RESEARCH ARTICLE

Prevalence of IDH1/2 Mutations in Different Subtypes of Glioma in the North-East Population of Morocco

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Abstract

Background: Genetic alterations in gliomas have increasing importance for classification purposes. Thus, we are especially interested in studying *IDH* mutations which may feature potential roles in diagnosis, prognosis and response to treatment. Our aim was to investigate *IDH* mutations in diffuse glioma patients diagnosed in university hospital centre of Fez in Morocco. Materials and Methods: *IDH1* codon 132 and *IDH2* codon 172 were direct-sequenced in 117 diffuse glioma samples diagnosed and treated in University Hospital Hassan II between 2010 and 2014. Results: The R132H *IDH1* mutation was identified in 43/117 tumor samples and R172K *IDH2* mutation was detected in only one anaplastic oligodendroglioma. *IDH* mutations were observed in 63.2% of astrocytomas, 73.3% of diffuse oligodendrogliomas and 12.90% of glioblastomas. Conclusions: Our results confirmed other studies published earlier for other populations with some small discrepancies.

Keywords: *IDH* mutations - glioma - immunohistochemistry - sequencing

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Introduction

Diffuse gliomas represent the most common and lethal primary malignant brain tumors. The 2007 World Health Organisation classification distinguishes gliomas based on their morphology and architecture (Louis et al., 2007). Grade I gliomas occur mainly in childhood and are generally curable with complete surgical resection. Diffuse gliomas can be classified from low grade (grade II) through anaplastic (grade III) to malignant (grade IV). Glioblastoma, the most malignant glioma, carries the worst prognosis.

So far histomorphological evaluation remains the key tool for the diagnosis but it is generally not sufficient to predict the clinical outcome or response to therapy. Thus, new molecular markers must be used as additional tools for diagnosis and/or treatment guidance. Among them, isocitrate dehydrogenase (*IDH*) gene mutation seems to be a good diagnostic marker but also a powerful prognostic marker for gliomas patients.

Indeed several meta-analyses have shown that *IDH* mutations are associated with better OS and better PFS, especially for patients with WHO grade III glioma. (Xia et al., 2015; Chen et al., 2016).

The discovery of somatic *IDH* mutations in gliomas was first reported in 2008 from the genome-wide sequencing performed on 22 patients with glioblastoma (Parsons et al., 2008). This study shows 12% of *IDH1* mutations affecting specially enzyme's active site localized on codon 132. In successive studies, *IDH* mutations have also been found in low-grade gliomas (Hartmann et al., 2009; Yan et al., 2009) as well as in acute myeloid leukemia (Patel et al., 2011).

IDH1 and *IDH2* are homodimeric enzymes involved in the Krebs cycle that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-kG) while reducing NADP+ to NADPH. *IDH1* is the most important NADPH producer in most human tissues, particularly the brain. NADPH is important for the regeneration of reduced glutathione that have the capacity to protect cells against oxidative stress (Lee et al., 2002). The generation of NAPDH by *IDH1* is involved in the protection against

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lipid peroxidation and oxidative DNA damage (Waitkus et al., 2016).

It has been shown that *IDH* mutations cause not only a function loss of the oxidative decarboxylation of the enzyme but also a gain of function because the mutated enzyme becomes capable of reducing alpha-ketoglutarate to 2-hydroxyglutarate. This "oncometabolite" competitively inhibits the alpha-ketoglutarate dependent enzymes which play an important role in gene regulation and tissue homeostasis. Expression of mutant *IDH* alters cellular differentiation and promotes the development of the tumor (Clark et al., 2016).

It is also important to note that the *IDH* mutations are thought to be one of the earliest genetic alterations in the gliomagenesis prior to malignant transformation and may affect a common glial precursor cell population (Watanabe et al., 2009; Leu et al., 2016).

IDH1 and *IDH2* mutations are observed in somatic cells. Mutations in these genes are heterozygous and missense changes and always mutually exclusive. These alterations generally affect codon R132 in the *IDH1* gene (>90%) and its homologous R172 in the *IDH2* gene, with mutations in the *IDH2* gene are less common in gliomas (3 to 5%) (Hartmann et al., 2009; Sanson et al., 2009).

Our aim in this study is to identify the *IDH* mutations and their frequencies in 117 Moroccan patients with glioma. Indeed, the Moroccan population is a non-caucasian population for which we have very limited data. Furthermore, tumors without *IDH1* mutation were analyzed another time for the presence of *IDH2* mutation. The 117 tumors include Grades II and III astrocytomas, oligodendrogliomas, oligoastrocytomas and glioblastomas.

Materials and Methods

Patients

The present retrospective study includes 117 glioma patients diagnosed and treated at University Hospital Hassan II of Fez between 2010 and 2014. Tumor tissues were obtained from the archives of the department of Pathology at the University Hospital. Research use of tissues and anonymization of data were in accordance with local ethical approvals. Hematoxylin and eosin-stained slides were independently reviewed by three neuropathologists who were blinded to the diagnosis of the patients. The tumors were classified according to the 2007 World Health Organization classification of Brain tumors. Furthermore, MRI imaging was reviewed by an experienced Radiologist for diagnosis comfirmation. In case of diagnostic disagreement between the observers, the patient was excluded from the study.

Immunohistochemistry

IHC was performed with the antibody clone H09 (Dianova) which detects specifically *IDH1* R132H protein. Deparaffinization, rehydratation and heat-induced epitope retrieval were performed with standard procedures.

IDH1 immunostaining was observed by two different neuropathologists. When, cell's cytoplasm shows diffuse and strong staining, it was scored as positive.

For each technique, we integrated positive and negative controls.

The FISH technique using "Vysis 1p36/1q25 and 19q13/19p13 probe" is still under development. Therefore, two antibodies were used when reviewing pathological slides to assess diagnosis: alpha-internexin and OLIG-2 (Oligodendrocyte transcription factor 2).

DNA extraction

Tumor DNA was isolated from formalin fixed paraffin embedded tumor samples following macrodissection of tumor tissue and normal brain tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen).

PCR amplification and Sequencing analysis

Exon 4 of *IDH1* and *IDH2* genes was sequenced. The PCR primers were used as follows: *IDH1* Forward primer 5'-AGA AGA GGG TTG AGG AGT TCA A-3' with reverse primer 5'-CAC ATA CAA GTT GGA AAT TTC TGG-3' and for *IDH2* 5'-TTG GCA GAC TCC AGA GCC CA-3' with reverse primer 5'-GCC CGG TCT GCC ACA AAG TC-3'.

The PCR was performed using Platinium®Taq DNA polymerase (Invitrogen). The PCR conditions were 94°C for 5 minutes; 40 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 1 minute; and extension at 72°C for 10 minutes.

The PCR products were purified using illustraTMExoProStarTM 1-Step according to the manufacturer's instructions.

The purified PCR products were subjected to direct sequencing using the previous primers and the BigDye Terminator V3.1 Sequencing Kit (Applied Biosystems) on a 3500Dx automated sequencer (Applied Biosystems).

Results

Our population included at the beginning122 patients (76 males and 46 females). The median age is 41 years ranged from 3 to 90 years old. The tumors show widespread anatomic distribution with a predominance of frontal lobe involvement.

Histological analysis was used to differentiate between different subtypes of gliomas. After revision, 7 cases of glioblastomas were reclassified as grade III oligodendrogliomas and one as grade III oligoastrocytoma. 2 cases were excluded from the study because of diagnostic disagreement between the three neuropathologists. It is noteworthy that three other cases were also excluded due to diagnostic discordance between the radiologist and the neuropathologists. The histologic diagnoses of the 117 cases included in the study were as follows : 23 of tumors were grade II gliomas (13 astrocytomas, 7 oligodendrogliomas, 3oligoastrocytomas), 32 grade III gliomas (6 astrocytomas, 23 oligodendrogliomas, 3oligoastrocytomas) and 62 Grade IV (glioblastomas) (Table 1).

To confirm the diagnosis of oligodendrogliomas and glioblastomas in all cases of doubt, immunohistochemistry analysis was performed with the use of the alpha-internexin and OLIG-2 antibodies. This technique has allowed us to

Table 1. IDH1 Mutation Frequencies According to Histological Subtypes in Different Grades of Gliomas

Histologic Subtype		Sex ratio M/F	Median age	No. of Patients with IDH1 mutation/Total No.	%
Grade II	A	9/4	32.5	1/8	61.5
	O	3/4	30	3/7	42.9
	OA	3/0	17	1/3	33.3
Grade III	A	1/5	33.5	4/6	66.7
	O	18/5	39.5	18/23	78.3
	OA	2/1	56	1/3	33.3
Grade IV	GBM	36/26	50.5	8/62	12.9
Total		72/45	41	43/117	36.8

A :Astocytoma, O : Oligodendroglioma, OA : Oligo-astrocytoma, GBM : Glioblastoma, M : Males, F : Females, No. : Number.

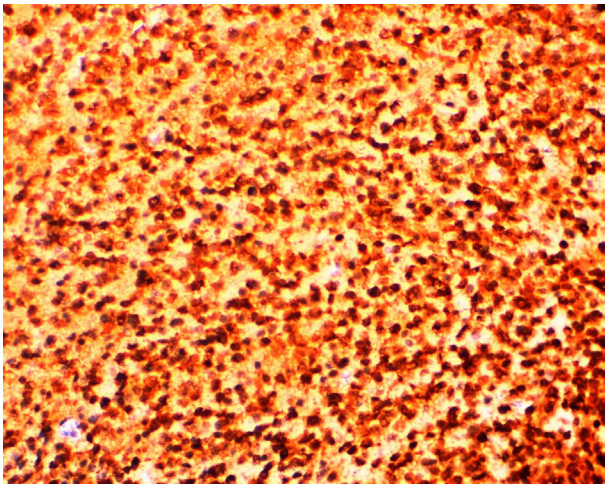

Figure 1. Anaplastic Oligodendrogliome Labeled with the Antibody anti-IDH1R132H (x100). This microscopic observation shows a strong and diffuse cytoplasmic staining of tumor cells from a patient with grade III oligodendroglioma and carring the R132H IDH1 mutation

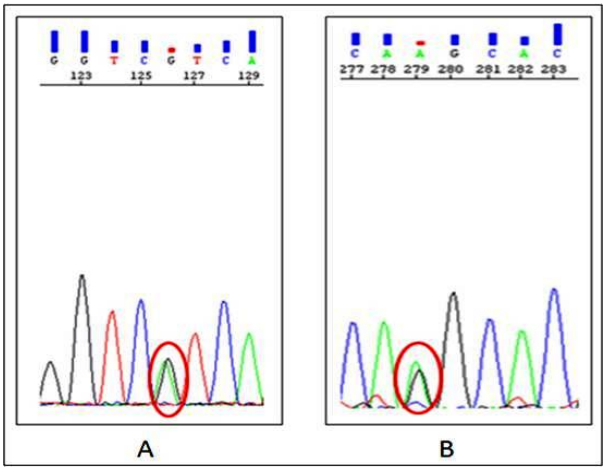

Figure 2. Somatic Mutations of the IDH1 Gene (R132H) in Glioblastoma. (A) and the IDH2 gene (R172K) in anaplastic oligodendroglioma (B)

confirm the reclassification of 7 glial tumors (passing from glioblastoma group to anaplastic oligodendroglioma) and to remove one ependymoma tumor.

The antibody clone H09 (Dianova) was used to detect *IDH1* R132H mutation product. m*IDH1* R132H immunostaining was found in 43/43 patients presenting the R132H mutation (sensitivity 100%) (Figure 1). No cases with non mutated *IDH1* gene was stained (Specificity 100%)

The analysis of exon 4 of *IDH1*and *IDH2* genes was performed in all tumors. For *IDH1*, we identified mutation in 43 cases (36.8%) located at codon 132 (CGT → CAT) and corresponded to an Arginine-Histidine substitution (R132H) (Figure 2A). Different grade of tumors show mutation in this gene as follows: 12/19 (63.2%) in Astrocytomas grade II and III, 21/30 (70%) in Oligodendrogliomas grade II and III, 2/6 (33.3%) in Oligoastrocytomasand 8/62 (12.9%) in Glioblastomas, while one *IDH2* mutation was detected in an anaplastic oligodendroglioma (Figure 2B). Detailed results are presented in Table 1.

Discussion

Recent years have seen appearing a large number of molecular markers. Some of them are used to classify tumors and have a better diagnosis while others are used to predict the prognosis of patients.

Recent publications have shown that the determination of *IDH* status is essential for the diagnosis and prognosis of glial tumors. *IDH* mutational status may help to differentiate glioma from reactive gliosis especially in the case of small biopsies. Furthermore, *IDH* mutations aid in the discrimination of various tumors such as grade II gliomas from pilocytic astrocytomas or pleomorphic xanthoastrocytomas, or secondary glioblastomas from primary glioblastomas or oligodendrogliomas and oligoastrocytomas from other brain tumors with oligodendroglioma-like morphology (Capper et al., 2009; 2010; 2011; Camelo-Piragua et al., 2010). Noteworthy, a number of studies have demonstrated that the presence of *IDH* mutations predicts significantly longer survival and progression-free survival for patients with gliomas (Weller et al., 2009; Van den Bent et al., 2010; Houillier et al., 2010; Mellai et al., 2011; Lv et al., 2011; Leeper et al., 2015; Eckel et al., 2015; Cancer Genome Atlas Research Network et al., 2015; Xia et al., 2015; Chen et al., 2016).

The *IDH* mutations with other genetic alterations are currently guiding treatment decisions for diffuse gliomas and they will probably, in the future, allow the adjustment of treatment with the molecular profile (Juratli et al., 2015).

On another side, an *IDH1* inhibitor (AGI-5198) has been developed and has shown to bind to and inhibit mutant *IDH1*. Other studies have tried to target protein function upstream or downstream of mutant *IDH*. The

results may yield promising novel therapeutic strategies (Davis et al., 2013; Rohle et al., 2013, Agnihotri et al., 2014). Moreover, the promising results of recent studies which tried to investigate the possibility of immunotherapeutic targeting of *IDH* mutations suggest that the mutant *IDH*-targeted can elicit potent antitumor immune response (Schumacher et al., 2014; Pellegatta et al., 2015; Waitkus et al., 2016).

IDH mutations have been found significantly correlated with glioma grade. A high frequency of these mutations has been especially described in grade II and III gliomas, as well as in secondary glioblastomas. These rates were confirmed by various studies on American (Killela et al., 2014; Christensen et al., 2011), Chinese (Li et al., 2012; Chuan et al., 2014), Japanese (Shibahara et al., 2011; Mukasa et al., 2012), French (Sanson et al., 2009), Brazilian (Pessoa et al., 2015) and other population (Wick et al., 2009; Gravendeel et al., 2009; Nobusawa et al., 2009; Bleeker, 2010).

In Morocco, only one study was published in 2013. The authors presented the results of molecular analysis of *IDH1*/2 and TP53 genes in 34 Moroccan patients with primary glioblastoma and reported their clinical and epidemiological characteristics (Hilmani S et al., 2013). The screening of all 34 glioblastomas for R132-*IDH1* and R172-*IDH2* mutations revealed the absence of these mutations in the population study.

However, our study is consequently the first experience which includes various subtypes of diffuse gliomas diagnosed in Moroccan patients. This work will give an idea about the distribution of these alterations in the North African population.

We detected 43 cases of *IDH1* mutations (R132H) in our series and only one case of *IDH2* mutation (R172K) was identified in a grade III oligodendroglioma wild-type for *IDH1*. Grades II and III Astrocytomas carried *IDH* mutations in 63.2%. Our work confirms previous studies which detected the same mutations in 63% (Sanson et al., 2009), 78.4% (Killela et al., 2014) and 78.3% (Christensen et al., 2011). Grade II and III Oligodendrogliomas carried *IDH* mutations in 73.3% comparable to 63% (Sanson et al., 2009) and 71% (Mukasa et al., 2012) in earlier studies. Moreover, the rate observed in glioblastomas in our study is 12.9% comparing to 16.8% (Zhang et al., 2016) and 10% (Patrick et al., 2014; Mukasa et al., 2012) in other publications. However, in Grade II and III Oligoastrocytomas, the *IDH* mutation was found in lower proportion (33.3%; 2/6 cases) than those described in other publications: 86.2% (Killela et al., 2014), 75% (Shibahara et al., 2011) and 68% (Sanson et al., 2009).

Furthermore, grade III glial tumors showed the highest frequency of *IDH* mutation (53.49%; 23/43 cases). This result is consistent with the Brazilian study that identified 66.7% of Grade III glioma patients carrying this mutation (Pessoa et al., 2015).

Thus, our results are fully concordant with the literature data with slight difference in oligoastrocytomas subgroup. This disparity is mainly due to the fact that the number of patients in this subgroup remains low (6 cases) and does not allow yet significant conclusions. The inclusion of a higher effective is required for more accurate results.

In conclusion, In this study, the results obtained are in concordance with the literature. We included the largest number of Moroccan patients with diffuse gliomas that has been analyzed for the presence of *IDH* mutations. We found that 63.2% of Astrocytomas, 73.3% of Oligodendrogliomas grade II and III and 12.9% of glioblastomas carried *IDH* mutation. These proportions are comparable to those found in European, Asian and American studies. Further studies are in progress to evaluate the prognostic role of *IDH* mutations in Moroccan patients with malignant gliomas. This study will provide a reliable basis for clinicians and oncologists in Morocco and raise awareness of the importance of integrating molecular biology data in their therapeutic approach.

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