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Interleukin 10, Tumor necrosis factor α, and Interferon ɣ levels in herpes zoster patients in Babylon– Iraq

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Abstract : Varicella-zoster virus (VZV) is the causative agent of varicella (chickenpox) and herpes zoster (shingles). Primary VZV infection is thought to happen via the inhalation of virus either in respiratory droplets or from shedding varicella lesions or by direct contact with infectious vesicular fluid. The immune responses act to eliminate replicating virus during varicella, but not all virus is cleared during this time, with some presumed to access nerve axons in the skin, enabling transport to neurons in sensory ganglia where the virus is able to establish a lifelong latent infection. When immunity declines, the virus reactivates causing herpes zoster (shingles). Fifty patients with herpes zoster attending Marjan Hospital in Babylon – Iraq, and thirty healthy control were subjected for this study. Serum samples were collected from patient and from healthy control. All samples were investigated for measuring the level of IL10, IFN ɣ and TNFα cytokine by ELISA to investigate their role in the immune-regulatory mechanisms involved in reactivation of latent VZV. The results showed that the highest proportion of herpes zoster infection within age group (41-60) by 23 (46%). Also there is significant rise in the levels of IL10 (12.12±5.59)pg/ml of shingles patients when compared with the healthy control group(4.74±0.90) pg/ml, while the levels of IFNɣ and TNFα were significantly lower in patients (184.31±21.95 ,51.55±5.14) pg/ml respectively when compared with healthy control group (218.03±26.21 ,62.35±6.74) pg/ml respectively.
Key words : Shingles, Cytokines ,IFNg, Varicella zoster virus , Herpes zoster, ELISA.

Introduction:

Varicella-zoster virus (VZV) is a double stranded DNA virus belongs to the *herpesviridae* family, highly contagious, exclusively infects human that infects a majority of the world's population. VZV causes two clinically significant diseases; varicella (chicken pox) and herpes zoster (shingles)[1-3].

The primary infection of VZV results in varicella. Varicella in the immunocompetent host is a mild disease, with lesion formation ceasing within 7 days[4]. The immune responses act to eliminate replicating virus during varicella, but not all virus is cleared during this time, with some presumed to access nerve axons in the skin, enabling transport to neurons in sensory ganglia, where the virus is able to establish a lifelong latent infection[5].

In about 10-20% of cases, VZV reactivates in life causes herpes zoster (HZ), also known as zoster or shingles[6,7].

VZV-specific cell mediated immunity may limit reactivation of latent VZV in sensory neurons and prevent the development of HZ by inhibiting the spread of VZV infection from these neurons[8].

Herpes zoster (HZ) manifests as an acute, painful vesicular rash and is often accompanied by chronic pain or postherpetic neuralgia[9,10]. HZ does not develop in all patients who had varicella. Unless the immune system is compromised, it suppresses reactivation of the virus and prevents herpes zoster outbreaks, but herpes zoster is more likely to occur in people whose immune systems are impaired due to aging, immunosuppressive therapy, psychological stress, or diseases that depress the cell-mediated response of the immune system, including human immunodeficiency virus infection and lymphoma[11].

Materials and Methods

This study includes fifty cases of herpes zoster infection were identified in patients admission to Marjan hospital in Babylon – Iraq, and about thirty healthy persons. The age of two groups range (14-80) years. Patients whose treated with antiviral therapy were excluded. Venous blood sample was obtained from herpetic and control groupsby using a disposable syringe. Five milliliter (ml) of blood were taken from each subject by venipuncture and pushed slowly into a gel disposable tubes . Blood in the gel disposable tube was allowed to clot for 10-15 minutes, then centrifugation of samples was done for 10-15 minutes at 1000 rpm, and then serum was separated into several 0.5 (ml) Eppendorf tubes, and then stored at (-20° C) until the time of analysis was carried out[12].

Enzyme Linked Immunosorbent Assay (ELISA) was used to evaluate the levels of IL10 (Elabscience), and IFNɣ and TNFα (Biolegand).

Results and Discussion

Shingles or herpes zoster is a viral infection caused by reactivation of latent varicella zoster virus[13]. Many studies suggest that weakened cellular immunity may be responsible for reactivation of the virus because of aging, stress, or genetic factors[14,15]. However, cytokines and soluble factors may be involved in the pathogenesis of herpes zoster. Cytokines play important roles in determining T cell responses[16].

Through reactivation of latent VZV from sensory ganglia and transport to the skin which followed by skin lesion formation, inflammatory response formed include immune mediators that associated with the release of paracrine secretion such as complement, interferon, histamine, substance p, and pro-inflammatory cytokines[17].

The results showed that the highest proportion of HZ infections within age group (41-60) by 23 (46%) followed by age group (21-40) by 13 (26%) compared with the rest of groups which were the proportion of age group (1-20) are 4 (8%) and age group (61-80) are 10 (20 %), in other word, the mean age of patients was (47.66±18.63) years old.

In this study, we found that high concentration of IL10 in shingles patients group compared with healthy control group, while the concentrations of IFNɣ and TNFα were significantly lower in patients compared with healthy control group as shown in figures (**1,2,3**).

Interleukin (IL10) is an immunoregulatory and anti-inflammatory cytokine that is important in protecting the host from infection associated immunopathology, autoimmunity, and allergy, mainly produced by monocytes and, Th2 lymphocytes, mast cells. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. ; however, it is also stimulatory towards certain T cells (Th2) and mast cells and stimulates B cell maturation and antibody production[18-21].

IL10 is capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells such as macrophages and Th1 cells[22,23].

IL10 is an immunomodulatory cytokine that suppresses cell-mediated immunity by inhibiting antigen presenting cells leading to T-cell anergy[24,25], also functions to minimize the development of Th1 responses by decreasing Th1 related cytokines (IL-12 and IFN-γ) and encouraging Th2 responses by increasing levels of Th2 related cytokines (IL-4, IL-5 and IL-13)[26,27]. Also, IL-10 potentiates humoral immune responses and can directly affect IgE regulation[28].

Polymorphisms of the IL-10 promoter gene may be associated with susceptibility to infectious disease. The promoter region of the gene for IL-10 is polymorphic, producing three different haplotypes (combinations of DNA sequences on one chromosome that are inherited together): GCC, ACC and ATA. IL-10 may be a factor in VZV reactivation leading to shingles, and genetic inheritance of a specific haplotype of the IL-10 promoter gene may predispose carriers to shingles. This idea, too, considers genetic variations in human hosts, rather than genetic variations in the VZV itself, to be behind increase or decrease shingles[14].

In the present study, serum IL-10 levels were higher in our patient during shingles infection than healthy control persons. This result agrees with study had been done by Smith-Norowitz *et al*.(2009) that concluded that (before shingles, low levels of IL-10 were detected in serum; during shingles, the serum level of IL-10 was increased 30-fold; it subsequently diminished at 5 months after shingles)[29].

Interferones (IFNs) are commonly grouped into two types. Type I IFNs are also known as viral IFNs and include IFN-α (leukocyte), IFN-β, (fibroblast), and IFN-ω. Type II IFN is also known as immune IFN (IFN-γ).

Human IFN-γ is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Originally characterized based on its anti-viral activities[30,31] IFN-γ also applies anti-proliferative and proinflammatory and immunoregulatory activities. IFN-γ can upregulate MHC class I and II antigen expression by antigen-presenting cells.

Interferon gamma (IFN-γ) is a potent cytokine produced following primary VZV infection[32]. Nonhuman primates experimentally infected with simian varicella virus (SVV), the counterpart of VZV, produce IFN-γ 7 to 11 days post infection[33,34]. IFN-γ also inhibits herpes simplex virus 1 (HSV-1) infection *in vitro*[35] and *in vivo*[36,37]. IFNγ shows a key role in preventing the virus from exiting the latency state[38]. Also, VZV reactivation correlates with a decline of VZV-specific IFN-γ-producing immune cells[39,40].

VZV infection of human neurons *in vitro* is productive, although infected cells appear healthy 2 weeks later[41-43].

In study had been done by Baird *et al*. (2015), tested whether a cytopathic effect (CPE) eventually develops in VZV-infected neurons and, if so, whether IFN-γ treatment suppresses viral growth and promotes neuronal survival after VZV infection. The study had been revealed the inhibition of VZV DNA accumulation and viral transcription in VZV-infected human neurons by IFN-γ. Generally, IFN-γ prolonged the life of VZV-infected neurons by inhibiting viral growth, reducing VZV genome content and transcript abundance, and decreasing production of infectious virus, that mean IFN γ has important role in latency of VZV[44].

In study has been done by Smith-Norowitz TA *et al.(*2009) revealed that before, during, and after shingles, low levels of IFN-gamma were detected in serum, but neither IL-1beta nor IL-4 were detected[29].

Tumor necrosis factor (TNF) is a proinflammatory cytokine that has a wide variety of functions. It can cause cytolysis of certain tumor cell lines; it is involved in the induction of cachexia; it is a potent pyrogen, causing fever by direct action or by stimulation of interleukin-1 secretion; it can stimulate cell proliferation and induce cell differentiation under certain conditions.

TNF-α is produced by activated macrophages, T and B lymphocytes, natural killer cells, astrocytes, endothelial cells, smooth muscle cells, some tumor cells, and epithelial cells[45].

TNF is considered a central cytokine in acute viral diseases, including those caused by influenza virus, dengue virus, and Ebola virus[46].

Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease[47], cancer[48], major depression[49], Psoriasis[50], and inflammatory bowel disease[51].

Excess TNF production is associated with a number of chronic inflammatory and autoimmune diseases. TNF is essential in the control and suppression of intracellular pathogens: it stimulates recruitment of inflammatory cells to the area of infection, and stimulates the formation and maintenance of granulomas that physically contain infection. In addition to stimulation of granuloma formation, TNF directly activates macrophages, which then phagocytose and kill pathogens.

Previous studies have revealed that TNF-α had a markedly greater effect on influenza virus replication[52], and can inhibit the replication of vesicular stomatitis virus, encephalomyocarditis virus, and herpes simplex virus in a dose-dependent manner and can prevent the development of cytopathic effects[53].

Inflammatory cytokines, like tumor necrosis factor and the members of the interferon family, are effective mediators of the innate antiviral immune response. The intracellular antiviral states resulting from treatment of cultured cells with each of these cytokines independently has been well studied; but, within complex tissues, the early inflammatory response is likely mediated by simultaneously expressed mixtures of these, and other, protective anti-viral cytokines. Such cytokine mixtures have been shown to induce potently synergistic anti-viral responses in vitro which are more complex than the simple summation of the individual cytokine response profiles. The physiological role of this 'cytokine synergy', however, remains largely unappreciated in vivo[54].

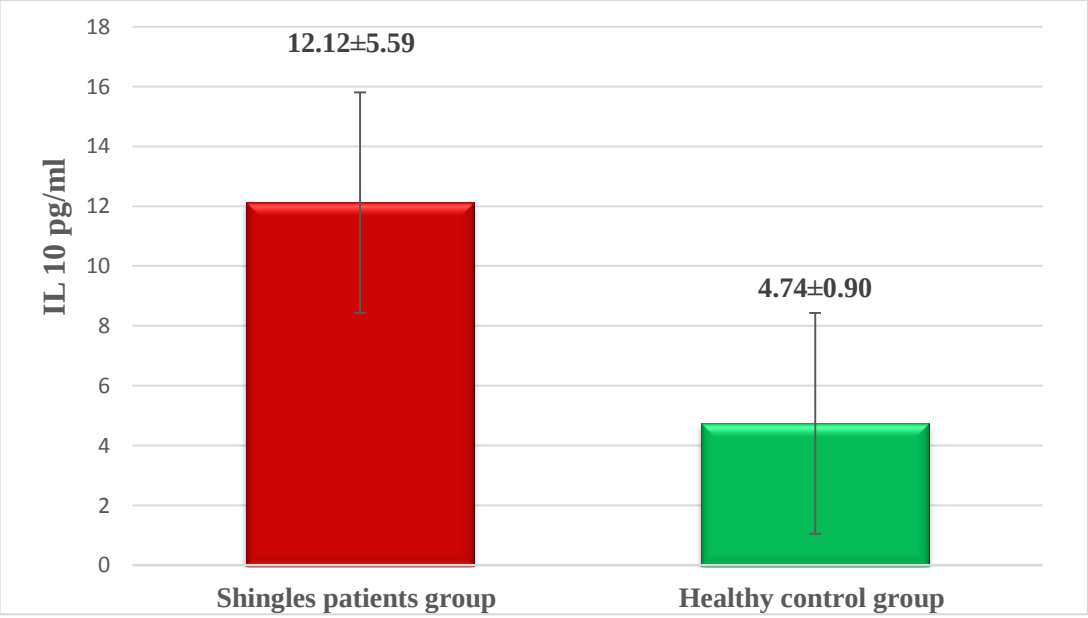
TNF inhibitors have been approved for the treatment of inflammatory bowel disease, psoriasis, and rheumatoid arthritis[55,56]. In contrast, the use of TNF inhibitors for the treatment of sepsis has not been successful, possibly due to the early release and short circulating half-life of the cytokine[45].

Many studies reported the increased risk of HZ infections in patient on anti-TNF-α therapy[57-63].

In fact, the incidence of HZ in the general population ranges from 3.2-4.2 cases per 1000 persons per year[64], while it is reported that rate is increased up to 10.60 per 1000 patient-years in patients on anti-TNF-α therapy[65].

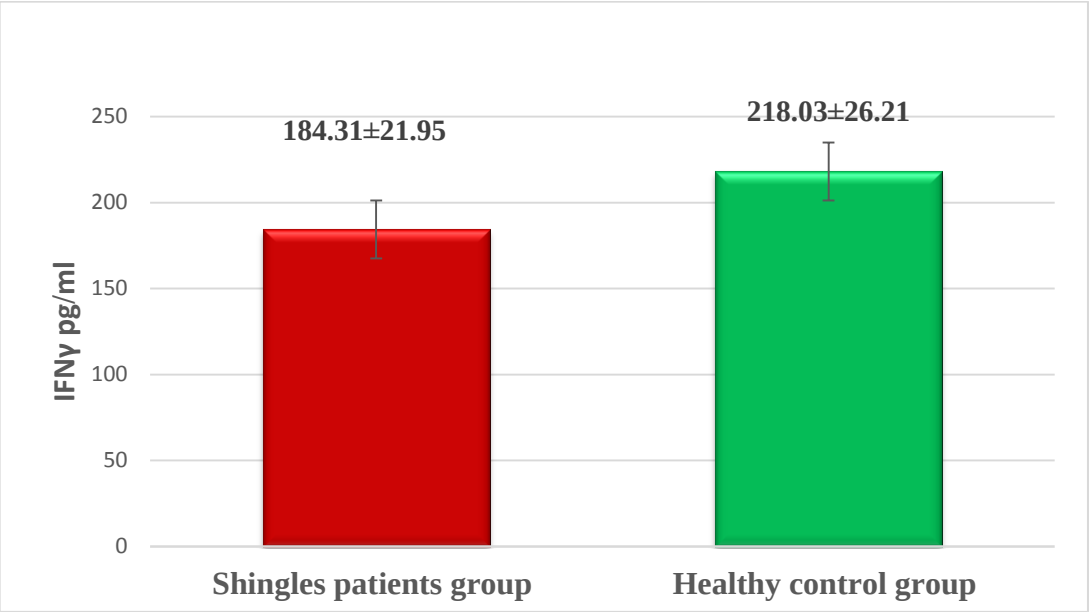
TNF-α inhibits replication of VZV and VZV antigen expression and it has been shown that blocking of TNF-α by monoclonal antibodies completely inhibits this antiviral activity. As a result, blocking of TNF-α may have a severe impact on host defense including viral infections[66].

There is a connection between development of shingles and involvement of cellular immunity in peripheral blood. In study done by Zhang M. *et al*.(2011) to explore the levels of T-helper cell Th1/Th2 type cytokine profiles in the blister fluid of the skin lesions from the patients with HZ ,the result was revealed that, TNF-α in the blister fluid from the patients' skin lesions were significantly lower than those from the control group, whereas the levels of Th2 cytokines IL-10 and IL-4 were significantly higher than those in the control group. It was concluded that a cytokine imbalance was present in the local lesions of patients with shingles during disease development[67].



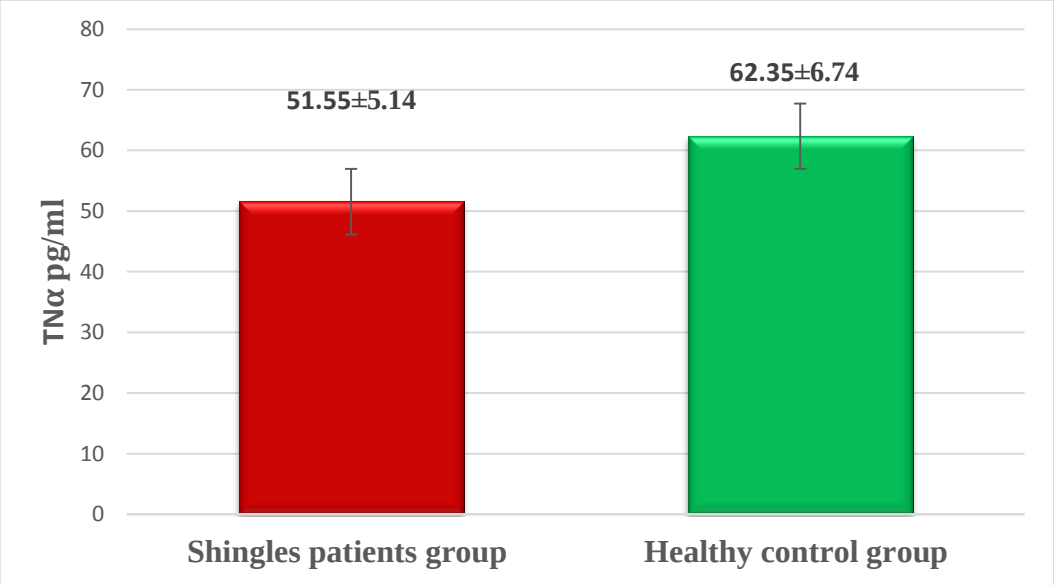
***significance (P < 0.01)**

Figure (1) Concentration of IL-10pg/ml level between shingles patients group and control group.



***significance (P < 0.01)**

Figure(2) Concentration of IFNγpg/ml between shingles patients group and control group.



***significance (P < 0.01)**

Figure (3): Concentration of TNFα pg/ml between shingles patients group and control group.

Statistical Analysis

Data were analyzed statistically by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).The normal distribution was conformed correlation analysis, independent t-test was used to estimate differences between two groups in continuous variable. Result are reported as mean and standard deviation (mean±SD) unless otherwise indicated. A p-value of ≤ 0.01 was considered as a lowest limit of significant[68,69].

Conclusions

The latent VZV reactivates leading to shingles when the levels of Th1 cytokines such IFN ɣ and TNFα decrease and levels of Th2 rise such as IL10 that suppress cell mediated immunity.

References

1. Arvin A. and Gilden, D.(2013) in Fields Virology 6thEdn (EdsKnipe, D., Howley, P.) 2015–2057 (Lippincott Williams and Wilkins)
2. Cohen JI.(2010). The varicella-zoster virus genome. Curr.Top. Microbiol. Immunol. 342, 1–14
3. Zhang Z, Selariu A, Warden C, Huang G, Huang Y, Zaccheus O, Cheng T, , Xia N and Zhuet H. (2010) .Genome-Wide Mutagenesis Reveals That ORF7 Is a Novel VZV Skin-Tropic Factor. PLoSPathog 6(7): e1000971.
4. Simon H. (2012).Shingles and chickenpox (Varicella-zoster virus).Retrieved from: http://umm.edu/health/medical/reports/articles/shingles-and-chickenpox-varicella zoster-virus.
5. Steiner I, Kennedy PGandPachner AR (2007). "The neurotropic herpes viruses: herpes simplex and varicella-zoster". Lancet Neurol 6 (11): 1015–1028.
6. Zerboni L.,NandiniSen , Stefan L Oliver and Ann M. Arvin. (2014). Molecular mechanisms of varicella zoster virus. Nature Reviews Microbiology 12,197–210
7. Hariharan K, Pillai BS and Bansal D.(2016). Herpes zoster reactivation after extracorporeal shock wave lithotripsy: A case report. Indian J Urol.;32(3):242-3.
8. Steain M., Sutherland J., Rodriguez M., Cunningham A., Slobedman B. and Abendroth A. (2014) Analysis of T-cell responses during active varicella-zoster virus reactivation in human ganglia. J Virol 88: 2704–2716.
9. Mueller NH, Gilden DH, Cohrs RJ, Mahalingam R and Nagel MA. (2008). Varicella zoster virus infection: clinical features, molecular pathogenesis of disease, and latency. Neurol Clin.;26(3):675–697. viii.
10. Andrei G and Snoeck R.(2013). Advances in the treatment of varicella-zoster virus infections.Adv Pharmacol;67:107-68
11. Gershon A., Gershon M, Breuer J, Levin M, Oaklander A and Griffiths P .(2010). Advances in the understanding of the pathogenesis and epidemiology of herpes zoster. J. Clin. Virol., 48(Suppl. 1), S2–S7.
12. Lewis S.M., Bgin B.J., and Bates A.(2006). Daci and Lewis practical hematology.10th ed. Churchill Livingston. USA; 1-5.
13. Hamborsky J (2015). Epidemiology and Prevention of Vaccine-Preventable Diseases (PDF) (13 ed.). Washington D.C. Public Health Foundation. pp. 353–374
14. Cho JW. (2007). Polymorphism of the IL-10 gene is associated with susceptibility to herpes zoster in Korea. Journal of Dermatological Science 45(3):213-215.
15. De Paschale M and Clerici P.(2016). Microbiology laboratory and the management of mother-child varicella-zoster virus infection.World J Virol. 12;5(3):97-124.
16. Tian Y, Mollo SB, Harrington LE and Zajac AJ.(2016). IL-10 Regulates Memory T Cell Development and the Balance between Th1 and Follicular Th Cell Responses. J Immunol;197(4):1308-21.
17. Young HoonJeon. (2015).Herpes Zoster and Postherpetic Neuralgia: Practical Consideration for Prevention and Treatment. *Korean J Pain*, 28(3): 177–184.
18. Akdis CA and Akdis M. (2015).Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. World Allergy Organ J.;8(1):17.
19. Li W, Dong H, Huang Y, Chen T, Kong X, Sun H, Yu X and Xu J.(2016). Clonorchissinensis Co-infection Could Affect the Disease State and Treatment Response of HBV Patients.PLoSNegl Trop Dis;10(6):e0004806.
20. Sanin DE, Prendergast CT, Bourke CD and Mountford AP.(2015). Helminth Infection and Commensal Microbiota Drive Early IL-10 Production in the Skin by CD4+ T Cells That Are Functionally Suppressive.PLoS Pathog.;11(5):e1004841.
21. Beichu Guo. (2016).IL-10 Modulates Th17 Pathogenicity during Autoimmune Diseases. J Clin Cell Immunol.; 7(2): 400.
22. Shalev I, Schmelzle M, Robson SC and Levy G. (2011) Making sense of regulatoryT cell suppressive function. SeminImmunol 23: 282-292.

23. Tang-Feldman YJ, Lochhead GR, Lochhead SR, Yu C and Pomeroy C (2011). Interleukin-10 repletion suppresses pro-inflammatory cytokines and decreases liver pathology without altering viral replication in murine cytomegalovirus(MCMV)-infected IL-10 knockout mice. Inflamm Res 60: 233-243.
24. Hoves S, Krause SW, Schütz C, Halbritter D, Schölmerich J, Herfarth H and Fleck M. (2006). Monocyte-derived human macrophages mediate anergy in allogeneic T cells and induce regulatory T cells. J Immunol 177: 2691-2698
25. Bijjiga E and Martino AT. (2013). Interleukin 10 (IL-10) Regulatory Cytokine and its Clinical Consequences. J Clin Cell Immunol S1: 007.
26. Laouini D, Alenius H, Bryce P, Oettgen H, Tsitsikov E and Geha RS.(2003). IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. J Clin Invest.;112(7):1058-66.
27. Cope A, Le Friec G, Cardone J and Kemper C. (2011). The Th1 life cycle: molecular control of IFN-γ to IL-10 switching. Trends Immunol 32: 278-286.
28. Urry Z, Xystrakis E and Hawrylowicz CM.(2006). Interleukin-10-secreting regulatory T cells in allergy and asthma.Curr Allergy Asthma Rep;6:363-371.
29. Smith-Norowitz TA, Josekutty J, Lev-Tov H, Kohlhoff S, Norowitz KB, Silverberg JI, Chice S, Durkin HG and Bluth MH.(2009). IgE anti-varicella zoster virus and other immune responses before, during, and after shingles. Ann Clin Lab Sci.;39(1):43-50
30. Katze MG, He Y and Gale M Jr. (2002). Viruses and interferon: a fight for supremacy. Nat Rev Immunol.;2(9):675-87.
31. Roff SR, Noon-Song EN and Yamamoto JK .(2014). The Significance of Interferon-γ in HIV-1 Pathogenesis, Therapy, and Prophylaxis. Front Immunol.;4:498.
32. Torigoe S, Ihara T and Kamiya H. (2000). IL-12, IFN-gamma, and TNF-alpha released from mononuclear cells inhibit the spread of varicella-zoster virus at an early stage of varicella. MicrobiolImmunol 44:1027–1031.
33. Haberthur K, Meyer C, Arnold N, Engelmann F, Jeske DR and Messaoudi I. (2014).Intrabronchial infection of rhesus macaques with simian varicella virus results in a robust immune response in the lungs. J Virol 88(21):12777-92.
34. Traina-Dorge V, Sanford R, James S, Doyle-Meyers LA, de Haro E, Wellish M, Gilden D and Mahalingam R. 2014. Robust pro-inflammatory and lesser anti-inflammatory immune responses during primary simian varicella virus infection and reactivation in rhesus macaques. J Neurovirol 20:526–530.
35. Bartee E and McFadden G .(2013). Cytokine Synergy: an underappreciated contributor to innate anti-viral immunity. Cytokine.; 63(3): 237–240.
36. Accardo A, Vitiello M, Tesauro D, Galdiero M, Finamore E, Martora F, Mansi R, Ringhieri P and Morelli G. (2014). Self-assembled or mixed peptide amphiphile micelles from Herpes simplex virus glycoproteins as potential immunomodulatory treatment. Int J Nanomedicine. 7;9:2137-48.
37. Bigley NJ.(2014). Complexity of Interferon-γ Interactions with HSV-1. Front Immunol. 2014 Feb 6;5:15.
38. Johnson HM. (2015). Gamma interferon: from antimicrobial activity to immune regulation. Front Immunol.;5:667.
39. Sarkadi J, Jankovics M, Fodor K, Kis Z, Takacs M, Visontai, Jankovics I and Gonczol E. (2015).High-level cellular and humoral immune responses in Guinea pigs immunized intradermally with a heat-inactivated varicella-zoster virus vaccine. Clin Vaccine Immunol.;22(5):570-7.
40. Kwon HJ, Bang DW, Kim EN, Wi CI, Yawn BP, Wollan PC, Lahr BD, Ryu E and Juhn YJ . (2016). Asthma as a risk factor for zoster in adults: A population-based case-control study. J Allergy Clin Immunol.;137(5):1406-12.
41. Grose C, Yu X, Cohrs RJ, Carpenter JE, Bowlin JL and Gilden D. (2013).Aberrant virion assembly and limited glycoprotein C production in varicella-zoster virus-infected neurons. J Virol 87:9643–9648. 14.
42. Sloutskin A, Kinchington PR and Goldstein RS. (2013). Productive vs non-productive infection by cell-free varicella zoster virus of human neurons derived from embryonic stem cells is dependent upon infectious viral dose. Virology 443:285–293.
43. Yu X, Seitz S, Pointon T, Bowlin JL, Cohrs RJ, Jonjic S, Haas J, Wellish M, Gilden D. (2013). Varicella zoster virus infection of highly pure terminally differentiated human neurons. J Neurovirol 19:75–81.
44. Baird NL, Bowlin JL, Hotz TJ, Cohrs RJ and GildenD .(2015). Interferon Gamma Prolongs Survival of Varicella-Zoster Virus-Infected Human Neurons In Vitro. J Virol.;89(14):7425-7.

45. Clark IA. (2007). How TNF was recognized as a key mechanism of disease. Cytokine Growth Factor Rev. 18:335–343.
46. Aggarwal BB. (2003). Signalling pathways of the TNF superfamily: a double-edged sword. Nat. Rev. Immunol. 3:745–756.
47. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J and Herrmann N (2010). "A meta-analysis of cytokines in Alzheimer's disease".Biol Psychiatry. 68 (10): 930–941.
48. Locksley RM, Killeen N, Lenardo MJ (2001). "The TNF and TNF receptor superfamilies: integrating mammalian biology". Cell. 104 (4): 487–501.
49. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010). "A meta-analysis of cytokines in major depression".Biol Psychiatry. 67 (5): 446–457.
50. Victor FC, Gottlieb AB (2002). "TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis". J Drugs Dermatol. 1 (3): 264–75.
51. Brynskov J, Foegh P, Pedersen G, Ellervik C, Kirkegaard T, Bingham A, Saermark T (2002). "Tumour necrosis factor alpha converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease".
52. Seo S. H. and Webster R.G .(2002). Tumor Necrosis Factor Alpha Exerts Powerful Anti-Influenza Virus Effects in Lung Epithelial Cells .J. Virol.;76 :3 1071-1076
53. Wang W, Xu L, Brandsma JH, Wang Y, Hakim MS, Zhou X, Yin Y, Fuhler GM, van der Laan LJ, van der Woude CJ, Sprengers D, Metselaar HJ, Smits R, Poot RA, Peppelenbosch MP and Pan Q.(2016). Convergent Transcription of Interferon-stimulated Genes by TNF-α and IFN-α Augments Antiviral Activity against HCV and HEV.Sci Rep.;6:25482
54. Boussemart L., Jacobelli S., Batteux F., Goulvestre C., Grange P., Carlotti A., Morini J.P., Gorin I., Ziza J.M., Avril M.F. and Dupin N.(2010). Autoimmune Bullous Skin Diseases Occurring under Anti-Tumor Necrosis Factor Therapy: Two Case Reports Dermatology;221:201–205
55. Sethi G, Sung B, Kunnumakkara AB and Aggarwal B. (2009). Targeting TNF for treatment of cancer and autoimmunity. Adv. Exp. Med. Biol. 647:37–51.
56. Kopf M, Bachmann MF and Marsland BJ.(2010). Averting inflammation by targeting the cytokine environment. Nat. Rev. Drug Discov. 9:703–718.
57. Wendling D, Streit G, Toussirot E and Prati C. (2008). Herpes zoster in patients taking TNFalpha antagonists for chronic inflammatory joint disease. Joint Bone Spine.;75:540–543.
58. Garcia-Vidal C, Rodríguez-Fernández S, Teijón S, Esteve M, Rodríguez-Carballeira M, Lacasa JM, Salvador G and Garau J. (2009). Risk factors for opportunistic infections in infliximab-treated patients: the importance of screening in prevention. Eur J ClinMicrobiol Infect Dis.;28:331–337
59. Strangfeld A., Listing J., Herzer P., Liebhaber A., Rockwitez K., Richter C. and Zink A. (2009). Risk of herpes zoster in patients with rheumatoid arthritis treated with anti-TNF-alpha agents. JAMA, 301, 737-44.
60. Tresch S, Trueb RM, Kamarachev J, French LE and Hofbauer GF. (2009). Disseminated herpes zoster mimicking rheumatoid vasculitis in a rheumatoid arthritis patient on etanercept. Dermatology.; 219:347–349.
61. Cruz MJ, Baudrier T, Ferreira O and Azevedo F. (2011). Herpes zoster at the site of infliximab infusion: case report. CutanOcul Toxicol.;30:236–238.
62. Winthrop KL, Baddley JW, Chen L, Liu L, Grijalva CG, Delzell E, Beukelman T, Patkar NM, Xie F, Saag KG, Herrinton LJ, Solomon DH, Lewis JD and Curtis JR..(2013). Association between the initiation of anti-tumor necrosis factor therapy and the risk of herpes zoster . JAMA.;309(9):887-95.
63. Winthrop K., Yamanaka H., Valdez H., Mortensen E., Chew R., Krishnaswami S., Kawabata T. and Riese, R. (2014). Herpes Zoster and Tofacitinib Therapy in Patients With Rheumatoid Arthritis. Arthritis & Rheumatology (Hoboken, N.j.), 66(10), 2675–2684.
64. Yawn BP, Saddier P, Wollan PC, St Sauver JL, Kurland MJ, Sy LS (2007). "A population-based study of the incidence and complication rates of herpes zoster before zoster vaccine introduction". Mayo Clin. Proc. 82 (11): 1341–1349.
65. McDonald JR, Zeringue AL, Caplan L, Ranganathan P, Xian H, Burroughs TE, Fraser VJ, Cunningham F and Eisen SA.(2009). Herpes zoster risk factors in a national cohort of veterans with rheumatoid arthritis.Clin Infect Dis.;48:1364–1371.
66. Di Costanzo L, Ayala F, Megna M, Gaudiello F, Patrì A and Balato N.(2013) The risk of herpes zoster in the anti-TNF-α era: a case report and review of the literature. Journal of Dermatological Case Reports.;7(1):1-4.

67. Zhang M, Wu N, Yang L, Zhang J, Sun X, Zhong S, Ma X and Wang Y.(2011). Study on the T-helper cell 1/2 cytokine profile in blister fluid of patients with herpes zoster and its clinical significance. JDermatol.;38(12):1158-62.
68. Danial, W. (1999). Probability and t distribution Biostatistics, 7th ed. A foundation for analysis in the health sciences.83-123.
69. Salman JM, Abdul-Adel E, Alkaim AF. Effect of pesticide glyphosate on some biochemical features in cyanophyta algae oscillatorialimnetica.International Journal of PharmTech Research.2016; 9: 355-365.

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