ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The effect of serum Interleukin (IL-33) Levels as a possible clinical marker correlated with some biochemical parameters of *Toxoplasmosis* in Iraqi female patients

*** Nawal M.J. AL- Shammaa**
Department of Chemistry Ibn-Alhaitham ,College of pure Sciences
Education University of Baghdad

Manuscript Info

Manuscript History:

Received: 11 January 2014
Final Accepted: 22 February 2014
Published Online: March 2014

Key words:
Toxoplasmosis, Immunoglobulin, Liver Enzymes , Alkaline phosphate

Corresponding Author

Nawal M.J.AL-Shammaa

Abstract

Toxoplasmosis caused by intracellular protozoan parasite, *Toxoplasma gondii*, is infects a wide variety of hosts including humans ,Various warm-blooded animals including birds and rodents can serve as an intermediate host. Interleukin (IL-33) is a member of the IL-1 family of cytokines, Interleukin- 33 (IL-33) is a novel anti-inflammatory cytokine suppressing the immune response .The present study was conducted on 50 patients from the Iraqi female patients with *toxoplasmosis* attending Baghdad teaching hospital ,in addition to 50 healthy controls. All subjects were (20-45) years old. Parameters measured in the sera of patients and healthy groups, were interleukin-33 (IL-33), Immunoglobulins IgG, ,IgA, IgM, aspartate aminotransferase AST , alanine aminotransferase, ALT and Alkaline phosphate ALP.I showed a highly significant increase in females patients compared with the healthy control was in IgA and a significant increase IgG and IgM but significant decreased AST and ALT. There was no significant positive correlation(+ve)between IL-33 and ALP in control and patient groups and a significant positive correlation(+ve) between IL-33 and AST ,ALT for control group while a significant negative correlation in patient groups .They were highly significant (+ve) correlation between IL-33 and IgA $p \leq 0.001$) in patient and negative correlation in control also there was a significant negative (-ve) correlation IgG in control and patient respectively and a significant positive correlation IgM in patients a significant negative in control groups.

I was noticed non significant increase ALP in patient groups compared with healthy controls.

Introduction

Toxoplasmosis caused by intracellular protozoan parasite, is infects a wide variety of hosts, including humans, *Toxoplasma gondii* is a microscopic protozoa that causes a disease called toxoplasmosis. The disease is found all over the world. Some estimates suggest that over 30 % of human population is infected. For example, in Germany and France most people carry the parasite, whereas in South Korea it is quite rare. More than 60 million people in the United States are said to be infected(1). Toxoplasmosis is usually asymptomatic, because our immune system keeps the parasite from causing illness. The disease is more problematic for pregnant women and people who have weakened immune systems. Cats are the primary host and humans and other warm blooded animals are just intermediate hosts(2). Some results indicate a strong correlation between schizophrenia and toxoplasmosis. According to some studies women with toxoplasmosis are more likely to cheat their husbands. Men with the parasite have shown to be more aggressive. Infected humans also have slower reaction times(3).The clinical presentations of *toxoplasmosis* can go asymptomatic or could cause signs and symptoms varied depending on the pathogenesis, immune status of the patient and clinical setting such as immune competent, congenital and immune compromised

toxoplasmosis. In the immune competent patients, it is usually asymptomatic or is associated with only mild and unspecific symptoms such as fever, enlarged lymph nodes, myalgia, stiff neck ,sore throat and abdominal pain. Individually, the immune response is clearly instrumental in restricting the parasite replication and avoiding overt disease ,but is absolutely unable to discharge the parasites infection. Furthermore, the parasite is of major medical relevance after primary maternal infection during pregnancy, eventually leading to congenital toxoplasmosis. (4). *T.gondii* infection induces several immunological changes in the body which are characterized by the production of the immunoglobulins IgM, IgG and IgA (5-6). The main form of transmission is from an infected mother to fetus through the placenta,the rate of placental transmission is between 17-25% when there is maternal infection during the first and second trimester 65% and when infection occurs during the third trimester from pregnant women (8-9)Toxoplasmosis can be diagnosed serologically in pregnant women by several testes that depend on the demonstration of anti-Toxoplasma antibodies in the serum (10). Interleukins are a subset of a larger group of cellular messenger molecules called cytokines, which are modulators of cellular behavior. Like other cytokines, interleukins are not stored within cells but are instead secreted rapidly, and briefly, in response to a stimulus, such as an infection agent (11).Once an interleukin has been produced,it travels to its target cell and bind to it via a receptor molecule on the cells surface .This interaction triggers a cascade of signals within the target cell that ltimately alter the cells behavior. Interleukins regulate immune responses(12)Its production is then up regulated in inflamed tissues, thus contributing to the further amplification of inflammatory responses. Studies of IL-33 in deficient mice will provide more information on intracellular functions of this cytokine. A large body of evidence supports the pathogenic role of IL-33 in asthma and possibly other inflammatory airway conditions. Furthermore, IL-33 has been shown to be involved in experimental models of arthritis and potentially has a pathogenic role in ulcerative colitis and fibrotic conditions, suggesting that IL-33 antagonists might be of interest for the treatment of asthma, rheumatoid arthritis and ulcerative colitis. However, IL-33 also appears to exert important functions in host defense against pathogens and to display cardioprotective properties, which might have implications for the clinical use of IL-33 blockade(13-14).Interleukin(IL-33),also called IL-1F11,first described as a proinflammatory cytokin in 2005 through a computationally derived profile of members of the IL-1 cytokine family, belongs to IL-1 family, and is most closely related in structure to IL-18 and IL-1β.IL-33mRNA is broadly expressed in many tissues of human and mouse, but it is more restricted at the level of cell types . IL-33 and some inflammatory and immune diseases provide a fundamental basis for further study of theoretic investigation and clinical appalications of IL-33.

The aimed of the present study is to investigate the possible role of IL-33 in pathogenesis of *toxoplasmosis* and its relation with immunoglobulins (IgA, IgG and IgM) AST ,ALT and ALP

Material and Methods

Study groups

The present study was performed on a group of 50 human from Baghdad of Iraqi females patients with Toxoplasmosis during April-September 2013.They were diagnosed by physician at the hospital .In addition a group of 50 healthy females were enrolled in the study as a control group.

Sampling

Blood samples of 5 ml were drawn from all subjects enrolled in this study, and kept in plain tubes left to clot at room temperature for 15 min. Then centrifuged at 3500 g for 10 min to separate the serum.

Interleukin-33(IL-33) determinations:

Interleukin-33(IL-33) has been estimated by using enzyme immunoassay (ELISA)technique using the manufacturer instruction as supplied with kit from Ray Bio®. Immunoglobulins (IgM ,IgA and IgG) determined by Riaimmunodiffusion (RID) method [20] using kit from LTA s.r.l. (Italy). Serum IL-33 levels were measured using specific enzyme-linked immune sorbent assay (ELISA) kit. Human IL-33 for *in vitro* quantitative measurement) ,according to the manufactures protocol

Serum (AST) and (ALT)activity were measured using colorimetric method according to (Retiman and Frankel)(16)utilizing a ready made Kit for determination of serum aspartate and alanine amino transferase respectively .The procedure was done according to the manufactured instruction as kit supplied from serum Alkaline phosphate(ALP) activity was measured using enzymatic colorimetric method where a ready made kit is used

Statistical analysis

The data was expressed as mean ± SD. The comparison between patients group and control group were analyzed by using student t-test. Pearson's correlation coefficient was used to examine between IL-33 and immunoglobulins in patients group. P-value of ≤ 0.001 and ≤ 0.05 were considered highly significant and significant respectively

Results and Discussion

Table(1)shows the levels of IL-33 concentration are (90.68±67.85pg/ml), (18.71±9.41pg/ml) in sera of patients and control respectively .This table shows highly significant increase in females patients compared with the healthy

control was in IgA ,and a significant increase IgG and IgM but significant decreased AST and ALT concentration .While non significant increase ALP in patient groups.

Table(1) :The Concentration Values(Mean ±SD)of IL-33,IgG,IgA,IgM,AST,ALT, and ALP level in patient and control group

Group parameters	Controls n=50	Patients n=50	**P value**
IL33pg/ml	18.71±9.41	90.68±67.85	≥0.05
IgG mg/dl	736.66±76.59	1045±124.53	≤0.05
IgA mg/dl	84.33±5.88	201.83±60.66	≤0.001
IgM mg/dl	53.16±7.49	185.66±9.83	≤0.05
AST IU/L	15 ±2.96	5.25±1	≤0.05
ALT IU/L	15.16±2.31	4.5±0.65	≤0.05
ALP IU/L	7±2.36	9.13±3.04	≥0.05

S Significant ($P \leq 0.05$),HS highly significant($P \leq 0.001$), NS no significant($P \geq 0.05$)

There was no significant positive correlation(+ve)between IL-33 and ALP in control and patient groups and a significant positive correlation(+ve) between IL-33 and AST ,ALT for control group while a significant negative correlation in patient groups shown in figures (1,2,3).

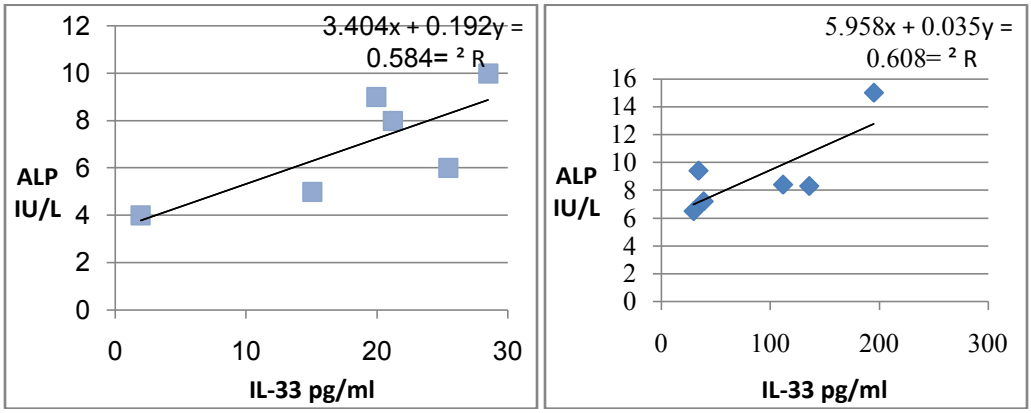

Figure (1): Correlation between serum level IL-33 and ALP in controls and patients group

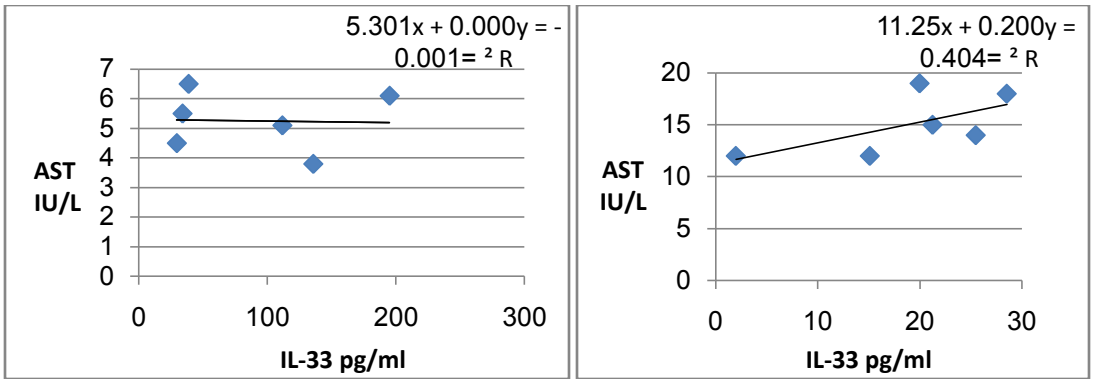

Figure (2): Correlation between serum level IL-33 and AST in controls and patients group

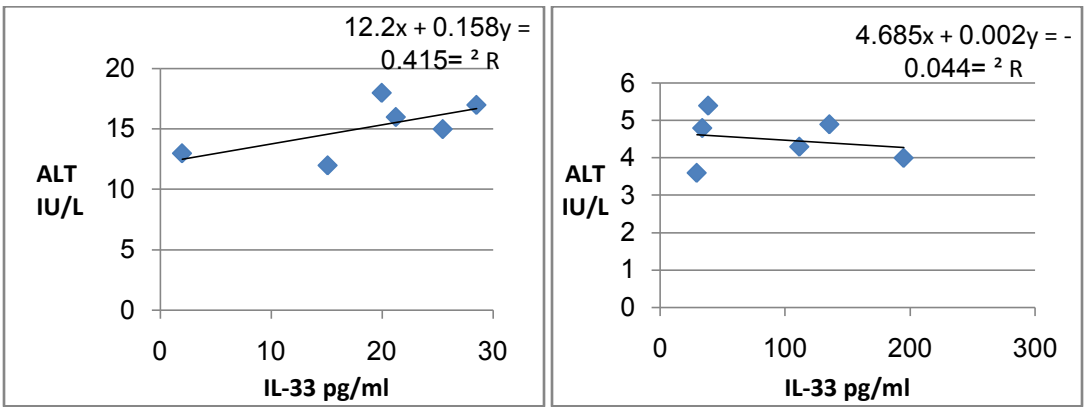

Figure (3): Correlation between serum level IL-33 and ALT in controls and patients group

They were highly significant (+ve) correlation between IL-33 and IgA (r=-0.710 p≤0.001) in patient and negative correlation (r=-0.156P≤0.001)control shown in figure (4) respectively, while there was a significant negative (-ve) correlation (r=-0.39,p≤0.05)(r =-0.45≤p0.05)IgG in controls and patients respectively and positive correlation IgG (r=0.125p≤0.05)in patients, a significant negative(r=-0.488 p≤0.05) in control figure (5,6) for patients group.

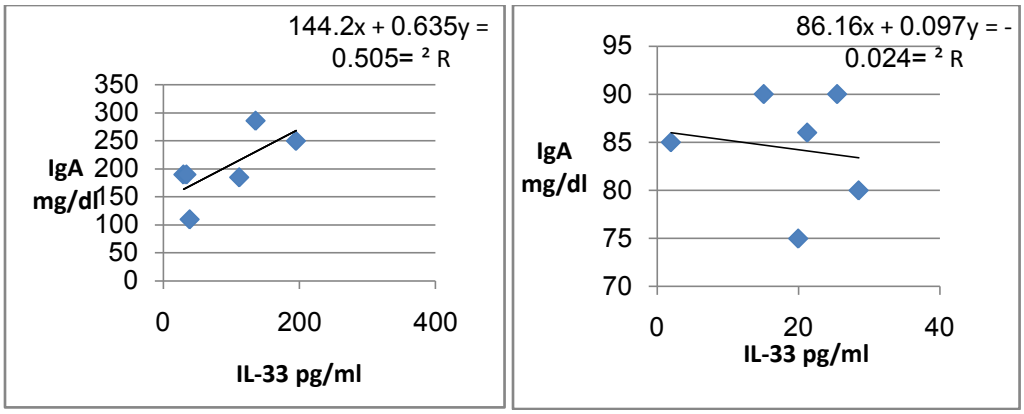

Figure (4): Correlation between serum level IL-33 and IgA in controls and patients group

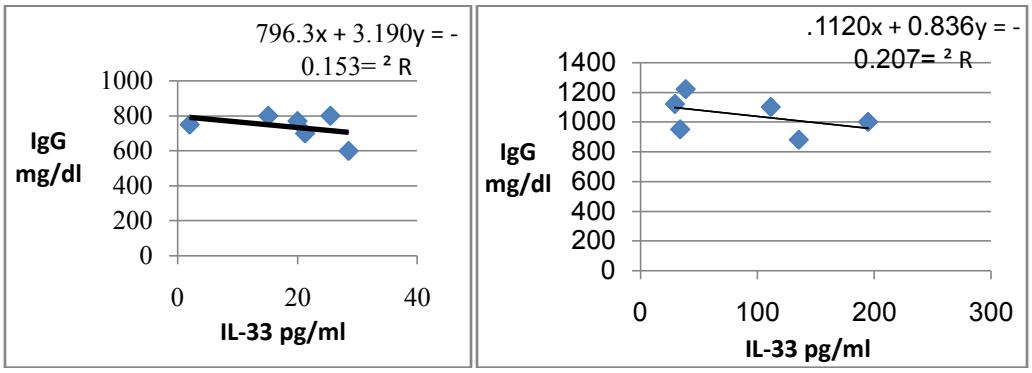

Figure (5): Correlation between serum level IL-33 and IgG in controls and patients group

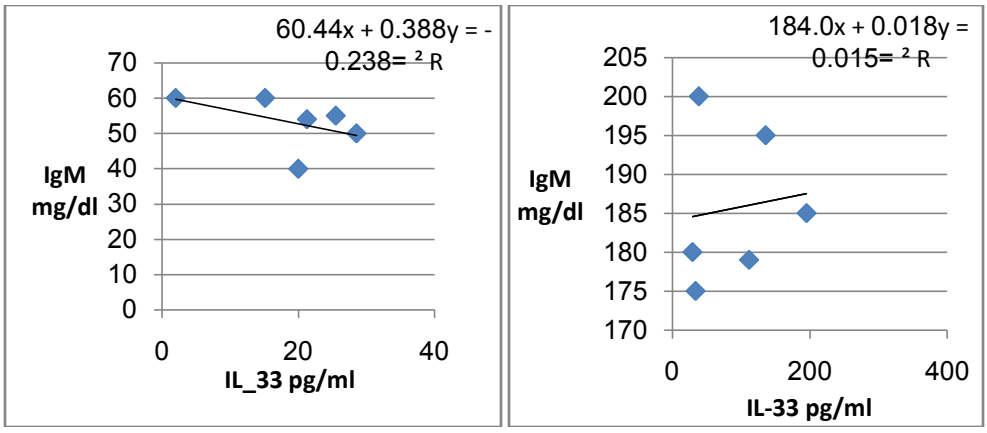

Figure (6): Correlation between serum level IL-33 and IgM in controls and patients group

These data suggest that monocytes play a critical role in controlling the parasite and become infected, but there is little known about the mechanisms mediating innate immunity to the parasite in human cells.IL-1 is an inflammatory cytokine that has been described as a "master regulator" of inflammation, since it can activate downstream inflammatory genes (13). *T. gondii* has been shown to induce IL-1 in multiple human cell types, including

monocytes, foreskin fibroblasts, and retinal pigment epithelial cells *in vitro*(14–17). Exogenous administration of IL-1 is protective against lethal *T. gondii* infection *in vivo* (18). Additionally, IL-1 is required for IL-12- mediated resistance to *T. gondii* in an *in vivo* SCID mouse model (19). These data suggest that *T. gondii*-induced IL-1 mediates protection against the parasite, but pathways that collectively, this research suggests that monocytes and IL-1 contribute to immunity to *T. gondii*. In the current study, I provided important mechanistic insight into the regulation of IL-1_ during *T. gondii* infection of primary human monocytes and THP-1 cells by defining both parasite and host factors in volvedin IL-1_ synthesis, processing, and release. I demonstrated that *T. gondii* induced IL-1 production is dependent on the classical inflamma some components caspase-1 and ASC. Monocyte productionof IL-1_ required active parasite invasion, and among the type I, II, and III strains of *T. gondii*, only the type II strain induced substantial levels of IL-1. Additionally, we demonstrated a role for a specific parasite factor, dense granule protein GRA15, in *T. gondii* induction of IL-1 Sinceead to IL-1 production in human cells during *T. gondii* infection are not well understood . In this study, IL-33 secretion is potently induced through a mitogen activated protein kinase (MAPK)-dependent activatorprotein-1 (AP-1) pathway by IL-1β, TNF, and LPS, whileST2L expression is up regulated by IL-4 and IFNγ by activation of STAT6 and STAT1, respectively. IL-33 was shown to enhance the expression of proinflammatory (28) .For many years, the interleukin-1 receptor family member ST2 was an orphan receptor that was studied in context of inflammatory and autoimmune disease . This study to compared between them, but other study also found positive IgA and IgM in single samples were analyzed with regard to three parameters, IgG in IU/mL by MEIA and ELFA and IgM and IgG-avidity indexes by ELFA(29). The parameters were compared to neonatal infection determined by neonatal IgM, in order to assess the risk of mother-to-child transmission. The absence of neonatal IgM in the 32 infants born to mothers with high avidity, low indexes of IgM and even very high levels of IgG reinforces the importance of high avidity in evaluating the risk of transmission in any gestational age. The presence of neonatal IgM in five infants born to mothers with low avidity and one stillbirth reinforces the importance of low avidity concerning the risk of transmission when it is measured in IgM-positive pregnant women(30).

High indexes of IgM (M1, M2 and M5) also support the importance of IgM levels concerning the risk of transmission. M3 and M4 were under 20 weeks and their neonates were IgM negative ,probably because the risk of transmission is lower in the first half of pregnancy(31). The results in the present study showed that the serum level of IL-33 was significantly higher in female patients with autoimmune disease (*Toxoplasmosis*)than in healthy control .However, in 2005, a new cytokine interleukin-33 was identified as a functional ligand for Toll-interleukin -1 receptor super family (ST2).IL-33/ST2 signaling is involved in T-cell mediated immune responses .Recently, an unanticipated role in cardiovascular disease has been demonstrated, IL-33/ST2 not only represents a promising cardiovascular biomarker but also a novel mechanism of intra myocardial fibroblast-cardiomyocyte communication that may prove to be a therapeutic target for the prevention of heart failure (29). On the other hand ,several studies have reported on the inhibitory effects of ST2 in inflammatory and fibrotic disesses, suggesting that IL-33 /ST2 is a unique cytokine with potential pro-and anti-inflammatory effects(30).Many previous studies have demonstrated that IL-33 may have a pleiotropic function in different diseases, and it could represent a novel target for the treatment of a range of diseases .Other study suggests that locally produced IL-33 may contribute to the pathogenesis of joint inflammation and destruction(22)

These results indicate that IL-33 may contribute to the pathogenesis chronic autoimmune diseases(31).IL-33 has a protective role in atherosclerosis by reducing macrophage foam cell formation suggesting that IL-33 may to be a potential therapeutic agent against atherosclerosis(32).Furthermore ,experimental data suggest a protective role for ST2 during Acute Pancreatitis (AP).Highlighting the potential regulatory role of mast cells and the possibility of the ST2 pathway as a new therapeutic target in AP(33). From this study I found negative correlation (-ve) between IL-33 and IgG ,while there was positive correlation(+ve) between IL-33 and IgA, IgM and ALP with IL-33 when compared between patients group(34).

From the present study can be stated that IL-33 increase concentration in sera of *toxoplasmosis* female of Iraqi patients. This is due to interleukins that regulate information transfer among different types of leukocytes during various stages of immune or inflammatory response (35). This is confirmed by increase IgG, IgA, IgM and ALP but decrease in AST and ALT concentration. This finding may indicate that autoimmune disease Like *Toxoplasmosis* might influence cytokine production in these patients (36).

References

1. Remington JS, Desmonts G. Toxoplasmosis. In :Remington JS, Klein JO, editors. Infection Diseases of the Fetus and Newborn Infant. 4th eds.Philadelphia W.B. Saunders Company 1995;143- 263

2. Dubey JP. Advances in the life cycle of Toxoplasma gondii. Int J Parasitol 1998;28:1019-24.4.MontoyaJG,Liesenfeld O. Toxoplasmosis. Lancet 2004;363:1965-76.
3. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet 2004;363:1965-76.
4. Gaby Palmer & Cem Gabay insights Interleukin-33 biology with potential into human diseases *Nature Reviews Rheumatology* 7, 321-329 (June 2.
5.Bacigalupo, M. A., P. Bazzini, L. Farina, and A. Ius. 1996. Evaluation of three immunoassays for detection of *Toxoplasma*-specific immunoglobulin G and M. Eur. J. Clin. Chem. Clin. Biochem. 34**:**503–505.(2011) | doi:10.1038/nrrheum.2011.53
6. Hayde, M., H. R. Salzer, G. Gittler, H. Aspock, and A. Pollak. 1995. Microparticle enzyme immunoassay (MEIA) for *Toxoplasma* specific immunoglobulin G in comparison to the Sabin-Feldman dye test. A pilot study. Wien Klin. Wochenschr. 107:133–136.
7. Liesenfeld, O., C. Press, J. G. Montoya, R. Gill, J. L. Isaac Renton**,** K. Hedman, and J. S. Remington. 1997. False-positive results in immunoglobulin M (IgM) *Toxoplasma* antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. J. Clin. Microbiol. 35:174–178.
8. Petithory, J. C., I. Reiter Owona, F. Berthelot, M. Milgram, J. De Loye, and E. Petersen. 1996. Performance of European laboratories testing serum samples for *Toxoplasma gondii*. Eur. J. Clin. Microbiol. Infect. Dis. 15:45– 49
9. Sandin, R. L., C. C. Knapp, G. S. Hall, J. A. Washington, and I. Rutherford. 1991. Comparison of the Vitek Immunodiagnostic Assay System with an indirect immunoassay (Toxostat Test Kit) for detection of immunoglobulin G antibodies to *Toxoplasma gondii* in clinical specimens. J. Clin. Microbiol. 29:2763–2767.
10. Valtaud, E., C. Lacroix, M. H. Rodier, G. Toullat, and J. L. Jacquemin. 1991. Critical study of ELISA technique and high sensitivity direct agglutination test for the screening of anti-*Toxoplasma* IgG. Ann. Biol. Clin. Paris 49:397–400.
11.Interleukin(IL)(protein)-Britannica Online Encylopedia(2013).
12.National Cancer Institute at the national institute of health from online 1-800-4-cancer.Live help online chat.
13.Kaviratne M, Hesse M, Leusink M, Cheever AW, Davies SJ, McKerrow JH, Wakefield LM, Letterio JJ, Wynn TA: IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent. J Immunol 2004, 173:4020–4029.
14. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA: IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptorrelated protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005, 23:479–490.
15.Shivanda Nayak B:"Manipal Manual ofClinical Biochemistry"."3rd ed.,Medical publishers LTD.,New Delhi,2007,96-100.
16.Reitman S,and Frankel S Amer(1957)J Clin.Path.,28:56.
17.Sorlie,D.E.(1995)Medical biostatistics&epidemiology: Examination &board review:First ed.Norwalk,Connecticut,Appleton&Lange:47-88.
18.Arroyo-Villa I,Bautista-Caro MB Balsa A, et al:Frequency of Th17 CD4+T cell in early rheumatoid arthritis:A marker of anti-CCP seropositivity.,Arthritis Res Ther.(2012) 7(8):e42189.
19.Eggleton P.,Bremer E.,Tarr JM, et al : Frequency of Th17 CD20+cells in the peripheral blood of rheumatoid arthritis patients are higher compared to healthy subjects.Arthritis Res Ther.(2011)13(6):R208.
20.Gabay C, Mclnne BL :The biological and clinical importance of the" new generation" cytokines in rheumatic diseases.J.Arthritis Research& Therapy,(2009)11:230.
21.Yuan FL:IL-33:A promising therapeutic target for rheumatoid arthritis? Expert Opin Ther Targets.;(2011) 15 (5):529-34.
22.Gaby P .Dominique T. Celine L., et al: Inhibition of Interleukin-33 Signaling Attenuates the Severity of Experimental Arthritis.Arthritis and rheumatism;(2009)60(3):738-749.
23. Sugimoto R, Enjoji M, Nakamuta M, Ohta S, Kohjima M, Fukushima M, Kuniyoshi M, Arimura E, Morizono S, Kotoh K, Nawata H: Effect of IL-4 andIL-13 on collagen production in cultured LI90 human hepatic stellate cells. Liver Int 2005, 25:420–428.
24. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA: IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptorrelated protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005, 23:479–490.
25. Marvie P, Lisbonne M, L'Helgoualc'h A, Rauch M, Turlin B, Preisser L, Bourd-Boittin K, Theret N, Gascan H, Piquet-Pellorce C, Samson M: Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. J Cell Mol Med 2010, 14:1726–1739
26. Coyle AJ, Lloyd C, Tian J, Nguyen T, Erikkson C, Wang L, Ottoson P, PerssonP, Delaney T, Lehar S, Lin S, Poisson L, Meisel C, Kamradt T, Bjerke T,Levinson D, Gutierrez-Ramos JC: Crucial role of the interleukin 1

receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. J Exp Med 1999, 190:895–902.
27. Nishida A, Andoh A, Imaeda H, Inatomi O, Shiomi H, Fujiyama Y: Expression of interleukin 1-like cytokine interleukin 33 and its receptor complex (ST2L and IL1RAcP) in human pancreatic myofibroblasts. Gut 2010, 59:531–541.
28. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G: The myofibroblast: one function, multiple origins. Am J Pathol 2007, 170:1807–1816.
29.Mclaren JE,Michael DR,Salter RC,et .al:IL-33reduces macrophage foam cell formation.J Immunol,;(2008)185(2):1222-9.
30.Kakkar R,and Lee RT(2008):TheIL-33/ST2 pathway :therapeutic target and novel biomarker.Nat Rev Drug Discov.;7(10);827-40.
31.Wang S.,Ding L,Liu SS,et al(2012):IL-33:A potential Therapeutic Target in Autoimmune Diseases,J Investing Med (2012);60(8):1151-6.
32.Lanny Gov,Alborz Karimzadeh,Norikivo Ueno,et.al.2013.Haman Innate Immunity to Toxoplasma gondii Is Mediated by Host Capase-1 and Asc and Parasite RA15.mBio4(4)doi:10.1128/mBio.0.0255-13.
33.Ouziel R:The ST2 pathway is involved in acute pancreatitis: a translational study in humans and mice,Am J pathol,;(2012)180 (6):2330-9.
34 Liesenfeld, O., C. Press, R. Flanders, R. Ramirez, and J. S. Remington. 1996. Study of Abbott Toxo IMx system for detection of immunoglobulin G and immunoglobulin M *Toxoplasma* antibodies: value of confirmatory testing for diagnosis of acute toxoplasmosis. J. Clin. Microbiol. 34:2526–2530.
35. Liesenfeld, O., C. Press, J. G. Montoya, R. Gill, J. L. Isaac Renton, K. Hedman, and J. S. Remington. 1997. False-positive results in immunoglobulin M (IgM) *Toxoplasma* antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. J. Clin. Microbiol.174-178.
36. Olsen, M. A., and P. P. Root. 1994. Comparison of four different immunoassays for detection of *Toxoplasma*-specific immunoglobulin G. Diagn. Microbiol. Infect. Dis. 19:19–24