al.,* 2010; Olugbenga *et al.,* 2016). Viral seromarkers include anti-HCV for Hepatitis C virus infection, HBsAg for Hepatitis B virus infection, anti-HBe indication HBV infection and clearance of HBeAg; HBeAg indicating replication and active infection of HBV and anti-HIV/P24antigen indicating HIV infection(Prabina *et al.,* 2019; Lee *et al.,* 2010; Olugbenga *et al.,* 2016).

This work was designed to determine viral seromarkers (HIVp24 antigen and antibodies to HIV1/HIV2, anti-HCV, HBsAg, HBeAg, and anti-HBe) and inflammatory responses in patients with decreased plasma Superoxide dismutase.

Materials and Methods

Study area

This work was carried out in Owo, Ondo State –Nigeria. Owo is a major city in Ondo State-Nigeria. Owo was the capital of a Yoruba city-state between 1400 and 1600 AD. Owo hosts Federal Medical Centre, Achievers University, Rufus Giwa Polytechnic, Secondary and Primary Schools. It is the headquarters of Owo local government area in Owo/Ose Federal constituency of Nigeria.

Study Population

Sixty three (63) patients (Male-33; Female-30; Age- 27 – 71 years) with decreased plasma Super Oxide Dismutase (SOD) who were negative to Acid Fast Bacilli and Thick-Giemsa Film blood technique (for the detection of *Plasmodium*) were recruited as test volunteers while Seventy (70) volunteers (Male-35; Female-35; Age- 27 – 71 years) with normal Super Oxide Dismutase (SOD) who were negative to Acid Fast Bacilli and Thick-Giemsa Film blood technique (for the detection of *Plasmodium*) were studied as control subjects.

Inclusion Criteria

1. Volunteers with decreased plasma SOD and normal blood glucose were included as test subjects
2. Volunteers with normal SOD and blood glucose were included as control subjects

Exclusion criteria

1. Test and control subjects who were positive to Acid Fast Bacilli and Thick-Giemsa Film blood technique (for the detection of *Plasmodium*) with abnormal blood glucose level were excluded from the study. Malaria and tuberculosis are more prevalent in the area according to the reports of Dada *et al.*,(2016) that overall prevalence percentage of malaria was found to be high in Ondo State Hospital Akure (20.7%). Adeniyi *et al.,* (2017) reported that the most prevalent five respiratory diseases were tuberculosis (TB), pneumonias, chronic obstructive pulmonary diseases (COPD), asthma and lung cancer (53%, 21.1%, 13.7%, 8.4% and 1.4% respectively).

Specimen Collection

Five milliliters of venous blood was obtained from each of the subjects into lithium heparinized bottles . The Plasma was extracted for biochemical and ELISA assays while the whole blood was used for the identification of *Plasmodium*. Sputum samples was obtained from each of the subjects consecutively for three days for Ziehl Neelsen staining.

Analysis of Biochemical and Immunological parameters

Superoxide Dismutase (S.O.D) Activity

This was determined using Abcam kit (ab65354). Manufacturer's instructions were strictly followed and applied.

HIVp24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum

These were determined in the subjects using Bio-Rad Genscreen™ ULTRA HIV Ag-Ab qualitative enzyme immunoassay kit (72278 883666) designed for the detection of HIV p24 antigen and antibodies to HIV-1 and HIV-2 in human serum/plasma by Enzyme Linked Immunosorbent Assay(ELISA). Manufacturer's instructions were strictly followed and applied.

Anti-HCV ELISA

Anti-HCV was determined in the subjects using Bio-Rad Monolisa™ Anti-HCV PLUS Version 3 screening kit(72315 883663) designed for the detection of anti-HCV antibodies (Hepatitis C

Virus) in human plasma or serum by ELISA technique. Manufacturer's instructions were strictly followed and applied.

Detection of HBsAg, HBeAg, HBeAb by ELISA

1. Hepatitis B surface antigen (HBsAg) test was determined in the test and control volunteers by a one step MONOLISA AgHBs PLUS enzyme immunoassay technique of the sandwich type (72408 883665_2013_12) for the detection of HBsAg in serum or plasma using the reagent kit of BIO –RAD Raymond Poincare, Marnes La Coquette.
2. **HBeAg and anti-HBe tests** were determined in the test and control volunteers by immunoassay technique based on ELISA principle for the detection of Hepatitis B e antigen(HBeAg) and antibody(Anti-HBe) in human plasma and sera using the reagent kit (0318) of: DIA.PRO Diagnostic Bioprobes Srl Via Columella, Milano – Italy
3. Manufacturer's instructions were strictly followed and applied.

Measurement of Blood glucose

Blood glucose was determined in the subjects by glucose oxidase method using the reagent kit of RANDOX(GL364). Manufacturer's instructions were strictly followed and applied.

TNF-α ELISA using Human ABCAM ELISA Kit (ab181421)

Plasma TNF-α was determined in the subjects by ELISA. Manufacturer's instructions were strictly followed and applied.

IL-10 ELISA using Human IL-10 ELISA Kit (ab46034)

Plasma IL-10 was determined in the subjects by ELISA. Manufacturer's instructions were strictly followed and applied.

Detection of Acid Fast Bacilli in sputum and Identification of *Plasmodium* in blood

Acid Fast Bacilli test and Identification of *Plasmodium* using sputum and blood samples respectively were carried out by the method described by Cheesbrough, (2006)

Ethical Consideration

The proposal of this work was reviewed and approved by Ethical and Research committee of Federal Medical Centre, Owo – Nigeria (FMC/AUOS//19/0199). The consent of each of the participants was also obtained.

Method of Data analysis

The results obtained was subjected to statistical analysis to determine mean, standard deviation student 't' test and probability values using SPSS IBM20.0.

Results

The frequency of viral seromarkers obtained in this study include 11.1%(7) Anti-HCV; 20.6%(13) Anti-HBe ; 20.6%(13) HBeAg ; 20.6%(13) HBsAg ; 3.2% (2) HIVag(p24)/Ab ; 0%(0) Anti-HCV + HIVAg/Ab ; 3.2% (2) HBsAg + HBeAg + HIVAg/Ab and 20.6%(13) HBeAg + HBsAg + anti-HBe. Seromarker of hepatitis B virus was found to be more prevalent. 3.2% (2) of the patients expressed HBsAg + HBeAg + HIVAg/Ab (Table 1; Figure 1).

The results obtained in patients with decreased plasma Superoxide dismutase showed that the frequency of viral seromarkers of hepatitis B virus were more than the seromarkers of hepatitis C virus and Human Immunodeficiency Virus while seromarkers of both HBV and HCV were more

than those of HIV. Only coinfection of HIV and HBV (3.2% (2)) was found in the patients expressed as anti-HBe, HBsAg + HBeAg + HIVAg/Ab (Table1; Figure1).

There was no significant difference in the plasma value of TNF-α (pg/ml) and IL-10 (pg/ml) in the results obtained in patients who expressed HBeAg + HBsAg + anti-HBe and Anti-HCV ($p>0.05$; Table 1,2; Figure 1)

There was a significantly higher plasma value of TNF-α (pg/ml) in patients who expressed HIVAg(p24)/Ab than in patients who expressed HBeAg + HBsAg + anti-HBe ($p<0.05$; Table 1,2; Figure 2). However there was no significant difference in the plasma value of IL-10 (pg/ml) in patients who expressed HIVAg(p24)/Ab and HBeAg + HBsAg + anti-HBe($p>0.05$; Table 1,2; Figure 1).

There was a significantly higher TNF-α and lower plasma IL-10 in patients who expressed HBsAg + HBeAg + HIVAg/Ab than those who expressed HBeAg + HBsAg + anti-HBe ($p<0.05$; Table 1,2; Figure 1).

There was a significantly higher TNF-α in patients who expressed HIVag(p24)/Ab than those who expressed Anti-HCV in their plasma ($p<0.05$; Table 1,2; Figure 2). However there was no significant difference in the plasma value of IL-10 (pg/ml) in patients who expressed HIVAg(p24)/Ab and Anti-HCV ($p>0.05$; Table 1,2; Figure 2).

There was a significantly higher TNF-α and lower plasma IL-10 in patients who expressed HBeAg + HBsAg + anti-HBe, HIVag(p24)/Ab, HBsAg + HBeAg + HIVAg/Ab , Anti-HCV than the Control subjects($p>0.05$; Table 1,3; Figure 1).

There was a significantly higher TNF-α and lower plasma IL-10 in patients who expressed HBeAg + HBsAg + anti-HBe, HIVag(p24)/Ab, HBsAg + HBeAg + HIVAg/Ab , Anti-HCV than the Control subjects ($p<0.05$; Table 1,2; Figure 2) except that no significant difference was obtained in plasma IL-10 in patients with HBeAg + HBsAg + anti-HBe and Control($p>0.05$; Table 1,3; Figure 1).

There was a significant increase in plasma TNFα and a significant decrease in IL-10 in patients who expressed seromarkers of HBV, HCV and HIV which was more pronounced in patients who expressed HIV seromarkers and also in those who co-expressed HIV and HBV seromarkers($p<0.05$).

There was a significant decrease in the plasma SOD in patients who expressed Anti-HCV, anti-HBe , HBeAg , HBsAg, HIVAg-Ab, HBsAg+HBeAg+HIV and HBeAg+HBsAg+ anti-HBe compred with the results obtained in the control($p<0.05$; Table 1,3, 4; Figure 1)

Discussion

The frequency of viral seromarkers obtained in this study include 11.1%(7) Anti-HCV; 20.6%(13) Anti-HBe ; 20.6%(13) HBeAg ; 20.6%(13) HBsAg ; 3.2% (2) HIVag(p24)/Ab ; 0%(0) Anti-HCV + HIVAg/Ab ; 3.2% (2) HBsAg + HBeAg + HIVAg/Ab and 20.6%(13) HBeAg + HBsAg + anti-HBe. Seromarker of hepatitis B virus was found to be more prevalent. 3.2% (2) of the patients expressed HBsAg + HBeAg + HIVAg/Ab.

The results obtained in patients with decreased plasma Superoxide dismutase showed that the frequency of viral seromarkers of hepatitis B virus were more than the seromarkers of hepatitis C

virus and Human Immunodeficiency Virus while seromarkers of both HBV and HCV were more than those of HIV. Only coinfection of HIV and HBV (3.2% (2)) was found in the patients expressed as anti-HBe, HBsAg + HBeAg + HIVAg/Ab

There was a significant increase in plasma TNFα and a significant decrease in IL-10 in patients who expressed seromarkers of HBV, HCV and HIV which was more pronounced in patients who expressed HIV seromarkers and also in those who co-expressed HIV and HBV seromarkers.

Expression of viral seromarker is an indication of active viral infection or immunity. It may also indicate viral replication acute or chronic viral infections. Expression of more than one viral seromarkers is an indication of coinfection with others virus **(**Barbara *et al.,* 1991; Mel *et al.,* 2005; Adeniyi *et al.,* 2017).

In hepatitis B virus infection HBsAg is produced in the liver in excess by the virus and secreted into the blood, where it serves as a marker for active infection and infectivity (Mel *et al.,* 2005).

Expression of HBeAg in the plasma of the patients can be associated with high levels of HBV replication, greater infectivity and an increased risk of hepatic fibrosis (Mel *et al.,* 2005) .

Presence of anti-HBe in plasma or serum indicates HBV infection, clearance or decreased plasma level of HBeAg in the serum, but this may not alter the sequelae of chronic infection (Adeniyi *et al.,* 2017). Generally, hepatitis B e-antigen (HBeAg) is expressed in the serum of patients with a new acute infection which is associated with higher HBV DNA levels an indication of increased infectiousness.

Antibody to HCV (Anti-HCV) in serum is an indication of HCV infection. The antibody is produced upon HCV infection and appear in the serum after seroconvertion (Barbara *et al.,* 1991).

Antibody to HIV is a non-neutralizing/non-protective antibody expressed in HIVinfection and expressed in the serum after seroconversion. HIVp24 antigen is more reliable antigen for the diagnosis of HIV infection. Presence of HIVp24 antigen and anti-HIV indicates HIV infection. HIVp24 antigen is detectable ealier than antibodies to HIV (Simon and Chitrangada, 2016).

Expression of viral seromarkers in patients with decreased SOD can be associated with the fact that viral infection can bring about oxidative stress in the affected individuals(Schwarz ,1996 ; Romá-Mateo *et al.,* 2005;Segal, 2005 Halliwell, 2007; Valko *et al.,* 2007; Hwang,. 2013; Joseph *et al.,* 2015). Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage(Schwarz ,1996 ; Romá-Mateo *et al.,* 2005;Segal, 2005 Halliwell, 2007; Valko *et al.,* 2007; Hwang,. 2013; Joseph *et al.,* 2015). This is due to the production of toxic peroxides and free radicals that can damage cell, and its components. The damage is usually caused by reactive oxygen species (ROS), e.g. $O2^-$ (superoxide radical), OH (hydroxyl radical) and H2O2 (hydrogen peroxide) (Schwarz ,1996 ; Romá-Mateo *et al.,* 2005;Segal, 2005 Halliwell, 2007; Valko *et al.,* 2007; Hwang,. 2013; Joseph *et al.,* 2015). However, reactive oxygen species are also beneficial, as they are used by the immune system to attack and kill pathogens (Schwarz ,1996 ; Romá-Mateo *et al.,* 2005;Segal, 2005 Halliwell, 2007; Valko *et al.,* 2007; Hwang,. 2013; Joseph *et al.,* 2015).

Decreased plasma level of SOD in the patients can be explained by the activities of antioxidants like SOD to inhibit oxidation which is a chemical reaction that can produce free radicals that can damage the cells of patients (Kalra *et al*., 1994; Ezimah *et al.*,2005). There was a significant decrease in the plasma SOD in patients who expressed Anti-HCV, anti-HBe , HBeAg , HBsAg, HIVAg-Ab, HBsAg+HBeAg+HIV and HBeAg+HBsAg+ anti-HBe compared with the results obtained in the control. Excessive utilization of SOD as antioxidant against oxidation induced by viral infection may be responsible for the reduction in the plasma level of SOD. In another way, low plasma SOD an antioxidant that protects the body against free radical toxicity may be responsible for the patients' susceptibility to viral infections(Schwarz 1996; Valko *et al*., 2007) as indicated by the expression of Anti-HCV; Anti-HBe ; HBeAg ; HBsAg ; HIVag(p24)/Ab ; HBsAg + HBeAg + HIVAg/Ab and HBeAg + HBsAg + anti-HBe.in this study. Low SOD is also a form of immune response in viral infection(Kalra *et al*., 1994; Beck, 2001; Ezimah *et al.*,2005).

Increased TNFα and reduced plasma IL-10 in patients who expressed viral seromarkers in this study indicates pro and anti-inflammatory responses(Beck, 2001; Sang and Robert, 2002; Kim and Solomon, 2010; Shi *et al.,* 2013).

Increased TNFα can be due to its pro-inflammatory bioactivities in viral infection leading to the excessive production to act on liver for acute phase response, phagocytosis and for inhibition of viral replication as (Beck, 2001; Sang and Robert, 2002; Kim and Solomon, 2010; Shi *et al.,* 2013).

Reduced plasma IL-10 in this work can be due to the anti-inflammatory activities of the cytokine as IL-10 can inhibit pro-inflammatory cytokines such as TNFα. IL-10 has been reported to suppress cytokine secretion, antigen presentation and CD4+ T cell activation (de Waal *et al.*,1991; Aste-Amezaga *et al.,* 1998;Akdis *et al.,* 2000; Joss *et al.,* 2000).

Conclusion

Patients with decreased SOD expressed viral seromarkers including significant inflammatory response indicated by an increase in TNFα and decreased IL-10. There was also a higher frequency of viral seromarkers of hepatitis B virus than the seromarkers of hepatitis C virus and Human Immunodeficiency Virus while seromarkers of both HBV and HCV were more than those of HIV. Only coinfection of HIV and HBV (3.2% (2)) was found in the patients expressed as anti-HBe, HBsAg + HBeAg + HIVAg/Ab.

References

Adeniyi BO, Awokola BI, Irabor I, Obaseki DO, Ayeni EO, Alele BK and Erhabor GE (2017) Pattern of Respiratory Disease Admissions among Adults at Federal Medical Centre, Owo, South-West, Nigeria: A 5-Year Review. Ann Med Health Sci Res. ; 7: 96-101

Akdis CA, Joss A, Akdis M, Faith A, Blaser K .(2000) "A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding". FASEB Journal. ; 14 (12): 1666–8. doi:10.1096/fj.99-0874fje. PMID 10973911.

Aste-Amezaga M, Ma X, Sartori A, Trinchieri G .(1998) "Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10". Journal of Immunology.; 160 (12): 5936–44. PMID 9637507.

Barbara Hosein, Chyang T. Fang, Mark A. Popovsky, John Ye, Meilun Zhang and Chang Yi (1991)Wang. Improved Serodiagnosis of Hepatitis C Virus Infection with Synthetic Peptide Antigen from Capsid Protein. Proceedings of the National Academy of Sciences of the United States of America Vol. 88, No. 9, pp. 3647-3651

Beck MA.(2001) Antioxidants and viral infections: host immune response and viral pathogenicity. J Am Coll Nutr. 2001 Oct;20(5 Suppl):384S-388S; discussion 396S-397S.

Brennan FM, Green P, Amjadi P, Robertshaw HJ, Alvarez-Iglesias M, Takata M(2008) . "Interleukin-10 regulates TNF-alpha-converting enzyme (TACE/ADAM-17) involving a TIMP-3 dependent and independent mechanism". European Journal of Immunology.: 38 (4): 1106–17. doi:10.1002/eji.200737821. PMID 18383040.

Cannon JG . (2000)"Inflammatory Cytokines in Nonpathological States". News Physiol. Sci.: 15: 298–303. PMID 11390930.

Dada EO, Okebugwu QC and Ibukunoluwa MR (2016) Prevalence of Malaria in Gbalegi, Idanre Local Government Area and State. HIV Curr Res, 1:1 DOI: 10.4172/2572-0805.1000110Hospital, Akure, Ondo State, Nigeria

de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE.(1991) "Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes". The Journal of Experimental Medicine.; 174 (5): 1209–20. doi:10.1084/jem.174.5.1209. PMC 2119001. PMID 1940799.

Ezimah AC, Onyeyili PA, Nwobu GO, Nwankwo EA.(2005) Glutathione peroxidase levels in Nigerians with AIDS. J Med Lab Sc; 14: 11-13.

Halliwell, Barry (2007). "Oxidative stress and cancer: have we moved forward?". Biochem. J. 401 (1): 1–11. doi:10.1042/BJ20061131. PMID 17150040.

Hayyan M, Hashim MA, Al Nashef IM (2016). "Superoxide Ion: Generation and Chemical Implications". Chem. Rev.: 116 (5): 3029–3085. doi:10.1021/acs.chemrev.5b00407. PMID 26875845.

Hwang O. (2013) "Role of oxidative stress in Parkinson's disease". Exp Neurobiol. 2013; 22 (1): 11–7. doi:10.5607/en..22.1.11. PMC 3620453. PMID 23585717.

Joseph N, Zhang-James Y, Perl A, Faraone SV .(2015) "Oxidative Stress and ADHD: A Meta-Analysis". J Atten Disord.; 19 (11): 915–24. doi:10.1177/1087054713510354. PMC 5293138. PMID 24232168.

Joss A, Akdis M, Faith A, Blaser K, Akdis CA . (2000)"IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway". European Journal of Immunology.; 30 (6): 1683–90.

Kalra J, Mantha SV, Prassad K.(1994) Oxygen-free radicals: Key factors in clinical diseases. Lab Medica International ; 11: 16-21.

Kim SY, Solomon DH.(2010) Tumor necrosis factor blockade and the risk of viral infection. Nat Rev Rheumatol. Mar;6(3):165-74. doi: 10.1038/nrrheum.2009.279. Epub 2010 Feb 9.

Lee MH, Yang HI, Lu SN, Jen CL, Yeh SH, Liu CJ, Chen PJ, You SL, Wang LY, Chen WJ, Chen CJ. (2010) Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. J Clin Oncol. Oct 20;28(30):4587-93. doi: 10.1200/JCO.2010.29.1500. Epub 2010 Sep 20.

Locksley RM, Killeen N, Lenardo MJ (2001). "The TNF and TNF receptor superfamilies: integrating mammalian biology". Cell. 104 (4): 487–501. doi:10.1016/S0092-8674(01)00237-9. PMID 11239407.

McCord JM, Fridovich I . (1988) "Superoxide dismutase: the first twenty years (1968-1988)". Free Radical Biology & Medicine.: 5 (5–6): 363–9. doi:10.1016/0891-5849(88)90109-8. PMID 2855736.

Mel Krajden, MD, Gail McNabb, and Martin Petric,(2005) The laboratory diagnosis of hepatitis B virus. Can J Infect Dis Med Microbiol. Mar-Apr; 16(2): 65–72. doi: 10.1155/2005/450574

Monica Cheesbrough (2006) District Laboratory Practice in Tropical Countries: Part 2 Second Edition. Cambridge University Press The Edinburgh Building, Cambridge CB2 8RU, UK Published in the United States of America by Cambridge University Press, New York www.cambridge.org

Mosser DM, Zhang X .(2008) "Interleukin-10: new perspectives on an old cytokine". Immunological Reviews.: 226 (1): 205–18. doi:10.1111/j.1600-065X.2008.00706.x. PMC 2724982. PMID 19161426.

Olugbenga Adekunle Olowe, Victor Olatunji Mabayoje, Olusola Akanbi,1 Olusolabomi Jose Adefioye,1 Rita Ayanbolade Olowe,1 Emmanuel Kehinde Fadeni,3 Adeolu Sunday Oluremi, and Oluyinka Oladele Opaleye(2016). HIV P24 antigen among HIV antibody seronegative blood donors in Osogbo Osun State, South Western Nigeria. Pathog Glob Health. ; 110(4-5): 205–208. doi: 10.1080/20477724.2016.1205311

P Prabina, S Jayanthi, C Krishna Murthy, S Babu Kumar, AS Shameem Banu, SR Sakunthala,and J Peruma.(2019) A Study on Hepatitis B Viral Seromarkers and Associated Risk Factors among the Patients Suffering from Acute and Chronic Hepatitis B Infection. Int J Appl Basic Med Res. 2019 Oct-Dec; 9(4): 206–211. Published online Oct 11. doi: 10.4103/ijabmr.IJABMR_263_18

Romá-Mateo C, Aguado C, García-Giménez JL, Ibáñez-Cabellos JS, Seco-Cervera M, Pallardó FV, Knecht E, Sanz P .(2005) "Increased oxidative stress and impaired antioxidant response in Lafora disease". Mol. Neurobiol.;51 (3): 932–46. doi:10.1007/s12035-014-8747-0. hdl:10261/123869. PMID 24838580.

Sang Heui Seo and Robert G. Webster (2002) Tumor Necrosis Factor Alpha Exerts Powerful Anti-Influenza Virus Effects in Lung Epithelial Cells. J Virol. ; 76(3): 1071–1076. doi: 10.1128/JVI.76.3.1071-1076.2002

Schwarz KB. (1996)Oxidative stress during viral infection: a review. Free Radic Biol Med. ;21(5):641-9.

Segal AW. (2005)"How neutrophils kill microbes". Annu. Rev. Immunol.; 23: 197–223. doi:10.1146/annurev.immunol.23.021704.115653. PMC 2092448. PMID 15771570.

Shi, X., Zhou, W., Huang, H. *et al*.,. (2013) Inhibition of the inflammatory cytokine tumor necrosis factor-alpha with etanercept provides protection against lethal H1N1 influenza infection in mice. Crit Care 17, R301 (2013) doi:10.1186/cc13171

Simon Bystryak , Chitrangada Acharya (2016) Detection of HIV-1 p24 antigen in patients with varying degrees of viremia using an ELISA with a photochemical signal amplification system Clin Chim Acta1;456:128-136.. doi: 10.1016/j.cca.2016.02.022. Epub 2016 Mar 3.

Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N(2010). "A meta-analysis of cytokines in Alzheimer's disease". Biol Psychiatry.: 68 (10): 930–941. doi:10.1016/j.biopsych.2010.06.012. PMID 20692646.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J .(2007) "Free radicals and antioxidants in normal physiological functions and human disease". Int. J. Biochem. Cell Biol. ; 39 (1): 44–84.

Table 1: Frequency of viral seromarkers, mean and standard deviation of plasma TNF-α, IL-10 SOD and glucose obtained in the patients

Viral Seromarkers	**Frequency**	**TNF-α (pg/ml)**	**IL-10 (pg/ml)**	**Plasma Glucose (mg/dl)**	**Plasma SOD, (U/mL)**	**AFB test**	***Plasmodium* test**
Anti-HCV	11.1 %(7)	4.3 ± 0.1	2.7 ± 0.1	101 ± 3.0	53 ± 1.0	Negative	Negative
Anti-HBe	20.6%(13)	4.0 ± 0.2	2.9 ± 0.2	98 ± 2.0	55 ± 2.0	Negative	Negative
HBeAg	20.6%(13)	4.5 ± 0.1	2.5 ± 0.1	99 ± 3.0	55 ± 1.0	Negative	Negative

HBsAg	20.6%(13)	4.3 ± 0.1	2.7 ± 0.2	98 ± 2.0	54 ± 1.0	Negative	Negative
HIVAg(p24)/Ab	3.2% (2)	5.3 ± 0.1	2.2 ± 0.15	100 ± 2.0	52 ± 2.0	Negative	Negative
Anti-HCV + HIVAg/Ab	0	0	0	0	0	-	-
HBsAg + HBeAg + HIVAg/Ab	3.2% (2)	7.3 ± 0.2	2.0 ± 0.1	98 ± 2.1	51 ± 1.0	Negative	Negative
HBeAg + HBsAg + anti-HBe	20.6%(13)	4.3 ± 0.1	2.7 ± 0.1	99 ± 1.5	55 ± 1.5	Negative	Negative
Control	70	2.3 ± 0.1	4.7 ± 0.1	99 ± 2.0	98 ± 1.3	Negative	Negative

Note:

Anti-HCV - antibody to HCV

Anti-HBe – Envelope Antibody to HBV

HBeAg- Envelope Antigen to HBV

HBsAg- HBV surface antigen

HIVAg(p24)/Ab- HIV antigen and antibody

TNF-α (pg/ml)- Tumor necrosis factor alpha

IL-10 (pg/ml)- Interleukin 10

Table 2 : Comparative analysis of TNF-α and IL-10 obtained in the patients

	HBeAg + HBsAg + anti-HBe Vs. Anti-HCV	**HBeAg + HBsAg + anti-HBe Vs. HIVag(p24)/Ab**	**HBeAg + HBsAg + anti-HBe Vs. HBsAg + HBeAg + HIVAg/Ab**	**HIVag(p24)/Ab Vs Anti-HCV**

TNF-α (pg/ml)	**'t'**	0	-7.07107.	-13.41641.	7.07107.
	'p'	0.5	0.00971*	0.002755*.	0 .00971*
IL-10 (pg/ml)	**'t'**	0	2.23607.	4.94975.	-2.23607.
	'p'	0.5	0.077423.	0 .019238*	0 .077423.

Note

*Significant ($p<0.05$)

't'- Students' 't' value

'p'- probability value

Table 3: Comparative analysis of TNF-α and IL-10 obtained in the patients and control

	HBeAg + HBsAg + anti-HBe Vs. Control	**HIVag(p24)/Ab Vs. Control**	**HBsAg + HBeAg + HIVAg/Ab Vs. control**	**Anti-HCV Vs. Control**

TNF-α (pg/ml)	**'t'**	14.14214.	21.2132.	22.36068.	14.14214.
	'p'	0 .002481*	0.001107*.	0.000997*	0.002481*
IL-10 (pg/ml)	**'t'**	2.23607.	-11.18034	-19.53874.	-14.14214.
	'p'	0 .077423.	0.003953*.	0.001305*	0.002481*

***Significant (p<0.05)**

Table 4: Comparative analysis of plasma SOD obtained in test and controls

		Anti-HCV Vs Control	**anti-HBe Vs. Control**	**HBeAg Vs Control**	**HBsAg Vs Control**	**HIVAg-Ab Vs Control**	**HBsAg+HBeAg+HIV Vs Control**	**HBeAg+HBsAg+ anti-HBe Vs Control**
Plasma SOD,(U/mL)	**'t'**	-31.82	-19.23	-38.91	-31.11	-20.57	-33.23	-19.23
	'p'	0.0005***	0.001**	0.0003***	0.0005***.	0.001**	0.0005***	0.001**

***Significant**

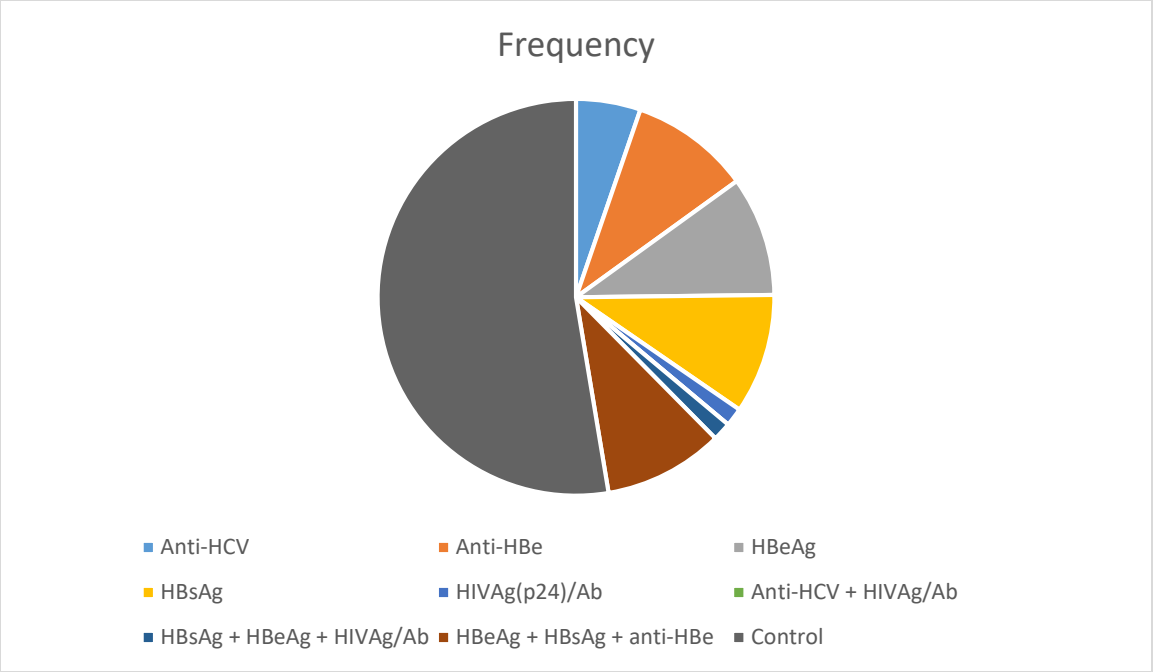

Figure 1 : Comparative description of viral seromarkers, plasma TNF-α and IL-10 obtained in the patients and control