Iraqi Journal of Biotechnology, 2023, Vol. 22, No. 1, 148-153

Role of *BIRC5* Gene Polymorphisms with Serum Level of Survivin in Iraqi Patients with Multiple Sclerosis

Masra M. Abdulkarim, Da'ad A. Hussain

Institute of Genetic Engineering and Biotechnology, University of Baghdad

Received: March 1, 2023/ Accepted: April 11, 2023/ Published: June 4, 2023

Abstract: Multiple sclerosis, often known as MS, is a disease of the central nervous system that is brought on by damage to the myelin sheath, which is a protective covering that surrounds and shields nerve cells in the brain and spinal cord. Undoubtedly, the immunological mechanisms are involved in the pathogenesis of this disease, but the initiating factors remain obscure. This study aimed to role the baculoviral inhibitor apoptosis protein (IAP) repeat containing 5 BIRC5) rs17878467 C/T) gene to regulate survivin levels in Iraqi patients diagnosed with multiple sclerosis. This study included hundred volunteers: fifty patients with multiple sclerosis, 18 (36.00%) males and 32 (64.00%) females. The age mean of MS patients was (38.14) years. While the fifty remaining samples were taken from healthy people 27 (54.00%) males and 23 (46.00%) females, the age mean was (41.04) years. Molecular study was performed in order to investigate the two single nucleotide polymorphisms (SNPs) for BIRC5 gene in chromosome 17 (rs9904341 and rs17878467) for studied groups which have determined using high resolution method (HRM) genotyping assay by real time polymerase chain reaction (RT-PCR) using the Qiagen rotor gene Q real-time PCR system. This study concluded that the rs17878467 C/T genotype was associated with increased risk for MS in Iraqi patients with highly significant difference. It was concluded that serum level of the survivin protein was higher in multiple sclerosis patients than the control group with statistically significant differences that can be considered a diagnostic marker.

Keywords: Multiple sclerosis, BIRC5 gene, Survivin, Apoptosis

Corresponding author: (Email: masramasra002@gmail.com).

Introduction

Multiple sclerosis, sometimes known as MS, is an autoimmune illness that causes chronic inflammation and demyelination of nerve cell axons, especially in the white matter of the brain and spinal cord (1). The average age of a patient with multiple sclerosis is between 20 and 45 years old, and the disease is found disproportionately often in young individuals (2).

It characterized as a disease marked by many sclerotic plaques in the spinal cord and brain of afflicted individuals. MS an etiology is unknown, but theories suggested the genetic factors, Vitamin D deficiency, cigarette smoking, and microbial agent as risk factors. Multiple sclerosis is a disease thought to be mediated by the immune system. However, there have been no particular antigens (proteins that excite the immune system) identified in multiple sclerosis (3). The immune cells seek out and assault the central nervous system (CNS), primarily causing damage to the myelin sheath, which is the axonal membrane that surrounds the nerve fibers, disrupting nerve impulses. The presentation and severity of symptoms will vary depending on the site of myelin sheath damage and which regions of the spinal cord or brain are affected; this will lead appearance of neurologic symptoms (4). MS is often

divided into four main types based on the length of the disease: relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS), primary-progressive MS (PPMS), and progressive relapsing MS (PRMS) (5).

Baculoviral inhibitor apoptosis protein (IAP) repeat containing 5 (*BIRC5*) is a gene that encodes a 16.5 kDa protein called survivin. This gene is located on the telomeric end of the human chromosome 17 (17q25.3), and it consists of an N-terminal Zn^{2+} binding BIR domain linked to a 65A amphipathic C-terminal-helix, in addition to 3 introns and 4 exons (6). It is a member of the family of genes known as inhibitors of apoptosis (IAP) and plays a role in the process of both apoptosis and cell proliferation (7).

The nucleus, the cytoplasm, the mitochondria, the exosomes, the outer surface of the cell membrane, and the extracellular matrix are all known locations for survivin to be found. Survivin's numerous roles are influenced by its subcellular location, its ability to reversibly dimerize, and its extensive posttranslational modification, which includes acetylation, ubiquitination, and notably phosphorylation (8). In mammalian cells, the protein survivin is involved in at least three different cellular processes: the control of mitosis, the regulation of apoptosis, and the cellular stress response (9). Survivin is a protein that has multiple functions, and in mammalian cells it participates in at least these three processes. During the developmental stage of an organism, survivin is expressed to a high degree, in contrast to differentiated tissues, where it is not expressed at all in healthy organisms. It has been shown that survivin regulates the immune system, specifically the formation and differentiation of effector CD4+ T cells, the hemostasis of CD8+ memory T cells, and the proliferation of activated T cells. It has been shown that the G/C single-nucleotide polymorphism (SNP) in the promoter of the *BIRC5* gene may affect the expression level of survivin (10). It has been reported that the-241C/T (rs17878467) SNP in the *BIRC5* promoter region modulates the production of survivin. While rs17878467 boosted the activity of the *BIRC5* promoter in HeLa cell lines, (11). Survivin playing important roles in the pathogenesis of autoimmune diseases. (12). Survivin is the smallest member belonging to one of the eight members of the family of proteins known as inhibitors of apoptosis (IAP). These proteins are critically important for the regulation of cell mitosis as well as the prevention of apoptosis. Both the process of organ regeneration following resection and the promotion of epithelial cell proliferation are dependent on the protein survivin (13). Research has shown that survivin goes through a process that controls its movement between the nucleus, mitochondria, cytoplasm, exosomes, and extracellular matrix. It is vital to have survivin because it is selectively produced in tumor tissues, where it upregulates mitosis and cell survival while downregulating apoptosis and cell death.

Survivin does this in a way that makes it a key player in the fight against cancer. Due to the fact that survivin both encourages cell growth and prevents apoptosis. The high correlation between the inflammatory condition and survivin expression highlight survivin as a viable diagnostic and therapeutic biomarker (14).

Materials and methods

The study includes 100 Iraqi individuals divided into two groups 50 patients and 50 control, 5ml of blood

samples were taken from each individual. to determine the role of survivin level in serum and gene polymorphism in etiopathogenesis of disease. The medical history from all patients has been taken. The specialist medical personnel at the clinic made the diagnosis using the new McDonald criteria (15). In certain advanced cases, cerebral spinal fluid (CSF) was also tested. The diagnosis was based on a clinical examination, magnetic resonance imaging (MRI) findings and evoked potential (EVP) test (16).

Blood specimens have been collected for patients with multiple sclerosis of each sample has been separated into two tubes; 2ml of whole blood have been placed in tube containing EDTA (Ethylene Diamine Tetracetic Acid) for DNA extraction for genetic section and the remaining 3ml of whole blood have been placed in Serum-separating tube; serum is separated and isolated from this 3ml of blood by centrifuging for 10 minutes at 3000 round per minutes. To detection of human survivin level we used standard sandwich enzyme-linked immune-sorbent assay technology was used as the foundation for the ELISA Kit-O15392.3. Genotyping of polymorphism (rs17878467) of the *BIRC5* gene was done, by using High Resolution Melting (HRM) SNP Genotyping Assay (17). HRM analysis with ramping by 2 sec from 65 to 95°C. Used master mixes were containing EVA-Green, HRM Master Mix Synthetic SNP sequences was tested using duplicates. The DNA was extracted, using DNA extraction kit EasyPure®Genomic (TransGen, biotech. EE101-01). Primer sequences were designed according to their reference sequence (rs) in the National Center for Biotechnology Information database (NCBI). The forward-primer CGCCATTAACCGCCAGATTTGAA and the Reverse-primer CCGCCACCTCTGCCAACG The thermal cycling program was as follows: enzyme activation in 94°C for 60 sec, (first one was denaturation 94°C for 5 sec and second step of annealing 62°C for 15 sec (40 cycle) and extension 72ºC for 20 sec).

Statistical analysis

The Statistical Analysis IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA and T-test was utilized in order to make a statistically significant comparison between means. The Chi-square test was utilized in order to make statistically meaningful comparisons between percentages (0.05 and 0.01 probability) estimates of the odds ratio and the confidence interval for this study GraphPad prism 9 program was used to draw the figures in this study. WINPEPI and SPSS program was used to detect the genotyping.

Results and discussion

Survivin level in multiple sclerosis patients and control group

Multiple sclerosis patients' survivin serum levels were significantly different from those of healthy subjects ($P < 0.05$). The mean± SE of survivin level of patients (mean± SE 546.2530), While the rate was low compared with healthy people (mean± SE 294.3823), as shown in (table 1). This highly Significant differences ratio shows the high correlation between the survivin level and disease.

Table (1): Comparison of survivin levels in patients with multiple sclerosis and the control group.

groups	No.	Mean	Std. Deviation	Std. Error Mean	*p-value*
Patients	50	546.2530	202.17025	28.59119	0.001**
Control	50	294.3823	59.35602	8.39421	

****: <0.01 level of highly significance.**

This result agrees with (18) found that the overexpression of survivin in a variety of immunopathological situations, such as autoimmune disorders, has created a new avenue for studying its significance as an etiologic and prognostic factor, diagnostic marker, and therapeutic target. The present study supports a similar study (19), which found the survivin biomarker for predicting the probability of developing rheumatoid arthritis (RA) and prognostic marker for joint deterioration in established RA patients. Another study (20) found strong associations between survivin expression and multiple sclerosis (MS), inflammatory bowel disease (IBD), psoriasis, and systemic lupus erythematosus (SLE). In addition, the increased in survivin level patients may be directly related to immunological changes that lead to reversible stimulation of an individual's immune response and thus increase disease activity. These findings are consistent with the observations reported in the studies by (21), they noted that survivin is one of the inhibitors apoptosis (IAP) proteins, apoptosis is crucial for the elimination of autoreactive cells; hence, any errors in this process lead to the development of autoimmune disorders, which explains the reason for elevated protein levels in patients with multiple sclerosis. (Figure 1) illustrates survivin level.

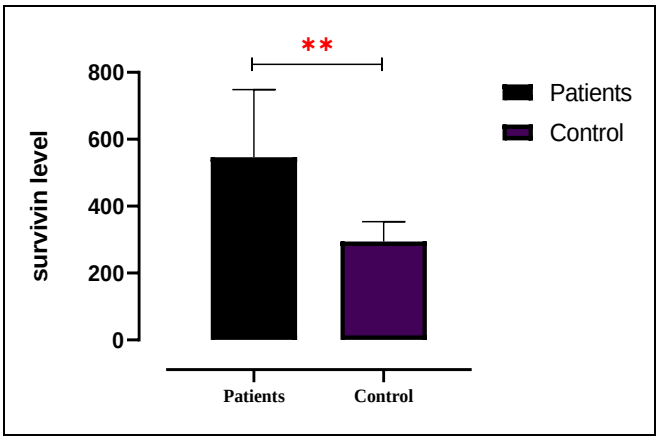

Figure (1): The survivin serum concentration in patients compared to healthy controls is depicted by bar graphs.

Genotyping findings

The genetic polymorphism and allele frequencies of *BIRC5* gene rs17878469 C/T was studied as shown in (Table 3). The results showed an increasing frequency of CC (homozygous) genotype in healthy controls compared to multiple sclerosis patients (52.00%, 20.00%) with (OR = 4.00, 95% CI = 0.10 - 0.60), while the frequency of (Heterozygous) CT genotype in MS Patients compared to control was (60.00%, 34.00% with OR = 6.7, 95% CI = 2.65 -15.76), and TT genotype frequency was (20.00%, 14.00% in patient and control groups respectively, with OR =0.4, 95% CI =0.08-2.4). The results showed that

there was a highly significant difference association between MS and rs17878469 polymorphism of *BIRC5* gene in the Iraq patients (P<0.05). The CC genotype was the reference genotype and TT genotype was mutant. The results shown of genetic analysis suggested that C allele of *BIRC5* gene polymorphism highest in control group compared with patients and T allele shown highest percentage in patients compared with controls. The three genotypes shown highly significant difference association with multiple sclerosis, this result disagrees with recent studies (22). The SNP frequency is significant in the samples under study, and the result varies according to the samples size. However, there are some contradictory conclusions. Limited sample sizes, low statistical power, ethnic variations, extensive geographic variation, interactions with other genetic or environmental factors, and clinical variability may all contribute to this difference (23). From these results it turns out that the genotype TT was risk factor of Ms. It is present in patients at a higher rate than healthy individual (24).

Table (3): Genotype and allele frequency of *BIRC5* gene polymorphism rs17878467 in Patient and Control group.

Genotype 17878467	Patients No. (%)	Control No. (%)	*P-value*	O.R.	C I (95%)
CC	10 (20.00%)	26 (52.00%)	0.002**	4.00	0.10 - 0.60
CT	30 (60.00%)	17 (34.00%)	0.001**	6.7	2.65 - 15.76
TT	10 (20.00%)	7 (14.00%)	0.008**	0.4	0.08 -2.4
Total	50	50	-	-	-
Allele Frequency					
C	0.61 (50)	0.73 (69)	-	Reference	-
T	0.39 (50)	0.27 (31)	0.006 **	1.2	0.7 - 2.2

NS: Non-Significant. *: Significant at 0.05 level. **: <0.01 level of highly significance

Conclusion

In light of all that was discovered, this was the first study, to the best of our knowledge, that attempted to look for regulatory mechanisms of survivin expression in MS patients' bodies, and it served that purpose. Research was done on the genetic variant known as rs17878467, which is an SNP in the *BIRC5* gene. and found there is relationship with risk of MS disease. On the other side, we found that the level of survivin increases in patients compared with control group, so can consider as a biomarker for MS.

References

1. Ghasemi, N.; Razavi, S. and Nikzad, E. (2017). Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. Cell Journal. 19(1):1-10.
2. Simpson, S.; Blizzard, L.; Otahal, P.; Van der Mei, I. and Taylor, B. (2011). Latitude is significantly associated with the prevalence of multiple sclerosis: a metanalysis. Journal Neurol Neurosurgery Psychiatry Jnnp. 82(10):1132-41
3. Ali, N.W. and Al.Kazaz, AK. (2018). Association between interlukin1B gene polymorphisms and keratoconus disease in a sample of Iraqi patients. Iraqi Journal of Biotechnology. 17(1):44-50.
4. Megan, M.; Pharm, D.; Samuel, F. and Hunter, MD. (2014). "Implications for Multiple Sclerosis in the Era of the Affordable Care Act: A Clinical Overview". The American Journal of Managed Care. 23(11):1152-1170.
5. Khondkarian, O.; Zavalishin, I. and Nevskaia, O. (1982). Classification of multiple sclerosis. Zhurnal Nevropatologii i Psikhiatrii Imeni SS Korsakova (Moscow, Russia: 1952). 83(2):164–166.
6. Li, D.; Hu, Ch. and Li, H. (2018). Survivin as a novel target protein for reducing the proliferation of cancer cells (Review). Biomedical Reports. (8): 399-406.

7. Rahban, D.; Mohammadi, F.; Alidadi, M.; Ghantabpour, T.; Pedram Abbasi Ghasem Kheyli, P.A.GH.; and Ahmadi, M. (2019). Genetic polymorphisms and epigenetic regulation of survivin encoding gene, BIRC5, in multiple sclerosis patients. BMC Immunology. 20(30):1-8.
8. Li, Y.; Lu, W.; Yang, J.; Edwards, M. and Jiang, Sh. (2021). Survivin as a biological biomarker for diagnosis and therapy. Expert Opinion on Biological Therapy. 21 (11): 1429–1441
9. Wheatley, S.P. and Altieri, D.C. (2019). Survivin at a glance. Journal of Cell Science. 132(7):1242-223826.
10. Ebrahimiyan, H.; Aslani, S.; Rezaei, N.; Jamshidi, AH. and Mahmoudi, M. (2018). (Survivin and autoimmunity; the ins and outs. Immunology Letters. (193):14–24.
11. Al-Faradhi, H.Q.I. (2015): Study of Some Immunological Parameters in Iraqi Patients with Multiple Sclerosis. A Thesis of Master (Ibn Al-Haitham) University of Baghdad.p:4-5.
12. Jang, JS.; Kim, KM.; Kang, KH.; Choi, JE.; Lee, WK.; Kim, CH.; Kang, YM.; Kam, S.; Kim, I.S. and Jun, JE. (2008). Polymorphisms in the survivin gene and the risk of lung cancer. Lung Cancer. 60(1): 31–40.
13. Cohran, V.; Managlia, E.; M Bradford, E.; Goretsky, T.; Li, T.; Katzman, R.B.; Cheresh, P.; Brown,J.B.; Hawkins, J.; Liu, Sh.X.L.; Plaen,I.G.; Weitkamp, J-H. and Barret, T.A. (2016). Epithelial PIK3R1 (p85) and TP53 regulate survivin expression during adaptation to ileocecal resection. American Journal Pathology. 186(7):1837–1846.
14. Lv, YG.; Yu, F.; Yao, Q.; Chen, JH. and Wang, L. (2010). The role of survivin in diagnosis, prognosis and treatment of breast cancer. Journal Thoracic Disease. 2(2):100-110.
15. Milo, R. and Miller, A. (2014). Revised diagnostic criteria of multiple sclerosis. Autoimmunity Reviews. (13):518-524.
16. Jasim, R.M. (2021). Relationship of Melatonin receptor gene polymorphism with the risk of polycystic ovary syndrome in a sample of Iraqi women. Institute of Genetic Engineering and Biotechnology/ Baghdad University. (Thesis). p:44-45.
17. Shomali, N.; Maashi, M.S.; Baradaran, B.; Sorkhabi, A.D.; Sarkesh, A.; Mohammadi, H.; Hemmatzadeh, M.; Marofi, F.; Shotorbani, S.S.; and Jarahian, M. (2022). Dysregulation of Survivin-Targeting microRNAs in Autoimmune Diseases: New Perspectives for Novel Therapies. Frontiers in Immunology.13(2):68-77.
18. Baraka, E.A.; El Din, M.S.; El Shambky, A.; Fouad, N.A. and Abdelkader, M.A. (2019). Serum and Synovial Survivin in Rheumatoid Arthritis: Relation to Disease Activity and Severity. Egypt Rheumatology Rehabilitation. 5(46): 221–228.
19. Khalaf, M.A.; Al-Saadi, BQ.H. and Mohammed, H.Q. (2022). Evaluation of TLR-3, TLR4, IL-7, and IL37 Immunological Markers in β-Thalassemia Major Iraqi Patients. Iraqi Journal of Biotechnology. 21(1): 115-123.
20. Mersha, TB. and Abebe, T. (2015). Self-reported race/ethnicity in the age of genomic research: its potential impact on understanding health disparities. Human Genomics. 9(1):1-22.
21. Oparina, N.; Erlandsson, M.C.; Fäldt Beding, A.; Parris, T.; Helou, K.; Karlsson, P.; Einbeigi, Z.; Bokarewa, M.I. (2021). Prognostic Significance of BIRC5/Survivin in Breast Cancer: Results from Three Independent Cohorts. Cancers. (13):2209.
22. Wright Willis, A.; Evanoff, BA.; Lian. M.; Criswell, SR. and Racette, BA. (2010). Geographic and ethnic variation in Parkinson disease: a population-based study of US Medicare beneficiaries. Neuroepidemiology. 34(3):143-51.
23. Waubant, E.; Lucas, R.; Mowry, E.; Graves, J.; Olsson, T.; Alfredsson, L. and Langer-Gould, A. (2019). Environmental and genetic risk factors for MS: an integrated review. Annals Clinical Translational Neurology. 6(9):1905-1922.
24. Al-Qazzaz, H.K. and Al-Awadi, S.J. (2022). Impact of DNA Methylation and Gene Expression of H19, SNRPN and LINE-1 Genes on Oligospermia. Iraqi Journal of Biotechnology. 21(1): 66-78.