ORIGINAL RESEARCH

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Investigation of a Glioblastoma Risk-Associated SNP of the *PTPRB* Gene in Familial Glioblastoma

Hatice SEVİM NALKIRAN[a], Sema YILMAZ RAKICI[b], İhsan NALKIRAN[a]

[a]Department of Medical Biology, Recep Tayyip Erdoğan University Faculty of Medicine, Rize, Türkiye
[b]Department of Radiation Oncology, Recep Tayyip Erdoğan University Faculty of Medicine, Rize, Türkiye

ABSTRACT Objective: To investigate the association of the single nucleotide polymorphism (SNP) rs2252784 in the protein tyrosine phosphatase receptor type B (PTPRB) gene with familial glioblastoma multiforme (GBM). **Material and Methods:** Genomic DNA was extracted from the peripheral blood samples of 2 sibling GBM patients, their 6 family members and 2 formalin-fixed paraffin-embedded (FFPE) tumor tissues. A 400 bp region was amplified and the restriction fragment length polymorphism (RFLP) technique was used to identify the rs2252784 SNP in exon 2 of the PTPRB gene. The GBM cell line T98G was used to validate the findings obtained from the tumor samples. **Results:** The analysis of DNA obtained from the blood samples of both GBM patients showed a wild-type (WT) genotype. However, the results of the PCR-RFLP analysis from FFPE tumor tissues showed that the first patient (proband) was heterozygous, and his sibling was homozygous for the rs2252784 variant. Discordant results between SNP analyses of the DNA samples isolated from the blood and FFPE tumor tissue were observed. The family members of the patients had either homozygous WT or heterozygous variants. **Conclusion:** The rs2252784 SNP was present in the tumor DNA of the patients but not in the DNA samples obtained from blood. This discrepancy might be the result of oncogenic DNA alterations associated with tumor formation. Paired analysis of tumors and blood samples from patients and patient-matched normal blood samples from GBM-affected families might provide additional insights into the underlying genetic alterations that occur during the development of a tumor in familial GBM.

Keywords: Glioma of the brain, familial; polymorphism, single nucleotide

Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor in adults.[1,2] It is associated with a high risk of morbidity and mortality. Approximately 5-10% of the patients have a family history of GBM.[3,4] Several rare inherited syndromes, such as neurofibromatosis type-1 (NF1) and type-2 (NF2), and Li-Fraumeni syndrome (LFS), are associated with an increased risk of GBM.[5] NF2 carriers undergo regular magnetic resonance imaging scans to facilitate early operation to maintain low morbidity.[6] Many studies have investigated the application of genetic testing for LFS in affected families. A follow-up strategy by applying whole-body magnetic resonance imaging facilitates the early detection of disease-related cancers.[7]

The Gliogene association was established in 2004 to study families with GBM and identify GBM-susceptibility genes in high-risk pedigrees with cases of familial GBM by constructing linkage maps of high-throughput data.[8] The data were collected on the members of 376 families who were affected by glioma from 14 centers, including Denmark, Sweden, the United States, and Israel, between January 2007 and February 2011. The data on the 17q12-21.32 locus associated with susceptibility to GBM were obtained after genotyping of 75 families.[9] In a study that examined germline or somatic gene alterations in familial clustering of GBM, germline mutations were detected in the p53 and cyclic AMP-dependent kinase number 2A *(CDKN2A*) genes. In the tissues obtained from cases without any syndrome, the genes encoding the proteins that participate in the transition from the G1 phase to the S phase of the cell cycle had defects. Besides the amplification and overexpression

Correspondence: Hatice SEVİM NALKIRAN
Department of Medical Biology, Recep Tayyip Erdoğan University Faculty of Medicine, Rize, Türkiye
E-mail: haticesevimtr@gmail.com

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of the *CDK4* and *CDK6* genes, mutations or deletions were also found in the *p53*, *RB1*, and *CDKN2A/B* genes.[10] Thus, understanding the phenotypic characteristics of non-syndromic families is important because a genetic cause cannot be elucidated in most cases presenting with a history of glioma. In early studies on breast cancer linkage studies, the classification of the age for the onset of cancer and the presence of related malignancies, such as ovarian cancer, played an important role in the detection of BRCA1/BRCA2.[11,12]

The gene encoding protein tyrosine phosphatase receptor type B (PTPRB) is located on chromosome 12q15.[13] PTPRB is a member of the protein tyrosine phosphatase family and has several fibronectin type III-like domains in its extracellular domain, a single intracellular catalytic domain containing phosphorylation sites at the C-terminal, and a transmembrane domain. PTPRB regulates various biological processes through the binding and dephosphorylation of several types of receptor tyrosine kinases (RTKs).[14] PTPRB can inhibit the activation of fibroblast growth factor receptor and the phosphorylation of ERK1/2, which can negatively regulate mammary gland branching morphogenesis.[15] Protein tyrosine phosphatases are signaling molecules that control various biological activities, such as cell growth, mitotic cell cycle, differentiation, and transition into neoplasm.[16] The Rs2252784 is a missense single nucleotide polymorphism (SNP) found on the *PTPRB* gene and causes a substitution of arginine (R) for lysine (K) at amino acid position 94 (R94K, p.Arg94Lys) in the peptide sequence.[17] No information is available in the ClinVar database for rs2252784. Additionally, information on inherited cancer-risk SNPs is limited. As PTPRB plays an important role in the RTK signaling pathways and probably contributes to tumor progression, we investigated the relevance of the SNP rs2252784 on the risk of developing familial glioma in an affected family.

MATERIAL AND METHODS

SAMPLE COLLECTION AND PARTICIPANTS

This study was conducted following the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Non-Invasive Clinical Research of Recep Tayyip Erdogan University, Rize, Türkiye, dated December 19, 2018, with the decision number 2018/193. Two siblings diagnosed with grade IV glioblastoma and their first-degree and second-degree family members (n=8) were informed of the aim and procedures in detail, and they were asked to provide written consent. Informed consent was obtained from each participant (informed parental consent was obtained when required). The proband (first patient) and his sibling (patient #2) are shown in the pedigree as II.1 and II.3 (Figure 1). Additionally, II.5 represents a sibling with no known clinical condition.

CELL CULTURE

The human glioblastoma cell line T98G (passage number 13 to 15) was used in this study and was cultured in high-glucose DMEM (Gibco, Life Technologies; Paisley, UK) containing 10% Fetal Bovine Serum (FBS) (Biowest, Nuaillé, France) at 37°C in the presence of 5% CO_2 in a cell culture incubator. As the T98G GBM cell line is well-characterized, we used it to compare the findings obtained using formalin-fixed paraffin-embedded (FFPE) tumor tissues.

DNA ISOLATION

The genomic DNA was isolated from the peripheral whole blood samples and T98G cells using the Quick-DNA Universal Kit (Zymo Research Corp., CA, USA) following the manufacturer's instructions. The Genomic DNA was extracted from the FFPE tissue using the FFPE DNA Isolation Kit (RTA Labo-

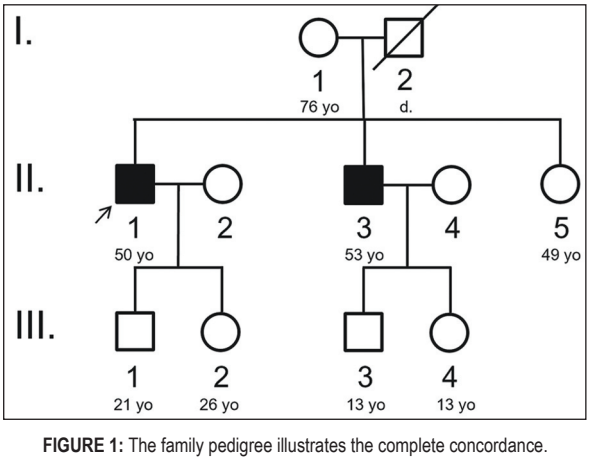


FIGURE 1: The family pedigree illustrates the complete concordance.

ratories, Türkiye) following the manufacturer's protocol. DNA quantification was performed by measuring the absorbance at 260 nm using a Thermo Scientific µDrop plate and the Multiskan GO microplate spectrophotometer (Thermo Scientific; Waltham, MA, USA).

AMPLIFICATION OF PCR PRODUCTS AND GENOTYPING

The following conditions were used for conducting the polymerase chain reaction (PCR): 25 ng of genomic DNA, 1.5 mM $MgCl_2$ (Qiagen, Germany), 0.2 mM of each dNTP (Boehringer Mannheim GmbH, Mannheim, Germany), 2.5 IU of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 10 mM of forward and reverse primers. Finally, ddH_2O was added to make up a final reaction volume of 25 µL. The forward (5' ACTGTTGTGTCTGATTCCTT 3') and reverse (5' ACCTTCATTTTTTGTATCCC 3') primer sets for the *PTPRB* gene were used to generate 402 base pair (bp) long amplicons. The amplification of the target sequence was conducted using an Applied Biosystems Gene Amp PCR Systems 9700 (Foster City, CA, USA) using the following program: 95°C for 9 min, followed by 30 cycles at 95°C for 1 min, 56°C for 1 min, 72°C for 1 min, and final extension at 72°C for 7 min. The PCR-restriction fragment length polymorphism (RFLP) was performed for all samples to detect the R94K variant. Restriction assays were performed overnight at 37°C with 1 µL of AcuI (#R0641S New England BioLabs Inc.) in 10X NEBuffer using 10 µL of PCR product in a reaction volume of 50 µL. The restriction fragments were separated by electrophoresis using a 1.5% agarose gel stained with EtBr (Sigma-Aldrich, Steinheim, Germany). The gel was then examined and photographed using the Gel Documentation system (DNR Bio-Imaging System Ltd., Jerusalem, Israel).

RESULTS

We collected the peripheral blood samples and FFPE tumor tissue sections of 2 non-twin male siblings diagnosed with GBM at the ages of 50 and 53 years (2 months apart) to obtain their genomic DNA. The PCR and RFLP analyses of the samples were conducted to investigate the genotypes of the SNP rs2252784. We obtained DNA fragments of 233 and 171 bp for R94K in the homozygous RR genotype (wild-type, WT), a 402 bp band (uncut) (carrying the SNP) in the homozygous KK genotype, and 3 bands of 402, 233, and 171 bp for the heterozygous RK genotype (Figure 2). The RFLP genotyping analysis of the DNA extracted from peripheral blood samples showed that the proband (II.1), I.1, II.3, and II.5 had homozygous RR genotypes (WT for rs2252784). Individuals III.1, III.2, III.3, and III.4, with no known medical conditions, had heterozygous variants (Figure 2, Table 1).

The PCR fragments amplified using the DNA samples extracted from the FFPE tissues and the T98G cells were analyzed by the RFLP method to determine rs2252784 (R94K). The samples from II.1F, III.1, and the T98G cells showed 3 fragments of 402, 171, and 233 bp for the RK genotype (heterozygous variant) and a 402 bp band (uncut) for the KK genotype (homozygous variant) for the sample II.3F (Figure 3, Table 1). The SNP results showed discrepancies between the DNA samples extracted from the FFPE tumor tissues and blood samples. While the results of the PCR-RFLP analysis showed that the peripheral blood samples of both patients were homozygous wild-type, the DNA samples from the FFPE tissues showed a heterozygous variant genotype for II.1F and homozygous variant genotype for II.3F (Figure 3, Table 1).

DISCUSSION

The rs2252784 SNP located on the *PTPRB* gene was previously linked to the risk of familial GBM in a study that analyzed the blood and tumor samples of individuals from a family with 2 siblings diagnosed with GBM.[18] In this study, we evaluated this SNP associated with the risk of developing familial GBM by collecting blood and tumor samples from 2 male non-twin siblings with GBM and from their extended family members. We compared the data of the siblings with those of their extended family members (n=6) with no known disease. The SNPs at 8 loci that influence the risk of glioma near the *TERC*, *TERT*, *EGFR*, *CCDC26*, *CDKN2A7*, *CDKN2B*, *PHLDB1*, *TP53*, *RTEL1*, and *POLR3B* genes were determined by conducting genome-wide association studies.[19-23]

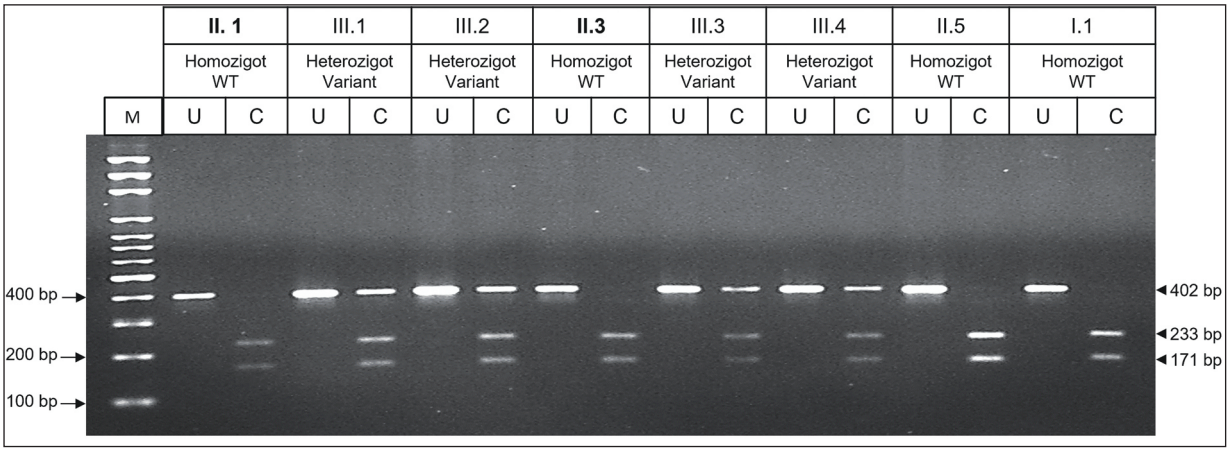


FIGURE 2: Results of PCR-RFLP for rs2252784 SNP specific analyses, peripheric blood samples.

A DNA fragment surrounding the SNP was amplified by PCR and digested with the AcuI restriction enzyme. Uncut amplicon and the products obtained after digestion were visualized on 1.5% agarose gel stained with EtBr. The amplicon is 402 bp in length, AcuI restriction enzyme cuts only the WT genotype and produces 2 fragments (171 and 233 bp). Individuals, II.1, II.2, II.5 and I.1 showed digested fragments, therefore, exhibited homozygous variant allele. All 3 fragments (171 and 233 and undigested 402 bp) were observed in individuals, III.1, III.2, III.3 and III.4.

U: Uncut; C: Cut: M: Marker; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; SNP: Single nucleotide polymorphism; WT: Wild-type.

The PTPRB protein influences carcinogenesis and cancer formation as a tumor suppressor. A study investigating the role of PTPRB in the tumorigenesis of non-small cell lung cancer (NSCLC) and the prognosis of NSCLC patients showed that the overexpression of PTPRB was associated with a decrease in the tumor growth rate and low PTPRB expression in patients was associated with advanced tumor stage.[24] The rs2252784 (R94K) SNP involves a change in the amino acid residue from R to K at position 94. This substitution occurs in the fibronectin type III 1 binding domain (amino acid positions 23-111) of the protein.[25] The PTPRB protein has many fibronectin type III repeats in its extracellular domain, which can interact with neuronal receptors and extracellular matrix components like tenascin C.[26] Although both residues are large, basic, and have similar physicochemical properties, any change in the protein-protein interactions might affect tumor formation-related molecular pathways.[25] The rs450045 SNP, a variant located on the *PTPRB* gene, is involved in SNP-SNP interaction in Tau-related pathology.[27]

TABLE 1: Rs2252784 genotype results of the patients and family members.

Individuals	Leukocytes	Tumor tissue
I.1	CC (RR)	-
II.1 (patient #1)	CC (RR)	CT (RK)
II.3 (patient #2)	CC (RR)	TT (KK)
II.5	CC (RR)	-
III.1	CT (RK)	-
III.2	CT (RK)	-
III.3	CT (RK)	-
III.4	CT (RK)	-

In this study, the results of the PCR-RFLP analysis of rs2252784 genotyping from peripheral blood samples showed that II.1 (patient#1), II.3 (patient#2) I.1 (sister), and II.5 (mother) were WT for rs2252784 (homozygous RR genotypes). Individuals III.1, III.2, III.3, and III.4 had heterozygous variants (RK). However, the DNA samples obtained from the FFPE tissue of patients II.1F and II.3F showed the RK (heterozygous variant) and KK genotypes (homozygous variant), respectively. The data were inconsistent when these findings were compared with the results of the DNA samples extracted from the blood of the patients. Additionally, we found that a malignant GBM cell line, T98G, was also a heterozygous variant, similar to the tumor tissue. The Rs2252784 SNP is a missense variant of the *PTPRB* gene and might contribute to GBM tumorigenesis.[18] The SNPs located on the phosphatase genes, such as *PTEN*, *PTPRB*, *PTPRD*, *PTPRN2*, *PTPRT*, and *PPP1R3A* can cause truncation mutations in metastatic

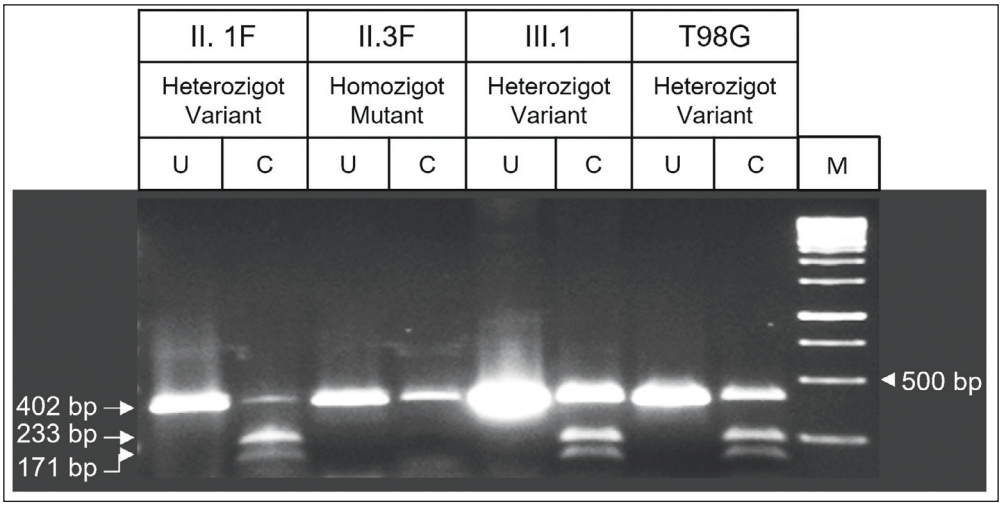


FIGURE 3: PCR-RFLP analyses for rs2252784 SNP in FFPE tumor tissue and T98G cell line.

An amplicon covering the SNP site was amplified by PCR and digested with the AcuI restriction enzyme. The PCR product and the products obtained after digestion were visualized on 1.5% agarose gel stained with EtBr. The full-length amplicon is 402 bp. The wild-type genotype is cut by the AcuI restriction enzyme and produces two fragments (171 and 233 bp). After the digestion step, II.1F, III.1 and T98G cell line showed all 3 fragments (171 and 233 and undigested 402 bp) therefore, exhibited homozygous variant alleles. II.3F was revealed to be a homozygous variant genotype. Individual III.1 was re-analyzed to confirm the expected fragments and loaded into the gel along with FFPE tissues. T98G as a human glioblastoma cell line was examined to evaluate its rs2252784 genotype.

U: Uncut; C: Cut; M: Marker; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; FFPE: Formalin-fixed paraffin-embedded; SNP: Single nucleotide polymorphism.

melanomas.[28] Missense variants in tumor suppressor genes, including *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A*, strongly affect cell proliferation and survival and are associated with susceptibility to several types of cancer, such as Fanconi anemia, acute lymphoblastic leukemia, breast cancer, and ovarian cancer.[29-32] In a study that investigated the predisposing variants in 2 related children with ganglioneuroma and neuroblastoma, a candidate gene (*CLEC12B*) for cancer predisposition was identified. This gene encodes a lectin C-type receptor and might regulate immune functions.[33]

The heterogeneous tumor samples in our study showed somatic genetic variations, which were different from the status of germline SNPs. The tumor (somatic) and patient (germline) genomes are both important in cancer research. Many recent scientific and clinical-translational studies have investigated the somatic genome.[34-36] Compared to spontaneous tumorigenesis and mechanisms of acquired drug resistance, the germline genome might play a less direct role in determining the outcome of cancer therapy, but it is extremely important in cancer risk prediction.[37]

Studies that investigated the concordance of genotyping results from blood and FFPE tissues reported that discordant findings may not significantly alter the results and FFPE tissues can be reliable sources for genotyping.[37,38] Guo et al. stated that discordant results might occur due to any changes in the processing or detection methods, such as fixation time or storage time of the FFPE tumor tissues, while analyzing the results.[39] Therefore, assessing the differences between somatic aberrations and germline aberrations is important. Very few studies have reported the discrepancies between the blood and FFPE tissue genotyping results; these studies mostly evaluated whether FFPE tissue samples can be used as reliable sources for genotyping analysis.[40,41] However, late events might occur in a subpopulation of cancer cells leading to the progression of glioma. The process of transformation of normal cells into malignant cells needs to be further studied.

CONCLUSION

Germline genetics strongly influences the tumor microenvironment and cell proliferation signaling pathway. SNPs linked to cancer risk play a functional role

through regulatory effects on the expression of target genes.[42] In the blood samples of individuals II.1 (patient#1), II.3 (patient#2), and II.5 (sibling with no known medical condition) rs2252784 was homozygous, indicating that missense variations were absent in these individuals. Backes et al. reported that a cumulative effect of several SNPs from different genes, such as *PTPRB* and *CROCC*, might be associated with GBM tumorigenesis. We could not associate heterozygous or homozygous variant genotypes of rs2252784 with the progression of familial glioma, probably because we studied only one SNP.[18] However, based on the data obtained from tumor samples, rs2252784 might be a pathogenic variant in the *PTPRB* gene and might be linked to the molecular changes initiating glioma tumor formation. Many genes are expressed in specific tissues, which makes it difficult to determine the regulatory role of cancer risk-associated SNPs that interact with genes that are not expressed in the most frequently analyzed tissue samples, such as blood.[43] Examining uncommon genetic alterations might provide new insights into cancer predisposition and progression in familial GBM. Large-scale genome-wide studies need to be conducted on several families with glioma patients to further elucidate the underlying molecular features that promote the development of glioma.

HIGHLIGHTS

- Familial glioma accounts for about 5-10% of all glioma cases.
- The status of rs2252784 in the *PTPRB* gene, which was previously associated with familial GBM risk, was analyzed in tumor and blood samples of 2 non-twin siblings diagnosed with GBM and in blood samples obtained from their extended family members.
- The WT genotype for the SNP of the *PTPRB* gene was detected in the blood samples of both GBM patients.
- In the FFPE tumor tissues, the first patient (proband) was heterozygous, and the sibling was homozygous for the rs2252784 variant.
- The change in the nucleotide for the rs2252784 SNP was not observed in the DNA samples obtained from blood. However, the DNA from the tumor of the patients showed heterozygous and homozygous nucleotide changes, which could be explained by the underlying genetic alterations related to the processes leading to tumor formation in familial GBM.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sema Yılmaz Rakıcı, Hatice Sevim Nalkıran;* ***Design:*** *Hatice Sevim Nalkıran, Sema Yılmaz Rakıcı;* ***Control/Supervision:*** *Hatice Sevim Nalkıran, Sema Yılmaz Rakıcı;* ***Data Collection and/or Processing:*** *Sema Yılmaz Rakıcı, Hatice Sevim Nalkıran;* ***Analysis and/or Interpretation:*** *Hatice Sevim Nalkıran, İhsan Nalkıran, Sema Yılmaz Rakıcı;* ***Literature Review:*** *Hatice Sevim Nalkıran;* ***Writing the Article:*** *Hatice Sevim Nalkıran;* ***Critical Review:*** *İhsan Nalkıran, Sema Yılmaz Rakıcı;* ***References and Fundings:*** *Hatice Sevim Nalkıran, Sema Yılmaz Rakıcı;* ***Materials:*** *Sema Yılmaz Rakıcı, Hatice Sevim Nalkıran.*

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