

<https://doi.org/10.33472/AFJBS.6.10.2024.861-868>



African Journal of Biological Sciences



Research Paper

Open Access

Serum Homocysteine Levels In Patients With Hashimoto's Thyroiditis

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Article History Volume 6, Issue 10, 2024

Accepted : 29 April 2024

doi: 10.33472/AFJBS.6.10.2024.861-868

Abstract :

Introduction : Hashimoto's thyroiditis is a chronic autoimmune thyroid disease characterized by presence of antibodies specific to thyroid antigen especially against thyroperoxidase (anti-TPO), antithyroglobulin (anti-Tg). Homocysteine is sulfur containing amino acid which is produced during catabolism of essential amino acid methionine. Autoimmune thyroiditis is often associated with autoimmune gastritis which has parietal cell antibodies (PCA) in the circulation and it directs against the proton pump (H⁺/K⁺ ATPase) and intrinsic factor resulting into atrophic gastritis. This atrophic gastritis reduces the availability of functional gastric mucosa and the ability to secrete intrinsic factor which causes malabsorption of vitamin B12 and so elevated level of homocysteine level. Hyperhomocysteinemia is connected with coronary heart disease (CHD) and cardiovascular diseases (CVD) by endothelial dysfunction and induces apoptotic cell death. Hence this study can help in early diagnosis of cardiovascular diseases in Hashimoto's thyroiditis.

Objective : Aim of the study is to evaluate the serum total homocysteine level and serum TSH level in patients with Hashimoto's thyroiditis and also to find if there is correlation between TSH level and homocysteine level.

Material and Method : A total 84 individuals were participated into the study : one group had 42 diagnosed patients of Hashimoto's thyroiditis of age group 20-55 and another group had 42 age, sex matched healthy controls. Serum total homocysteine level and serum TSH level were assayed by Enzymatic method and electrochemiluminescence method respectively.

Result : Mean age (35 ± 9.71 Vs. 38 ± 7.99 years, p=0.13). Thirty three (78.5%) cases and 30 (71.4%) control were females. Serum TSH level (6.49 ± 1.17 Vs. 1.5 ± 0.71 mIU/L, p<0.0001) which was significantly higher in cases as compared to control. Serum total homocysteine level (18.0 ± 3.66 Vs. 8.2 ± 2.61 μmol/l, p<0.0001) was significantly higher in patients as compared to control. Positive correlation was found between TSH level and total homocysteine level (r=0.96).

Conclusion : Positive correlation between TSH level and Homocysteine level in Hashimoto's thyroiditis contribute to increased cardiovascular risk and we can consider total homocysteine level as screening in Hashimoto's thyroiditis

Introduction :

Hashimoto's thyroiditis is a chronic autoimmune thyroid disease characterized by presence of antibodies specific to thyroid antigen especially against thyroperoxidase (anti-TPO), antithyroglobulin (anti-Tg), lymphocyte infiltration, and increased thyroid gland size[2,3,4]. According to American thyroid association, normal biological interval for serum TSH level ranges from 0.4-4mIU/L [19]. It is most frequent autoimmune disorder which causes hypothyroidism in 20-30% of patients[1]. It is often cause of subclinical hypothyroidism[25]. Clinically it is characterized mainly by systematic manifestation due to damage of the thyroid gland developing primary hypothyroidism[6].

In the thyroid gland, CD4+ T helper 1 cells induces CD8+ cytotoxic T helper 2 cells via proinflammatory cytokines[5]. These proinflammatory cytokines includes interleukin-6, interferon gamma, tumor necrosis factor alpha, interleukin-1b, etc[5]. Due to reactivity of autoantibodies and loss of immune tolerance, there is infiltration with T and B cells which causes destruction of thyroid cell by apoptosis[5]. The prevalence of Hashimoto's varies by geographical region which is 5.8 (95% CI 2.8-9.9%) in Asia [26].

Homocysteine is a sulfur containing amino acid which is present in blood, produced during catabolism of essential amino acid methionine[8]. Homocysteine metabolism takes place by intersection of two metabolic pathway i.e. remethylation and transsulfuration[7]. N-5-methyl-tetrahydrofolate(MTHF) act as methyl group donor to homocysteine for formation of methionine which is vitamin B12 dependant in remethylation[7,10]. Autoimmune thyroiditis is often associated with autoimmune gastritis[15]. Autoimmune gastritis is the disease with presence of parietal cell antibodies (PCA) in the circulation which directs against the proton pump (H⁺/K⁺ ATPase) and intrinsic factor[15,16]. It is a characterized by atrophy of corpus and fundus of stomach[16]. Resulting atrophic gastritis reduces the availability of functional gastric mucosa and the ability to secrete intrinsic factor which causes malabsorption of vitamin B12[17]. Eventually there is deficiency of Vitamin B12 causing elevated level of homocysteine[18].

Hyperhomocysteinemia is defined as a pathological condition of excessive plasma homocysteine level with normal levels ranges from 4 to 12.3 $\mu\text{mol/l}$ [13]. Elevated level of homocysteine is connected with coronary heart disease (CHD) and cardiovascular diseases (CVD) [8,9]. Recent study have shown that homocysteine causes endothelial dysfunction and induces apoptotic cell death contributing in development of atherothrombotic disease[11,12]. In a recent study, it is found that there is association between Hashimoto's thyroiditis and dyslipidemia[14]. Increased cardiovascular morbidity is related to dyslipidemia which are normalized after thyroid replacements [15]. Dyslipidemia may be partially liable for the high risk of vascular diseases in hypothyroidism. However dyslipidemia in patients with hypothyroid cannot be solely liable for increase in atherosclerosis and cardiovascular diseases[15]. Hence this study can help in early diagnosis of cardiovascular diseases in Hashimoto's thyroiditis. In this study we aimed to evaluate the serum total homocysteine level and serum TSH level in patients

with Hashimoto's thyroiditis, also to find if there is correlation between TSH level and homocysteine level.

Method and Material :

Cross sectional case control study were done in LTMMC & GH, SION from December 2023 to April 2024. Total 84 individuals were included into study. Inclusion and exclusion criteria were applied to both cases and control. 42 cases of already diagnosed Hashimoto's thyroiditis aged between 20-55 years were taken as inclusion criteria. Exclusion criteria includes of patients with history of heart disease, renal disease, bone disease, thyroidectomy, malignant tumor, autoimmune disease, patient on medication such as lipid lowering drugs, antithyroid drugs, hematinic drugs. 42 age and sex matched disease free healthy adult of age group 20-55 years were taken as control.

5 ml venous blood sample of all patients were collected in plain(red) vacutainers from thyroid opd, LTMMC, Sion. Blood samples initially centrifuged at 3000 rpm for 10 minutes. serum stored at -20° within 2 hrs of collection until analyzed. Analysis done for serum TSH level and serum total homocysteine level. Serum TSH level were determined by electrochemiluminescence method and serum total homocysteine level were determined by Enzymatic method . Serum TSH levels and serum total homocysteine levels of cases and control were compared. Mean values are interpreted with their 95% confidence interval. Statistical analysis was made using student's t test; $p < 0.05$ are considered as statistically significant.

Statistical Analysis :

All statistical data were analysed using Graphpad prism 5 software and microsoft excel 2021. Sample is collected by simple random sampling method. Total 84 individuals were taken which had divided into two groups. We used mean, standard deviation expressed with 't' test and to compare two groups (cases group and control group). All statistical analysis results with a two tailed p value < 0.05 were considered significant. Pearson correlation coefficients (r) were calculated to quantify the relationship between TSH levels and homocysteine levels.

Result :

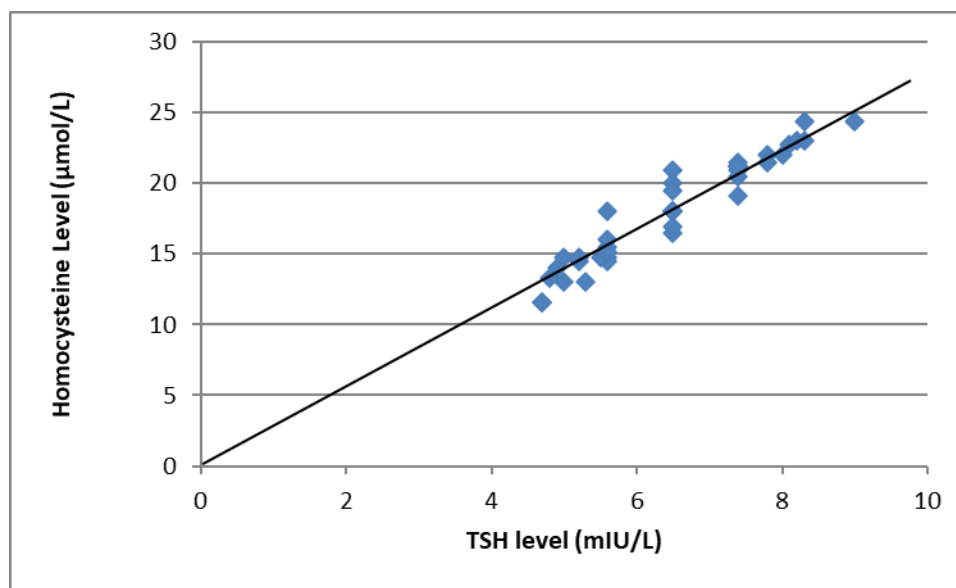
Total 84 individuals were taken into study. 42 were taken into control group and 42 were as cases . The baseline characteristics of case and control were found respectively: Mean age (35 ± 9.71 Vs. 38 ± 7.99 years , $p=0.13$). Thirty three (78.5%) cases and 30 (71.4%) control were females. Serum TSH level (6.49 ± 1.17 Vs. 1.5 ± 0.71 mIU/L, $p < 0.0001$) which was significantly higher in cases as compared to control. Serum total homocysteine level (18.0 ± 3.66 Vs. 8.2 ± 2.61 $\mu\text{mol/l}$, $p < 0.0001$) was significantly higher in patients as compared to control. The distribution of demographic and laboratory findings of study group are mentioned in table1.

In Hashimotos's thyroiditis, a positive correlation was found between TSH level and total homocysteine level ($r=0.96$) which shown by graph 1.

Table 1: Distribution of demographic and laboratory findings in study population.

| Variables | Hashimoto's thyroiditis n = 42 | Control n = 42 | p |
|--|-----------------------------------|------------------------------|----------|
| Age (years) | 35 ± 9.71 | 38 ± 7.99 | p=0.13 |
| Gender | F= 33(78.5%) M=9(21.4%) | F= 30(71.4%) M=12(28.15%) | |
| Serum TSH level (mIU/L) | 6.49 ± 1.17 | 1.5 ± 0.71 | p<0.0001 |
| Serum total Homocysteine level(μmol/l) | 18.0 ± 3.66 | 8.2 ± 2.61 | p<0.0001 |

Graph 1 :The relationship between TSH level and total homocysteine level



Discussion :

Increased homocysteine level may be due to genetic defect, defective metabolism, excessive intake of dietary methionine, deficiencies of vitamin B12, ,vitamin B6 and folate, and defective kidney function[20,21]. Homocysteine plays role in development of cardiovascular diseases by several mechanism such as increased proliferation of vascular smooth muscle cells, endothelial dysfunction, oxidative damage, increased synthesis of collagen, initiating an inflammatory response in vascular smooth muscle cells by increased production of CRP through NMDAr-ROS-ERK1/2/p38 NF-κB signal pathway as well as increase in the levels of cholesterol, triglyceride and LDL, and activity of HMG-CoA reductase[20,22,24]. Increased plasma homocysteine level affects platelet aggregation, vasomotor function and plasma anticoagulant function[20,22]. Hyperhomocysteinemia also causes nervous system disorder such as Alzheimer's disease and dementia, stroke[20,23,24].

In present study, elevated serum homocysteine levels were strongly associated with Hashimoto's thyroiditis. Serum homocysteine levels were significantly higher in patients with Hashimoto's thyroiditis compared to control group ($p < 0.0001$). This similar observation is mentioned in previous studies by Sengul E. et al in 2004, Bolal M. et al in 2020[25,28]. Study by Turhan S. et al in 2008 had shown that in majority of patients with subclinical hypothyroidism, Serum total homocysteine levels were not elevated and was not significantly higher in subclinical hypothyroidism[29]. In our study serum total homocysteine levels were significantly related to serum TSH level and there was positive correlation between serum TSH levels and serum total homocysteine levels. In coronary heart disease, relative risk increases by 40% for increased serum homocysteine level of 4 $\mu\text{mol/L}$ [27]. Increased serum total homocysteine level might be due result of increased formation of homocysteine, explained as deficiency of vitamin B12 is there in Hashimoto's thyroiditis [17]. Thus thyroid hormone may have influence on serum total homocysteine level through vitamin B12.

Conclusion :

Present study confirmed strong association between serum TSH level and serum total homocysteine level. Also it confirmed positive relation of serum TSH level and serum total homocysteine level. So, increased serum total homocysteine level may contribute to increased cardiovascular risk and we can consider total homocysteine level as screening in Hashimoto's thyroiditis for detection of cardiovascular risk, hence we can recommend vitamin B12 supplementation in Hashimoto's thyroiditis to prevent homocysteine induced cardiac complications.

Conflict of Interest: None declared.

Acknowledgement : Our acknowledgement to all the participants of our work. The authors have no conflicts of interest to disclose.

References :

1. Ragusa F, Fallahi F, Elia G, Gonnella D Et Al. Hashimoto's Thyroiditis: Epidemiology, Pathogenesis, Clinic And Therapy. Best Practice & Research Clinical Endocrinology & Metabolism.2019; 33 (6). Doi.Org/10.1016/J.Beem.2019.101367
2. Rallia M , Angelettia D, Fioreb M Et Al. Hashimoto's Thyroiditis: An Update On Pathogenic Mechanisms, Diagnostic Protocols, Therapeutic Strategies, And Potential Malignant Transformation.Autoimmunity Reviews 19.2020; Doi.Org/10.1016/J.Autrev.2020.102649
3. Pyzik A,Grywalska E,Matyjaszek-Matuszek B Et Al. Immune Disorders In Hashimoto's Thyroiditis:What Do We Know So Far?.Journal Of Immunology Research.2015; Doi.Org/10.1155/2015/979167

4. Chao G, Zhu Y Et Al. Frontiers In Endocrinology. Correlation Between Hashimoto's Thyroiditis-Related Thyroid Hormone Levels And 25-Hydroxyvitamin D. Front. Endocrinol. 2020 ; 11(4). Doi: 10.3389/Fendo.2020.00004
5. Bolal M, Ates I Et Al. The Relationship Between Homocysteine And Autoimmune Subclinical Hypothyroidism. International Journal Of Medical Biochemistry.2020;3(1):1-7. DOI: 10.14744/Ijmb.2019.13008
6. . Ralli M Et Al. Hashimoto's Thyroiditis: An Update On Pathogenic Mechanisms, Diagnostic Protocols, Therapeutic Strategies, And Potential Malignant Transformation. Autoimmunity Reviews.2020;19(10). <https://doi.org/10.1016/j.autrev.2020.102649>.
7. Jacob Selhub. Public Health Significance Of Elevated Homocysteine.Food And Nutrition Bulletin. 2008; 29 (2)
8. Peng Et Al. Elevated Homocysteine Levels And Risk Of Cardiovascular And All-Cause Mortality: A Meta-Analysis Of Prospective Studies Journal Of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) .2015;16(1):78-86. Doi:10.1631/Jzus.B1400183
9. Owecki M, Dorszewska J Et Al. Serum Homocysteine Levels Are Decreased In Levothyroxine-Treated Women With Autoimmune Thyroiditis. BMC Endocrine Disorders.2014;14(18).
10. Froese D.S, Fowler B, Et Al. Vitamin B12, Folate, And The Methionine Remethylation Cycle-Biochemistry, Pathways, And Regulation. Journal Of Inherited Metabolic Disease.2019;42:673–685. DOI: 10.1002/Jimd.12009
11. Güven A, İnanç F. Plasma Homocysteine Levels In Patients With Metabolic Syndrome. Eur J Gen Med 2004; 1(2): 38-42
12. Austin RC,Lentz SR Et Al.Role Of Hyperhomocysteinemia In Endothelial Dysfunction And Atherothrombotic Disease.Cell Death And Differentiation.2004(11):S56- S64. Doi:10.1038/Sj.Cdd.4401451
13. Lai W.K.C Ming , Kan Y Et Al. Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1–12 DOI: 10.1159/000437098
14. Cengiz H , Demirci T , Varim C. The Effect Of Thyroid Autoimmunity On Dyslipidemia In Patients With Euthyroid Hashimoto Thyroiditis. Pak J Med Sci. 2021 ; 37 (5) 1365-1370 Doi: <https://doi.org/10.12669/Pjms.37.5.3883>

15. Carvalho G.A.D, Muniz Figuera. T.M Effect Of Gastrointestinal Disorders In Autoimmune Thyroid Diseases. *Transl Gastrointest Cancer*. 2015;4(1):76-82. Doi: 10.3978/J.Issn.2224-4778.2014.07.03
16. Tozzoli A,B , Kodermaz A.G, Et Al. Autoantibodies To Parietal Cells As Predictors Of Atrophic Body Gastritis: A Five-Yearprospective Study In Patients With Autoimmune Thyroid Diseases. *Autoimmunity Reviews* 10. 2010: 80–83. Doi:10.1016/J.Autrev.2010.08.006
17. Collins A.B, Pawlak R. Prevalence Of Vitamin B-12 Deficiency Among Patients With Thyroid Dysfunction. *Asia Pac J Clin Nutr* 2016;25(2):221-226. Doi: 10.6133/Apjcn.2016.25.2.22
18. Remacha A.F, Souto J.C Et Al. Vitamin B12 Deficiency, Hyperhomocysteinemia And Thrombosis: A Case And Control Study. *Int J Hematol* . 2011; 93:458–464. DOI 10.1007/S12185-011-0825-8
19. TSH Reference Ranges Should Be Used To Safely Guide Thyroid Hormone Treatment In Hypothyroid Patients. *Clinical Thyroidology For Public*.2020;13(1):5-6.
20. Zhou Y , Chen Y . Association Between Plasma Homocysteine Status And Hypothyroidism: A Meta-Analysis. *Int J Clin Exp Med*. 2014;7(11):4544-4553
21. Mutairi F.A. Hyperhomocysteinemia: Clinical Insights. *Journal Of Central Nervous System Disease*. 2020; 12 : 1–8 . Doi.Org/10.1177/117957352096223
22. Ganguly P, Alam S.F. Role Of Homocysteine In The Development Of Cardiovascular Disease. *Ganguly And Alam Nutrition Journal*. 2015; 14(6)
23. Petras M, Tatarikova Z. Hyperhomocysteinemia As A Risk Factor For The Neuronal System Disorders. *Journal Of Physiology And Pharmacology*. 2014 ; 65 (1) 15-23
24. Sharma M Et Al. A Hyperhomocysteinemia: Impact On Neurodegenerative Diseases. *Basic & Clinical Pharmacology & Toxicology*. 2015; 117: 287–296. Doi: 10.1111/Bcpt.12424
25. Sengul E Et Al. Homocysteine Concentrations In Subclinical Hypothyroidism. *ENDOCRINE RESEARCH*. 2004 ; 30 (3) : 351–359
26. Hu X, Chen Y Et Al.. Global Prevalence And Epidemiological Trends Of Hashimoto's Thyroiditis In Adults: A Systematic Review And Meta-Analysis. *Front Public Health*. 2022.

Doi: 10.3389/Fpubh.2022.1020709. PMID: 36311599; PMCID: PMC9608544.

27. S Bamashmoos S.A Et Al. Relationship Between Total Homocysteine, Total Cholesterol And Creatinine Levels In Overt Hypothyroid Patients. Springerplus . 2013 ; 2:423

28. Bolal M, Ates I Et Al. The Relationship Between Homocysteine And Autoimmune Subclinical Hypothyroidism. Int J Med Biochem.2020;3(1):1-7 DOI: 10.14744/Ijmb.2019.13008

29. Turhan S , Sezer S Et Al. Plasma Homocysteine Concentrations And Serum Lipid Profile As Atherosclerotic Risk Factors In Subclinical Hypothyroidism. Ann Saudi Med.2008; 28(2): 96-101