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Possible Alterations in the Plasma Levels of Pro (TNF-alpha) and Anti-inflammatory (IL-10) Cytokines in Long-Term Insomnia

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Abstract
Long-term/chronic insomnia occurs in patients for over one month; it can affect the functions of the immune system and responses, which may involve cytokines. The aim was to determine alterations of Tumor necrosis factor alpha (TNF-alpha) and IL-10 cytokines in long-term insomnia. Thirty-three long-term/chronic insomnia patients (male, 22; female, 11) aged 47-61 years were initially recruited. Seven of them who were infected with microbial agents were excluded from the study. Of the 26 free of infectious agents only 21 (female, 5; male, 16) were successfully monitored. Age-matched apparently healthy non-insomnia subjects (male, 25; female, 25) free of the infectious agents were recruited as control. Plasma TNF-alpha, IL-10, HBsAg, anti-HCV, and HIVp24 antigen were assayed by ELISA method while determination of *Mycobacterium tuberculosis* was by Ziehl–Neelsen staining of sputum and *Plasmodium spp.* by thick blood Giemsa staining. Of the 33 insomnia patients initially recruited, 21.2% (7) were infected with microbial agents (*Plasmodium spp.*, 6.1% (2); HCV, 3.0% (1); HBV, 6.1% (2); *Mycobacterium tuberculosis*, 3.0% (1); *Plasmodium spp.* + HBV, 3.0% (1); and HIVp24, 0). Twenty-one chronic insomnia patients were finally investigated on cytokines. There were significantly higher mean plasma values of TNF-alpha and IL-10 cytokines in chronic insomnia patients before treatment than the values obtained in the control subjects ($p < 0.05$). The results also showed a significantly lower mean plasma value of TNF-alpha cytokine in chronic insomnia patients after treatment than the values obtained in the patients before treatment. There were no significant differences in the mean plasma values of IL-10 cytokines in chronic insomnia patients before and after treatment ($p > 0.05$). There was a significant increase in plasma TNF-alpha and IL-10 cytokines in long-term insomnia patients before treatment while plasma TNF-alpha significantly decreased after treatment, and no significant difference was obtained in the plasma IL-10 before and after treatment. TNF-alpha could be a good investigative index of insomnia.

Keywords: TNF-alpha; IL-10; Long term; Insomnia.

1. INTRODUCTION

Insomnia is a sleeping disorder also known as sleeplessness [1]. It could occur for days or weeks (short-term, transient, or acute insomnia) or last for more than a month (long-term or chronic insomnia). Insomnia can be caused by psychological stress, chronic pain, heart failure, hyperthyroidism, heartburn, restless leg syndrome, menopause, medications, caffeine, nicotine, alcohol, menstrual hormones, withdrawal from sedative drugs, hyperthyroidism, rheumatoid arthritis mental disorder, menopausal hormone [2]. Insomnia is more common in people aged 65 years and above than in younger people [3].

Insomnia can bring about poor immune system function [4]. The mechanism of insomnia is cognitive and psychological. Individuals with insomnia are characterized with increased urinary cortisol and catecholamines, increased cerebral utilization of glucose, and increased metabolism [4]. Insomnia influences immune response, which makes the immune system alter sleep patterns. Cytokines regulate immune response [4].

Tumor necrosis factor alpha (TNF-alpha) can be synthesized by cells like CD4+ lymphocytes, Natural killer (NK) cells, neutrophils, mast cells, eosinophils, and neurons [5, 6]. It promotes the inflammatory response; can stimulate phagocytosis; and result in acute phase response, the release of corticotrophin releasing hormone (CRH), suppression of appetite, and fever. It is implicated in depression [7]. Interleukin-10 (IL-10) is an anti-inflammatory cytokine. It inhibits the synthesis of pro-inflammatory cytokines and can enhance B cell survival, proliferation, and production of antibodies [8-10].

This work was designed to determine alterations in the plasma level of pro (TNF-alpha) and anti-inflammatory(IL-10) cytokines in long-term insomnia.

2. METHOD(S)

2.1. Study Area
The study area is Baptist Medical Centre, Saki, Nigeria, which is a faith-based hospital located in the northern part of the Oyo state in Nigeria. It is a 300-bed hospital and a referral center. It shares borders with Kwara State in Nigeria and Burkina Faso. It provides health care services and trains clinicians, medical laboratory technicians, and nurses.

2.2. Study Population
Thirty three (33) patients with long-term insomnia (male, 22; female, 11; 47-61 years) were initially recruited. Seven (7) of them were infected with microbes (*Plasmodium spp.*, 2; HCV, 1; HBV, 2; *Mycobacterium tuberculosis*, 1; *Plasmodium spp.* + HBV, 1) and as such were excluded from the study. Of the 26 free of infectious agents tested for, only 21 (female, 5; male, 16) were successfully monitored. Apparently healthy non-insomnia subjects within the same age group as the test volunteers (male, 25; female, 25) free of the infectious agents were studied as control. Sputum and blood samples were obtained from the subjects in the morning.
Duration of study: 4 months

2.3. Recruitment of Chronic Insomnia Patients
Insomnia patients of more than one month duration were recruited from the medical outpatient department of Baptist Medical Centre, Saki, Nigeria, before the commencement of treatment through the nurses and physicians in charge and by also utilizing the patients' medical histories.

2.4. Inclusion and Exclusion Criteria
Insomnia patients infected with HCV, HBV, *Mycobacterium tuberculosis*, *Plasmodium spp.,* and HIV; patients having fever, hyperthermia, episode of jaundice; and those on medication/treatment were excluded from the study.

2.5. TNF-alpha ELISA Using ABCAM Kit (USA)
Principle: A monoclonal antibody-specific TNF-alpha was coated on the wells of microtiter strips. Plasma, standards of known TNF-alpha concentrations, and control specimens were pipetted into the wells and made to react with a biotinylated monoclonal antibody specific for TNF-alpha, the enzyme streptavidin-HRP that binds the biotinylated antibody and TMB substrate to produce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of TNF-alpha present in the samples.

2.6. IL-10 ELISA Using ABCAM Kit (USA)
Principle: An antibody specific to human IL-10 coated on a microtiter well was allowed to react with standards, control, and samples pipetted into it. The mixture, after incubation and washing, was made to react with a biotinylated antihuman IL-10 antibody, HRP-conjugated streptavidin, and TMB substrate for color development, which is proportional to the amount of IL-10 in the sample at 450 nm. On addition of stop solution, the color changed from blue to yellow.

2.7. HIV1 p24 ELISA Using ABCAM Kit (USA)
Principle: Affinity-tag labeled binds the antibody, conjugated detector antibody immune captured and the sample analyte to formed a complex, which in turn immobilized via immunoaffinity of an antitag antibody coating the microtiter well, which react with a biotinylated antibody, TMB substrate, and HRP to produce a blue color. Upon the addition of the stop solution, the color changed from blue to yellow, the color intensity of which is proportional to the amount of bound analyte at 450 nm.

2.8. HBsAg ELISA Assay Using Diagnostic Automation/Cortez Diagnostics Inc., Kit (21250 Califa St., Suite 102-116, Woodland Hills, California, 91367 USA)
Principle: Monoclonal antibodies specific to HBsAg were precoated on polystyrene microwells, which reacted with the serum or plasma sample/standard/control, a second antibody, horseradish peroxidase conjugate, a chromogen that is tetramethylbenzidine (TMB), and urea peroxide to form a blue-colored product on the addition of sulfuric acid; the blue color then turns yellow. If the wells remain colorless, HBsAg result is negative.

2.9. Identification of *Plasmodium spp*. Using Giemsa Thick-Film Method
It was determined as described by Cheesbrogh [11].

2.10. Determination of *Mycobacterium Tuberculosis* Infection Using Sputum Ziehl–Neelsen Staining
The sputum samples were analyzed to determine Acid Fast Bacilli (AFB) as described by Cheesbrogh [11].

2.11. Anti HCV ELISA Assay

This was carried out using AccuDiag™ HCV Ab ELISA kit (USA).

Principle: A recombinant immunoreactive antigen corresponding to the core and the nonstructural regions of HCV were coated on microtiter, wells which was made to react with sample/control/standards, rabbit anti-human IgG antibodies conjugated to the enzyme horseradish peroxidase, a chromogen-tetramethyl-benzidine, and urea peroxide to /form a blue-colored solution that turns yellow after the addition of sulfuric acid. The color intensity is proportional to the amount of anti-HCV in the sample.

2.12. Statistical Analysis

The results obtained were subjected to statistical analysis to determine mean, standard deviation, student's t, and probability values at 0.05 level of significance by using SPSS 18.0.

2.13. Ethical Consideration

Ethical approval was obtained from the Research and Ethical committee of Baptist Medical Centre, Saki, Nigeria, before the commencement of the work. The consent of each of the test and control volunteers was also obtained.

3. RESULTS

Of the 33 insomnia patients initially recruited, 21.2% (7) were infected with microbial agents (*Plasmodium spp.,* 6.1% (2); HCV, 3.0% (1); HBV, 6.1% (2); *Mycobacterium tuberculosis*, 3.0% (1); *Plasmodium spp.* + HBV, 3.0% (1), and HIVp24, 0) (Figure 1).

Twenty one (21) patients with chronic insomnia were eventually investigated on cytokines.

There were significantly higher mean plasma values of TNF-alpha and IL-10 cytokines in chronic insomnia patients before treatment than the values obtained in the control subjects ($p < 0.05$; Table 1; Figure 2).

There were significantly lower mean plasma values of TNF-alpha cytokine in chronic insomnia patients after treatment than the values obtained in the patients before treatment ($p < 0.05$; Table 1; Figure 2).

The results showed no significant difference in the mean plasma values of TNF-alpha and IL-10 cytokines in chronic insomnia patients after treatment compared with the values obtained in the control subjects ($p > 0.05$; Table 1; Figure 2).

The results obtained also showed no significant difference in the mean plasma values of IL-10 cytokines in chronic insomnia patients before and after treatment ($p > 0.05$; Table 1; Figure 2).

4. DISCUSSION

Of the 33 insomnia patients initially recruited, 21.2% (7) were infected with microbial agents (*Plasmodium spp.*, 6.1% (2); HCV, 3.0% (1); HBV, 6.1% (2); *Mycobacterium tuberculosis*, 3.0% (1); *Plasmodium spp.* + HBV, 3.0% (1)). Infectious diseases in patients

Figure 1: Prevalence of *Plasmodium spp.*; HCV; HBV; *Mycobacterium tuberculosis*, 3.0% (1); and HIVp24 among the insomnia patients initially recruited.

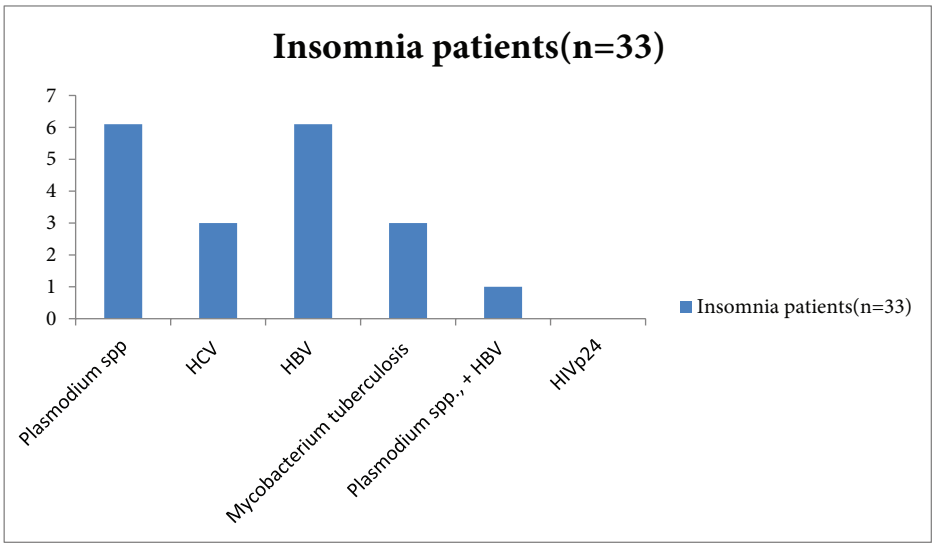

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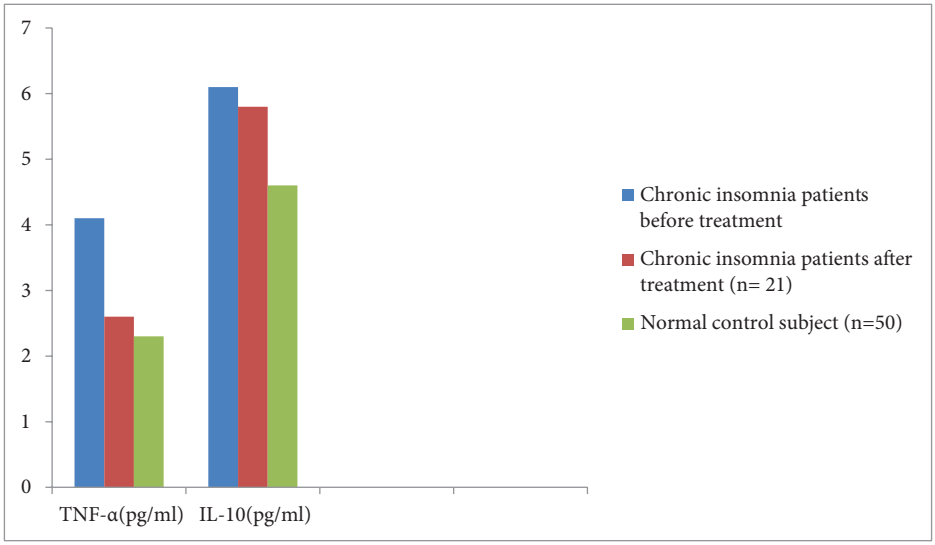
Table 1: Plasma TNF-alpha and IL-10 mean, standard deviation, student's t, and probability values obtained in the subjects.

		Chronic insomnia patients before treatment (*n* = 21)	Chronic insomnia patients after treatment (*n* = 21)	Normal control subject (*n* = 50)	Chronic insomnia patients before treatment vs. chronic insomnia patients after treatment	Chronic insomnia patients before treatment (*n* = 21) vs. normal control subject	Chronic insomnia patients after treatment vs. normal control subject
TNF-α (pg/mL)	Mean ± SD	4.1 ± 0.2	2. 6 ± 0.4	2.3 ± 0.3	-	-	-
	t value	-	-	-	0 3.3541	4.9923	0.6.
	p value	-	-	-	0.039279*	0.01893*	0.30472
	comment				Significant	Significant	Not significant
IL-10 (pg/mL)	Mean ± SD	6.1 ± 0.2	5.8 ± 0.3	4.6 ± 0.3	-	-	-
	t value	-	-	-	0.83205	4.16025	2.82843
	p value	-	-	-	0.246454	0.02660*	0.052786
	Comment	-	-	-	Not significant	Significant	Not significant

Figure 2: Comparative description of plasma TNF-alpha and IL-10 values obtained in the subjects.



with chronic insomnia could be due to the fact that insomnia can bring about poor immune system function, which may make the affected individuals susceptible to infectious agents [4].

There were significantly higher mean plasma values of TNF-alpha and IL-10 cytokines in chronic insomnia patients before treatment than the values obtained in the control subjects. The results showed no significant difference in the mean plasma values of TNF-alpha and IL-10 cytokines in chronic insomnia patients after treatment compared with the values obtained in the control subjects. The results also revealed no significant difference in the mean plasma values of IL-10 cytokines in chronic insomnia patients before and after treatment.

Significant increase in plasma TNF-alpha could be attributed to the report of [12] that TNF-alpha is a fatigue-inducing cytokine. The daytime shift TNF secretion is responsible for fatigue, causing difficulty in falling asleep [9] because under disease conditions, increase in pro-inflammatory cytokines plasma level correlates with increase in fatigue, which makes the affected patient more restless and tired [9]. These findings could also be associated with the reports of [6] that sleep and the circadian system can influence immune functions. In a normal sleep–wake cycle, the production of pro-inflammatory cytokines reach peaks during early nocturnal sleep whereas anti-inflammatory cytokine activity peaks during daytime wakefulness [6] Furthermore, TNF does not promote rapid eye movement sleep in physiological and inflammatory conditions [13]. Elevated proinflammatory cytokines have also been attributed to poor sleep quality in hemodialysis patients [14]. Increase in IL-10 before treatment could

be attributed to immune response of the cytokines as anti-inflammatory substance against the health effect of TNF-alpha, as IL-10 inhibits the production of pro-inflammatory cytokines like TNF-alpha [8-10].

There was a significantly lower mean plasma value of TNF-alpha cytokine in chronic insomnia patients after treatment than the values obtained in the patients before treatment. This could be attributed to convalescence as a result of the clinical intervention to reduce fatigue and sleeplessness [13, 14].

5. CONCLUSION

There was a significant increase in the plasma level of TNF-alpha and IL-10 in patients with chronic insomnia but there was a reduction in the plasma level of TNF-alpha. TNF-alpha could be a good investigative index of insomnia.

Author Contributions

This work was carried out with the collaboration of all the authors. Mathew F. Olaniyan was responsible for the design, sample collection, data analysis, literature search, and preparation of the research report. In addition, Alade A. Ogunlade carried out the sample collection, literature search, and preparation of the research report.

Conflict of Interest

None.

Funding

None.

References

1. Roth T. Insomnia: definition, prevalence, etiology, and consequences. J Clin Sleep Med. 2007; 3(5 Suppl):S7-10. PMC 1978319. PMID 17824495
2. Golub RM. Insomnia. JAMA. 2012; 307(24):2653. doi:10.1001/jama.2012.6219. PMID 22735439
3. Qaseem A, Kansagara D, Forciea MA, Cooke M, Denberg TD; Clinical Guidelines Committee of the American College of, Physicians. Management of Chronic Insomnia Disorder in Adults: a clinical practice guideline from the American College of Physicians. Ann Intern Med. 2016; 165(2):125-33. doi:10.7326/M15-2175. PMID 27136449
4. Wilson JF. In the clinic: insomnia. Ann Intern Med. 2008; 148(1):ITC13-1-ITC13-16. doi:10.7326/0003-4819-148-1-200801010-01001. PMID 18166757
5. Watson NF, Vaughn BV. Clinician's Guide to Sleep Disorders. CRC Press, Boca Raton, FL (2006), p: 10. ISBN 9780849374494
6. Besedovsky L, Lange T, Born J. Sleep and immune function. Pflugers Arch. 2012; 463(1):121-37.
7. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 2001; 104(4):487-501. doi:10.1016/S0092-8674(01)00237-9. PMID 11239407
8. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, *et al*. A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry. 2010; 68(10):930-41. doi:10.1016/j.biopsych.2010.06.012. PMID 20692646
9. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, *et al*. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010; 67(5):446-57. doi:10.1016/j.biopsych.2009.09.033. PMID 20015486
10. Eskdale J, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. Immunogenetics. 1997; 46(2):120-28. doi:10.1007/s002510050250. PMID 9162098
11. Monica C. District Laboratory Practice in Tropical Countries Part 2. 2nd ed. Published in the United States of America by Cambridge University Press, New York (2006). www.cambridge.org/9780521676311
12. Vgontzas AN, Zoumakis M, Papanicolaou DA, Bixler EO, Prolo P, *et al*. Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. Metabolism. 2002; 51(7):887-92. PMID: 12077736
13. Krueger JM. The role of cytokines in sleep regulation. Curr Pharm Des. 2008; 14(32):3408-16.
14. Taraz M, Khatami ME, Hajiseyedjavadi M, Farrokhian A, Amini M, *et al*.. Association between antiinflammatory cytokine, IL-10, and sleep quality in patients on maintenance hemodialysis. Hemodial Int. 2013; 17(3):382-90.