Mutational Analysis of the PTEN/MMAC1 Tumor Suppressor Gene in Sporadic Glioblastoma Multiforme

Heshmat Pour N[1], Tavassoli R[1], Mahzouni P[2]

Abstract

Background: In 1997 MMAC1 or the PTEN gene, was identified as a tumor suppressor gene on the long arm of chromosome 10.PTEN involves in the balance between proliferation, and differentiation, apoptosis and regulation of angiogenesis, and eventually mutation in this gene causes a strong potential for tumorigenesis cells. This study is the first report of the correlation between PTEN gene mutations with histological markers and the age of Glioblastoma patients with the purpose of using it to remove diagnostic ambiguities and identify the type of glioma.

Methods: In this study, we screened for PTEN mutations in exon 4-8 in glioblastoma patients in Isfahanian population. Genomic DNA ware extract from 60 formalin fixed frozen glioblastoma tumors, 4 normal tissue sample and 60 blood samples. Mutational analysis was performed using polymerase chain reaction, single strand conformation polymorphism (SSCP) and heteroduplex mobility techniques. In order to augment the accuracy, the experiments (PCR, SSCP, HMA) was repeated at least 3 times. The correlation between PTEN mutations with Clinical Pathologic markers (pleomorphism, Necrosis, cellularity, mitosis and endothelial proliferation) in Glioblastoma tumors and the age of patients were investigated With the help of statistical tests (t-test, Manvitni and Fisher's exact test).

Results: A total of 4 mutations (7%) were detected in 60 glioblastoma tumors. In this study, there was no significant relation between clinic pathologic markers and PTEN mutations (p-value > 0.05). Our data shows a positive association between the age of onset of cancer and PTEN mutations (p-value < 0.001) .

Conclusion: Our results suggested that PTEN mutations are Low in Iranian glioblastoma patients. People with PTEN mutations are more likely to develop glioblastoma before the age of 20.

Key words: Glioblastoma Multiform; PTEN gene; SSCP; HMA

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Introduction

About half of brain tumors are primary and the others are metastatic. In general, the most common and most malignant primary brain tumor is Glioblastoma multi form. The common age is 45-65, but rarely has it been reported below the age of 30. The word of glioma refers to a group of tumors whose origins are normal brain parenchymal cells [1]. WHO classification of nervous system divides tumors into four grades of grade I (pilocytic astrocytomas) with an average survival of 40 years to grade IV (Glioblastoma multi-form) with an average survival of 8-10 months after diagnosis. This grading is more based on the survival of patients than pathology characteristics of tissue, so the classification system of WHO is seen more as a grading of malignancy. Primary brain tumors include various histological species with different histology, clinical course, distribution and prognosis features [2]. The clinical Pathologic markers of gradation are Pleomorphism, Necrosis, mitosis, cellularity and endothelial proliferation that all these criteria are related to the manner of pathologist. So in some cases placing a tumor in a particular histological grade is problematic and other criteria are needed to help. Recently based on the molecular pathology,

immunohistochemical and clinical pathology studies, much progress has been made in understanding tissue generation CNS tumors [3,4]. This study also investigated the Correlation of PTEN mutations with clinicopathologic markers in Glioblastoma tumors. PTEN tumor suppressor gene or MMAC1 was identified on the region q23.3 of chromosome10 in 1997. PTEN/MMAC1 mutations increased in Glioma, prostate, melanoma, endometrial cancers and with less frequency in breast cancers [5]. PTEN gene contains 9 exons and encodes a 403 amino acid lipid phosphatase that dephosphorylates D3 of phosphatidylinositol 3, 4, 5 trisphosphat (Ptdlns(3, 4, 5)P3), producing phosphatidylinositol 4, 5 bisphosphate (Ptdlns(4, 5)P2) acting in opposite direction of phosphatidylinositol 3 Kinase (PI3K). PTEN is a member of the large protein tyrosine phosphatase family (PTP),which also shows Tyrosine phosphatase activity in vitro [6,7].Inactivation of PTEN results in the accumulation of PI3P, activation of a serine/threonine Kinase (AKT/PBK) and other signaling molecules including the mammalian target of rapamycin (mTOR), phosphatidylinositol-dependent Kinases (PDKs) and small GTPases of Rho family. Activation of AKT by PI3P modulates the activity of a variety of downstream proteins including BAD, MDM2, EGFR, P27 and FOXO transcription factors that are related to cell growth and survival [8, 9, 10]. The purpose of this study is to investigate the Correlation of PTEN mutations with clinicopathologic markers in Glioblastoma tumors and to find possible new treatment methods that can be used in screening Glioblastoma tumors from other brain tumors to obviate diagnostic ambiguities. Besides this study, the association between PTEN mutations with the age of onset of the disease was also investigated.

Materials and Methods

Tissue Samples

In this study, formalin fixed frozen glioblastoma tumors were randomly collected including 60 Glioblastoma tumors that were diagnosed during 2001-2005 in Zahra Hospital affiliated to Isfahan University of Medical Sciences. First slides, stained by hematoxylin - eosin method, and files related to any case were reviewed to collect the information related to the age of the onset of the disease in the forms for each patient. Also, 40 healthy blood samples and 5 samples of normal tissue from formalin fixed frozen glioblastoma tumors, confirmed by expert pathologist, were prepared to Confirmation mutation of single-nucleotide polymorphism and some Clinical Pathologic markers such as Pleomorphism, Necrosis, cellularity, mitosis and endothelial proliferation were determined for each tumor by expert pathologist.

Extracted DNA

DNA extraction from paraffin tissues was performed by standard methods in three stages: cutting and preparing tissue, wiping out paraffin from tissues and DNA extraction by means of phenol chloroform method. Genomic DNA was extracted from blood cells by using standard method of salt deposition Miller. To detect mutations in exons 4-8 of PTEN, PCR technique, Single Strand Conformation Polymorphism (SSCP) and Heteroduplex Mobility Assay (HMA) were used.

PCR Analysis

In order to proliferation genomic DNA, PCR amplification was performed. Primers were designed by using the Oligo program and their specific connection to the desired area was confirmed by Blast. In this study, in order to analyze DNA pieces better, lengths of exons more than 300 bp were divided into two pieces of A and B by using SSCP and HMA, and two pairs of primer were designed for each exon. DNA amplification was performed by using polymerase enzyme smarTaq, specific primers for exons 8-4 of PTEN gene and DNA samples were extracted after several optimization were obtained. PCR amplification was performed in 50 µl total reaction volume, the 2µl DNA Template (200-100ng), 1µl each primer (100ng)(table 1), 2.5µl MgCl2 (2.5 mM), 5 µl buffer 10X PCR, 1 µl dNTP (200 µM) and 0.4 µl enzyme Smar Taq (2 υ). The 5 exons were amplified in the following PCR conditions: an initial 5-minute denaturation at 940C followed by 35 cycles of 5-minute at 940C, 1minute at Tm for each primer (Table 1), 1minute at 720C, and a final extension of 10 minutes at 720C.Then with the help of DNA gel electrophoresis on 2% agaroz gel and buffer TBE 0.5X, voltage 5 V/Cm was conducted to determine the accuracy of PCR.

SSCP Analysis

After PCR amplification of DNA samples, SSCP technique was used to separate mutations.

The Formation of secondary structures in DNA single strand is independent of sequence length. In SSCP method, DNA single strand separation is done based on the difference in secondary structures. This method consisted of two stages: denaturing of PCR products and electrophoresis of denaturing products on nondenaturating polyacrylamid gel with neutral PH. In order to dilute and denature samples, first 10

microlitre of PCR products is added to 10 µL denaturing solution (95% formamid, 0.05% Bromophenol blue, 0.05% Xylene cyanol FF and 0.04% glycerol). Formamid prevents from recurrent denaturing of DNA single strands in subsequent stages and causes the increase of intensity and segregation of DNA single strands on polyacrylamid gel. Obtained mixture denatured for 5 minutes at 95 ° C temperature then immediately transferred to ice. 10% non denatureing polyacrylamid was used for electrophoresis of denaturing products [11]. After loading the samples on the gel, they were run at 4 ° C temperature and 175 V for 18 hours. The gels were stained with silver nitrate stainig method. The different single strand DNA sequences in gel were compared with normal sample.

HMA Analysis

A mismatch between a pair of bases in a DNA molecule is sufficient to cause the change of movement on the electrophoresis gel. The changes of single nucleotide and mismatch between the bases cause change in the structure and delay in the movent of duplex DNA molecules on the electrophoresis. In order to admit results of mutations screening with SSCP, we used HMA analysis. Genomic DNA of patients and healthy individuals were separately amplified with specific primers. PCR products from healthy individuals and patients were mixed for any exons 1:1 and were denatured for 5 minutes at 95oC and then slowly became cold for 30 minutes and were kept in 25 degree temperatures until electrophoresis. 10% non denatureing polyacrylamid gel was used for the Electrophoresis of the mixture products and it was runed at 4 ° C temperature and 175 V for 18 hours. The gels were stained with silver nitrate stainig method. This method detected more than 95 percent of the single nucleotide mismathches.

Statistical Analysis

The association between PTEN mutations with the age of onset of disease and some Clinical Pathologic markers were evaluated statistically. In this study, manvitni test, t-test and Fisher's exact test were used to compare two variable conditions.

Results

Qualitative Evaluation of PCR products with the help of agarose gel

Figure 1 shows an example of agarose gel 2% after the genomic DNA was extracted from blood and tissue samples. As it shows, due to its very high molecular weight gel,genomic DNA moves slowly and usually remains at the top of the gel.

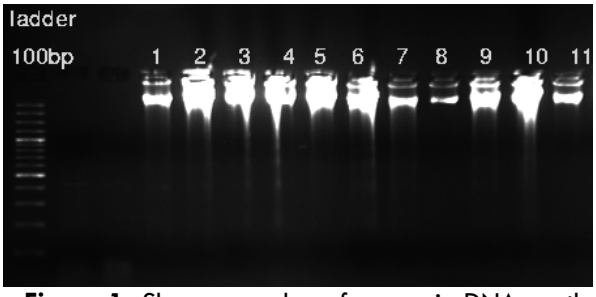

Figure 1. Shows samples of genomic DNA on the gel agarose 1% with 100 V for 45 minutes

PCR results

In this study, 100 blood and tissue samples were studied. First, genomic DNA was amplified by using PCR technique and appropriate primers for exons 4-8 PTEN gene. Two primer pairs were designed for exons 5, 6 and 8. The Results were reported for the exon number 5 with 5A and 5B, for the exon 6 with 6A and 6B and 8B for exon 8 with 8A. Thus after PCR, the size and quality of the product bands were examined by using agarose gel 2%. Optimization of PCR conditions for each primer pair were in such a way that bands of primer and additional bands get removed in order not to interfere in SSCP technique and the interpretation of results. Results from gel electrophoresis of PCR products of exon 5, region of exon 6B, 7 and region exon 8B gene PTEN is shown in Figures 2-5.

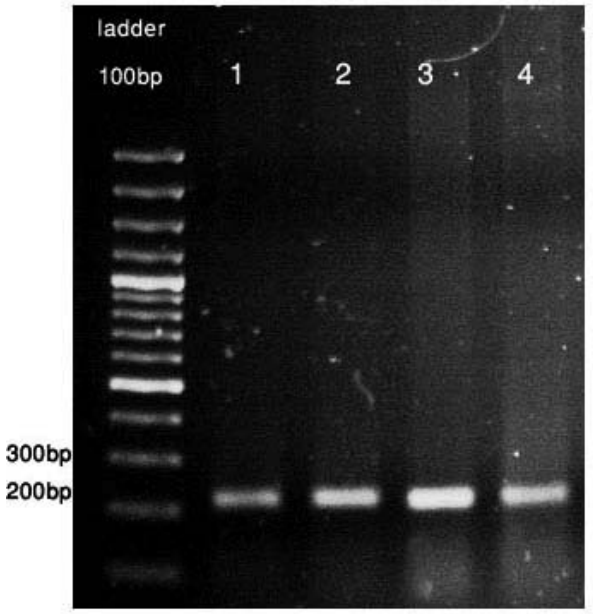

Figure 2. Shows PCR products of area 5B of exon 5. Row 1 healthy blood sample, rows 2 and 3

healthy tissue sample and row 4 tissue patient sample shows (DNA Marker 100bp).

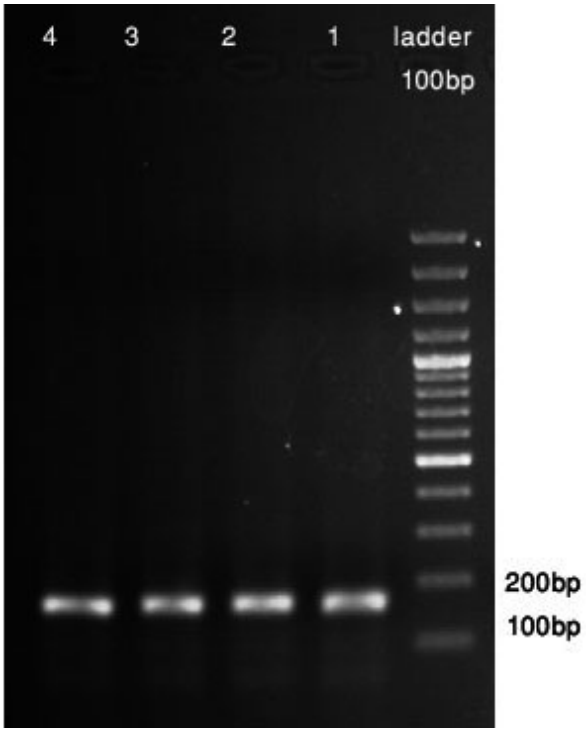

Figure 3. Shows PCR products of area 6B of exon 6. Row 1 healthy blood samples, rows 2 and 3 healthy tissue samples and row 4 patient tissue samples shows (DNA marker 100bp).

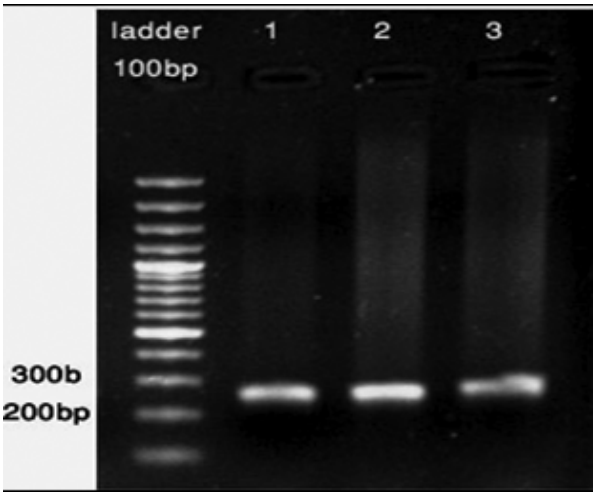

Figure 4. Shows PCR products of exon 7. Row 1 healthy blood samples, Row 2 healthy tissue samples and row 3 tumor tissue samples shows. (DNA marker 100bp).

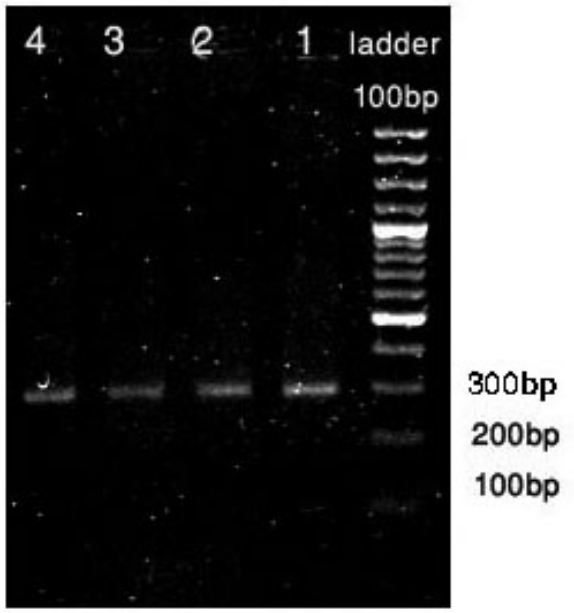

Figure 5. Shows PCR products of area 8B of exon 8. Row 1 healthy blood samples, rows 2 and 3 healthy tissue samples and row 4 patient tissue samples shows (DNA marker 100bp).

Detection the mutations by SSCP method

SSCP results of exon4, region 5B, region 8B PTEN gene is shown in figures 6-8.

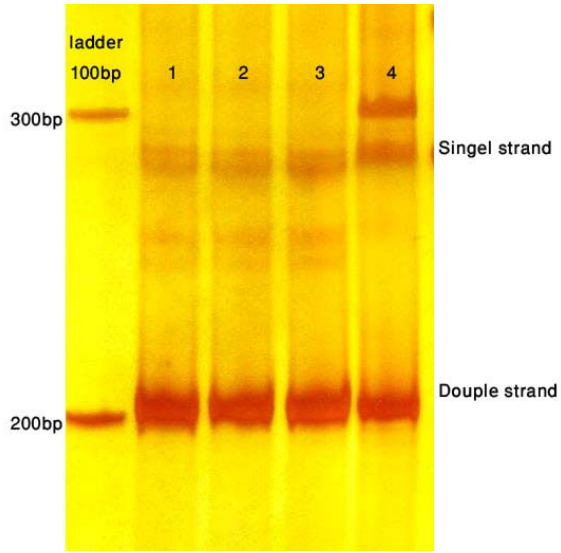

Figure 6. The study of mutations in exon number 4 by SSCP: row1 in the healthy blood sample, row 2 without mutation tumor tissue sample, row 3 healthy tissue sample and row 4 whit mutation tissue sample shows (DNA marker 100bp).

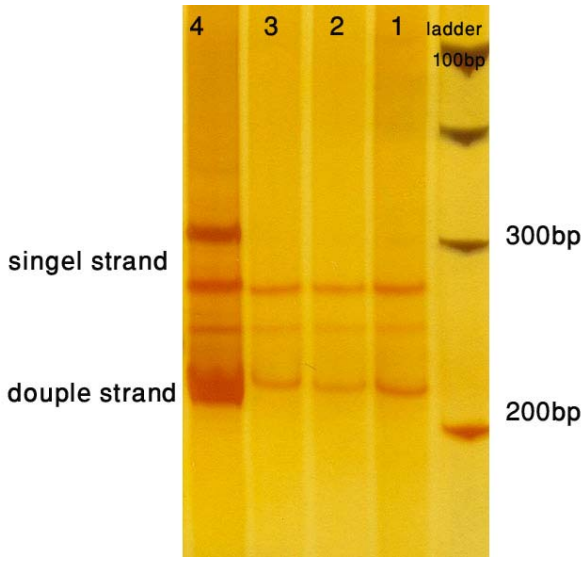

Figure 7. Study of mutation in region 5B of exon 5 by SSCP: row 1 healthy blood sample, row 2 without mutation patient tissue sample, row 3 healthy tissue sample and Row 4 with mutation tissue sample shows (DNA marker 100bp).

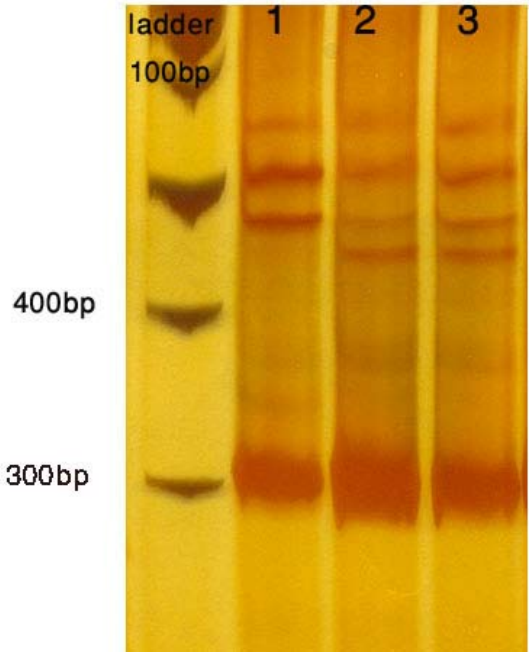

Figure 8. The study area 8B mutation exon 8 by SSCP: row 1 with mutation patient tissue sample, row 2 healthy tissue sample and row 3 without mutation patient tissue sample shows (DNA marker 100bp).

Changes observed in SSCP confirmed by HMA

Samples, whose pattern of bands in SSCP method was different from normal samples, were studied again by the HMA technique to ensure certainty. HMA results of exon 4, region exon 6B, rgion exon 5B and region exon 8B PTEN gene has been shown in figures 9-12.

In order to augment the accuracy, the experiments (PCR, SSCP and HMA) were repeated at least three times.

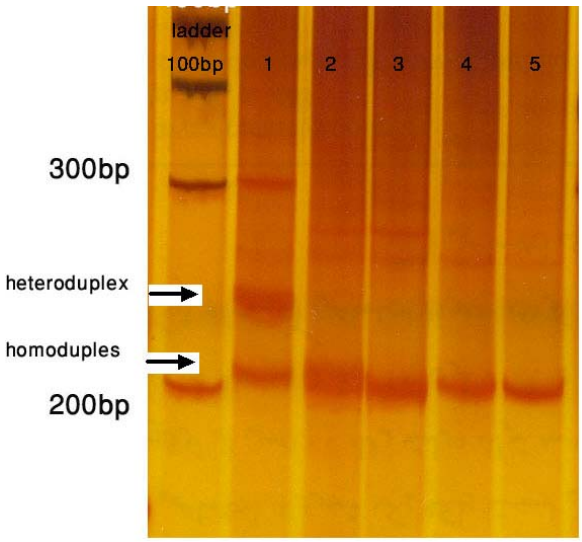

Figure 9. confirmed SSCP result in exon 4 by HMA technique: Row 1 whit mutation tumor tissue samples, row 2 healthy tissue samples of patient whit mutation and rows 3-5 healthy tissue samples (DNA marker 100bp).

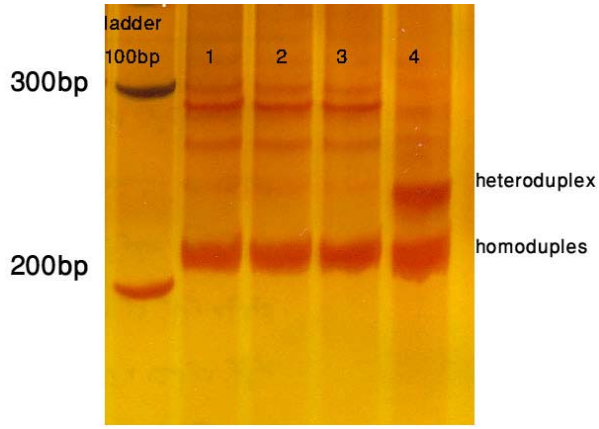

Figure 10. Confirmed SSCP result in region 5B of exon 5 by HMA technique: rows 1 and 2 patients without mutations tissue samples, Row 3 healthy tissue sample of whit mutation patient and Row 4 whit mutation tumor tissue sample shows (DNA marker 100bp).

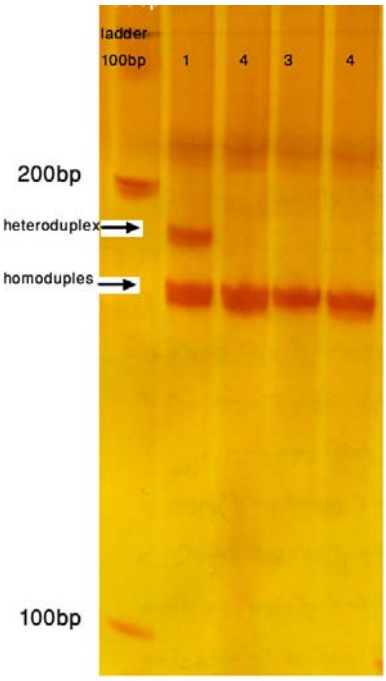

Figure 11. Confirmed SSCP result in region 6B of exon 6 by HMA technique: Row 1 whit mutation tumor tissue sample, row 2 healthy tissue sample of whit mutation patient and row 3 and 4 whitout mutation patient tissue sample shows (DNA marker 100bp).

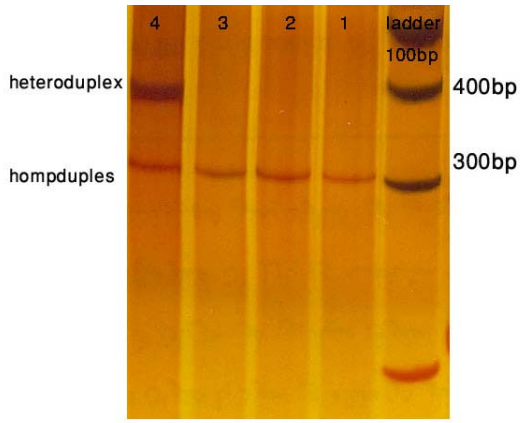

Figure 12. Confirmed SSCP result in region 8B of exon 8 HMA technique: row 1 and 2 without mutations patient tissue samples, Row 3 healthy tissue sample of whit mutation patient and Row 4 mutation patient tissue sample shows (DNA marker 100bp).

Percent of PTEN mutations in Glioblastoma tumors in Isfahan population

Among the 60 tissue samples of Glioblastoma 4 mutations were observed which is equivalent to almost 7 percent. Mutations observed in this study were located in exons 4, 5, 6 and 8.

Figure13. Distribution of PTEN mutation frequency percent in exons 4-8

The correlation between PTEN mutations with the age of patients

To study the correlation between the age of onset of cancer and mutations, the mean age of onset of cancer with mutations and without mutations were calculated. The mean age total patients were 18.91 ± 44.83 and the mean age of patients with mutation was 8.06 ± 11.75 and the mean age of patients without the mutation was 17.17 ± 47.20 (Figure 14). T-test showed significant difference between the mean age of patients with the mutation and patients without the mutation (P-value <0.001). In other words, with a certainty of 0.999, it can be said that mutations of PTEN gene is related to age of patients. This significant correlation was confirmed by Fisher's exact test with confidence of 0.999 (P-value: 0.001). Since 50 percent of patients were under 20 and 50 percent over 20 years, people were divided into two groups, above and below the age of 20 years. 50 percent of people below 20 years showed mutations in PTEN gene but no mutations were found in patients over 20 people (Table 2).

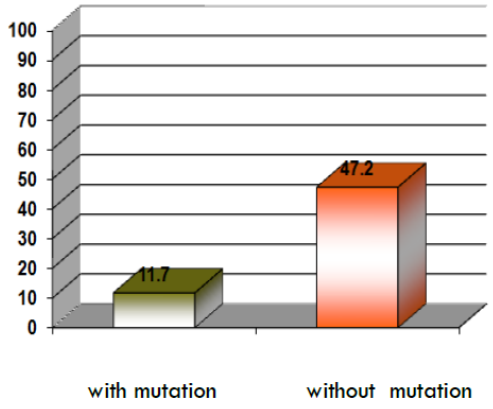

Figure 14. The mean age of patients with PTEN mutation and without PTEN mutation.

Table 1. Shows used Primers in this study

Primer	Primer sequence	Product length	Tm
Exon 4	F 5′CATTATAAAGATTCAGGCAATG3′	204	53
	R 5′GACAGTAAGATACAGTCTATC 3′		
Exon 5A	F 5′ACCTGTTAAGTTTGTATGCAAC 3′	229bp	58
	R 5′TTCCAGCTTTACAGTGAATTG 3′		
Exon 5B	F 5′GACCAATGGCTAAGTGAAGAT 3′	208bp	52
	R 5′TCCAGGAAGAGGAAAGGAAA 3′		
Exon 6A	F 5′CATAGCAATTTAGTGAAATAA 3′	212	57
	R 5′CGCCACTGAACATTGGAATA 3′		
Exon 6B	F 5′CAGTCAGAGGCGCTATGTGT 3′	165	55
	R 5′TGTTCCAATACATGGAAGGATG3′		
Exon 7	F 5′ TGACAGTTTGACAGT 3′	263	54
	R 5′AACCCTCTTTATAGG 3′		
Exon 8A	F 5′TGCAAATGTTTAACATAGGTGA 3′	262bp	54
	R 5′GTAAGTACTAGATATTCCTTGT 3′		
Exon 8B	F 5′AGTCTATGTGATCAAGAAATC 3′	300bp	54
	R 5′TCATCATGTTACTGCTACGTAAA3′		

Table 2. Shows distribution of PTEN mutation with *group age*

Age group \ Mutation		−	+	**Total**
≤20	Count	4	4	8
	Percent	50.0	50.0	100
20<	Count	52	0	52
	Percent	100	0	100
Total	Count	56	4	60
	Percent	93.3	6.7	100

Contact with mutations in different positions histological

In this study, all patients were of type glioblastoma molti form which in the WHO system were divided into four degrees and considered malignant. Four histological markers were studied in these patients.

After pathological examination, glioblastoma tumors were classified into 4 groupsof Pleomorphism, Necrosis, cellularity, mitosis and endothelial proliferation. Based on the cellularity Patients were classified in three types: two times normal, cellularity three times normal and cellularity more than three times the normal. The percent of cellularity intensity in people with mutation is more than people without mutations. But manvitni test didn't show a significant Correlation between the mutation rate and Cellularity (P-value <0.182). According to Pleomorphism Patients were classified in three types: mild, moderate and severe Pleomorphism. The manvitni test didn't show a significant Correlation between the mutation rate and Pleomorphism (P-value <0.36). Patients with necrosis were classified in three types: focal necrosis without palisading, focal necrosis with palisading and clear necrosis. According

Table 3. Shows distribution of PTEN mutation with *endothelial proliferation*

Mutation	Endothelial proliferation	Absence of endothelial proliferation	Endothelial proliferation two rows	endothelial proliferation more than two rows	Glomerular	Total
–	Count	2	4	27	23	56
	Percent	3.6	7.1	48.2	41	100
+	Count	0	1	2	1	4
	Percent	0	25.0	50.0	25.0	100
Total	Count	2	5	29	24	60
	Percent	3.3	8.3	48.3	40.0	100

Table 4. Shows distribution of PTEN mutation with *Necros*

Mutation	Necrosis	Necrosis focal without palisading	Necrosis with palisading	Necrosis whit clear focal	Total
–	Count	10	4	42	56
	Percent	17.9	7.10	75.0	100
+	Count	1	0	3	4
	Percent	25.0	0	75.0	100
Total	Count	11	4	45	60
	Percent	18.3	6.7	75.0	100

Table 5. Shows Distribution of PTEN mutation with *Pleomorphism*

Mutation	Pleomorphism	Mild Pleomorphism	Moderate Pleomorphism	Severe Pleomorphism	Total
–	Count	1	20	35	56
	Percent	1.8	35.7	65.5	100
+	Count	0	2	2	4
	Percent	0	50.0	50.0	100
Total	Count	1	22	37	60
	Percent	1.7	36.7	61.7	100

to the manvitni test there wasn't a significant Correlation between the mutation rate and necrosis (P-value <0.43). Also in this study patients were divided intofour groups according to endothelial proliferation: lack of endothelial proliferation, propagation endothelial proliferation to two rows, propagation endothelial proliferation more than two rows and endothelial proliferation with glomeroid. According to the manvitni test no significant Correlation between the mutation rate and endothelial was observed (P-value <0.241) (Tables 3-6).

Discussion

The first evidences about the role of PTEN gene as a tumor suppressor gene were obtained while PTEN mutations were studied in several cell lines and Glioma tumor [12]. The first studies on PTEN

Table 6. Shows distribution of PTEN mutation with *cellularity*

Mutation	*Cellularity*	Twice the normal	Three times the normal	More than three times the normal	Total
–	Count	6	10	40	56
	Percent	10.7	17.9	71.4	100
+	Count	0	0	4	4
	Percent	0	0	100	100
Total	Count	6	10	44	60
	Percent	10.0	16.17	73.3	100

mutations in Glioblastoma were by Li and colleagues. Li and his colleagues reported 5 nonsense mutations in the Glioma cells in 1997 [13]. Steck and his colleagues identified 11 mutations in Glioma in the same year [14]. Sakurada and colleagues, reported mutations of PTEN in 9 percent of Glioblastoma tumor in 1997[15]. At the end, Deimling and colleagues reported Loss of Heterozygosity (LOH) chromosomes 10 in 45 patients by studying 67 glioblastoma tumors in 2008 [16]. Zhou XP and his colleagues identified 13 mutations in Glioblastoma tumors by studying 47 patients [17]. In this study exons 8-4 PTEN gene were selected and Studied considering the focus of mutation rate in these exons based on the previous studies and with regard to this point that the selected exons code protein s with core functions therefore mutations in these regions cause loss of normal function of PTEN. In this Study like the other ones, SSCP technique was used to detect mutations. This technique has been used as a primary technique in the diagnosis of mutations and it is cheaper than sequencing. One of the weaknesses of this technique is the possibility of open DNA secondary structures formed by DNA single strand that we decrease this possibility by optimizing SSCP gel conditions such as temperature and glycerol rate. HMA technique was also used in the research to confirm SSCP results. Interpretation of HMA gel is easier than SSCP gel. In this study, the age of patients was 1-80 with the median age of 44.83 ± 18.91. About 7 percent of the patient samples in this study showed mutation in PTEN gene. The percent of mutations observed in this study are less than percent of mutations observed in previous studies. Sadati and his colleagues in 2004 [18] and Nasiri and his colleagues in 2005 [19] investigated PTEN mutations in prostate and breast cancers in Iran population and reported similar results with our study indicating a low prevalence of PTEN gene mutations in Iran population. In this study, the Correlation between PTEN mutations and four Clinical Pathologic markers (Pleomorphism, Necrosis, cellularity, mitosis and endothelial proliferation) was investigated. Our data did not show a positive association between clinic pathologic markers and PTEN mutations. In previous studies the Correlation between PTEN mutations and Clinical Pathologic markers were not investigated. The association between PTEN mutations and age of onset of disease was investigated. The mean age of total patients was obtained (18.91 ± 44.83) and the mean age of patients with mutation in PTEN gene (11.75±8.06) was considerably less than the mean age of total patients. The patients with mutations were 20, 15, 11, and were 1 year old. Based on manvitni test and Fisher exact test, probability of P-value <0.001 was Obtained showing significant Correlation between PTEN mutations and age of patients. People with PTEN mutations are more likely to develop glioblastoma before the age of 20. Izumoto and his colleagues reported significant Correlation between PTEN mutations and the age of patients with glioblastoma in the Japanese population in 2001 [20]. Understanding the association between clinic pathologic markers and molecular mechanisms has led to possible using of new treatment methods that can be used in screening glioblastoma tumors from other brain tumors to obviate diagnostic uncertainties.

Acknowledgment

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Conflict of Interest

None to declare.

Authors' Contribution

NH was the first author who has contributed his research work towards cancer study in his Master of Pharmacy study. MT has co-guided this research study and helped by carrying out the extraction procedure and other technical matters in the same. PM has selected this present research protocol and served as principle guide for the research study and has provided the space and supplied the samples for the present research work.

References

1. Mantravardi RVP. Brain stem glioma: an autopsy of 25 cases of cancer. Biology. 1982; 49: 1294-6.

2. Scherer HJ. Cerebral astrocytomas and their derivatives. Cancer. 1994; 40:159-98.

3. Lefranc F, Rynkowski M, DeWitte O, Kiss R. Present and potential future adjuvant issues in high-grade astrocytic glioma treatment. Adv Tech Stand Neurosurg. 2009; 34:3-35.

4. Zulc, KJ. Histological typing of tumours of the central nervous system. International Histological Classification of Tumours, Geneva, World Health Organization. cell. 1979; 21:211-6.

5. Sano T, Lin H, Chen X. Differential expression of MMAC/PTEN in glioblastoma multiforme: relationship to localization and prognosis. Cancer Research. 1999; 59:1820- 4.

6. Wang SI, Puc J, Li J. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Research. 1997; 57:4183- 6.

7. Zhen H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR et al. P53 and PTEN control neural and glioma stem/progenitor cell renewal and differentiation. Nature. 2008; 455(7216): 1129-33.

8. Sjostrom S, Andersson U, Liu Y, Brännström T, Broholm H, Johansen C, et al. Genetic variations in EGF and EGFR and glioblastoma outcome. Euro Oncol. 2010 Mar 2. [Epub ahead of print]

9. Peiffer SL, Herzog TJ, Tribune DJ, Mutch DG, Gersell DJ, Goodfellow PJ. Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. Cancer Research. 1995; 55:1922-6.

10. Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, Vogt PK. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth. 2001; 12: 363-9.

11. Steve E, Humphries V, Gudnason R, Whittall I. Single-strand conformation polymorphism analysis with high throughput modifications, and its use in mutation detection in familial hypercholesterolemia. Clinical Chemistry. 1997;43:427-35.

12. Joshi AD, Parsons DW, Velculescu VE, Riggins GJ. Sodium ion channel mutations in glioblastoma patients correlate with shorter survival. Mol Cancer. 2011; 11; 10:17.

13. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science. 1997; 275:1876-8.

14. Steck, PA, Pershouse, MA, Jasser, SA, Yung, WK, Lin H, Ligon, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. National Genetics. 1997; 15:356-62.

15. Sakurada A, Suzuki A. Sato M Infrequent genetic alterations of the PTEN/MMAC1 gene in lung, pancreas, kidney, and ovary. Jpn. J. Cancer Research.1997; 88: 1025-8.

16. Deimling A v, Ammon Kv, Schoenfeld D, Wiestler O, Seizinger B, Louis D. Subsets of Glioblastoma Multiforme Defined by Molecular Genetic Analysis. Brain Pathology. 2008; 3: 19 - 26.

17. Zhou XP, Li YJ, Hoang-Xuan K, Laurent-Puig P, Mokhtari K, Longy M . (1999a) Mutational analysis of the PTEN gene in gliomas: Molecular and pathological correlations. Cancer 84, 150-4.

18. Saadati M. MC Thesis. The University of Tehran; 2006.

19. Nasiri A. MC Thesis . The University of Isfahan; 2005.

20. Izumoto S, Ohnishi T, Kanemura H, Arita N, Maruno M, Moriuchi S . PTEN Mutations in Malignant Gliomas and their Relation with Meningeal Gliomatosis. Journal of Neuro-Oncology. 2001; 53: 21-6.