Immunohistopathological Profile and Biomolecular Level of Central Nerve Tumors in Kinshasa, DRC According to the Classification of the World Health Organization 2016

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Abstract: The goal is to determine immunohistochemical histopathological profile and biomolecular level of Central nerve Tumors in Kinshasa, DRC. According to the classification of the World Health Organization (WHO) 2016. Our study is described on a series of cases that were studied using standard histopathological techniques, before undergoing specific immunohistochemical techniques for the detection of anti-IDH1 antibodies in gliomas and biomolecular ones in the search for 1p19q deletion. Central nerve tumors account for 0.18% of all conditions, over a period of time. Meningothelial-type meningioma was the most common (60.9%). The most affected age group is over 50. The distribution of tumors by sex shows a predominance of woman (67.1%). The gliomas had constituted 29.6% of all nerve tumors recorded over the period considered and whose predominance was constituted by oligoastrocytomas with 42.1%. The immunohistochemical profile is characterized by a strong positivity with the anti-IDH1 antibody in case of grade II. Our study showed that central nerve tumors account for 0.18 of all conditions, gliomas are made up of 29.6% of all nerve tumors listed with a predominance of oligoastrocytomas. The immunohistochemical profile is characterized by a strong positivity with the anti-IDH1 antibody in case of grade II.

Keywords: Tumors, Glioma, Histopathologic, Immunohistochemistry, Biomolecular, Classification WHO 2016

1. Introduction

The gliomas are the most common primary brain tumors and group together different entities with very different prognosis. Anatomopathological examination is the standard for the diagnosis of these tumors.

However, pathologists may encounter diagnostic difficulties due, among other things to tumor heterogeneity. In this context, the confrontation between anatomopathological aspects and molecular biology are necessary.

We have recently witnessed major advances in the discovery of molecular alterations in these tumors, which led to the development of new molecular markers, some with a diagnostic role, others with a prognostic and therapeutic role [1, 2]

A better understanding of the mechanisms of these tumors and new therapeutic avenues are essential to improve the prognosis of patients with brain tumors.

In previous studies, the classification then used for gliomas that of the World Health Organization 2007. This classification was based on the histological aspects of gliomas. However, major inter-observer discrepancies have been demonstrated for the histological diagnosis of the same tumor type and especially for the diffuse infiltrating gliomas [3].

Due to these limitations, new approaches were needed to reduce this lack of reproducibility and improve the identification of prognostic factors.

In the different studies, it was shown that the molecular classification was better correlated with the prognosis than the histological classification [4-6].
A new WHO classification integrating molecular markers with histological data was finally established in 2016 [1]. This classification into more homogeneous subgroups makes it possible to better guide the different therapeutic options.

According to statistical data from the World Health Organization, brain tumor pathologies correspond to the following figures: 32.6 million recorded cases, 14 million diagnosed and 8.2 million deaths annually [7]. However, despite the significant therapeutic and diagnostic arsenal, the statistics of cancerous pathologies keep showing a continuous increase, as evidenced by the figures published by the World Health Organization [7].

In Democratic Republic of the Congo, few data related to the incidence of brain tumors are known. And this is due to the lack of a cancer registry.

Therefore, we proposed to conduct a descriptive study on a series of cases relating to histopathological profile and biomolecular level of central nerve tumors of patients admitted to Pathological Anatomy departments of Kinshasa University Clinics, Leboma laboratory and the National Biomedical Research Institute and this, over a period of 14 years from 2004 to 2018, representing a number of 64 cases.

The new classification 2016 groups together different tumor entities defined both by the histological aspect but also by the anomalies observed in immunohistochemistry and molecular biology.

It is therefore important to continue to improve knowledge on the pathophysiology of gliomas and to continue to improve the classification systems in order to better define the prognosis and especially the therapeutic management.

We are interested in coupling the histological classification of 2007 to the biomolecular classification of 2016.

2. Material and Methods

2.1. Material

Our study spans a period of 14 years, from 2004 to 2018. It is a consecutive series of 64 cases out of a total number of 34,406 biopsies, collected from Kinshasa University Clinics, (2004-2018): 11 cases of National Institute for Biomedical Research (l’INRB) (2004-2018): 17 cases and of LEBOMA laboratory (2013-2018): 36 cases.

The paraffin slides and blocks were taken from the archives of the aforementioned establishments and re-examined respectively according to the 2007 classification and the molecular classification of the WHO 2016.

All the fragments reached us fixed in 10% formalin. After dehydration, embedding in paraffin and microtome cutting, the preparations thus obtained were stained with hematoxylin-eosin-saffron.

The data collected was recorded and analyzed using SPSS version 12 software under Windows.

2.2. Methods

Our study is descriptive on a series of cases, having involved 64 blocks which were first of all studied according to standard histopathological techniques (or routine), before undergoing specific immunohistochemical techniques for the search of expression of anti-IDH1 and IDH2 antibodies in gliomas and biomolecular ones in the search for the deletion of the 1p19q genes.

Standard or routine histopathological Techniques: The biopsy tissue fragments fixed with 10% in formalin were dehydrated in alcohol, thinned with xylene before being impregnated in paraffin. The blocks obtained were cut with a microtome and fine ribbons of 3 to 5 microns were mounted on slides coated with albumin. After drying in an oven, these slides were stained with hematoxylin-Eosin before mounting a coverslip fixed by l’Eukit. In fact, after comes the optical microscope reading for the typing of our nerve tumors. The immunohistochemical study included the anti-IDH1 monoclonal antibody (clone H09), by resorting to Benchmark Ultra (Ventana-Roche), who is an immunohistochemistry automaton and according to the following steps: The tissue blocks fixed with 10% formalin and embedded in paraffin are cut with a microtome. The thin 4 micron ribbons were placed on the polysilane slides and dried in the oven for at least 12 hours at 37 degrees. We will then incubate the primary and secondary antibody then the developer according to the following steps:

1) Dewaxing with two xylene baths of 10 minutes each followed by rehydration with absolute alcohol (2 X 5 minutes) and a 5 minutes alcohol bath) 80 degrees then distilled water.
2) Pretreatment in a water bath heated to 96 degrees and in the buffer recommended by the manufacturer (EDTA or Citrate) for 30 to 40 minutes depending on the antigen to be unmasked.
3) After cooling for 20 minutes, circle the preparations with Dako-pen (Hydrophobic marker) then place 3% hydrogen peroxide for 20 minutes. Rinse the slides with distilled water and then in Tris-Buffer-Salin (TBS).
4) Incubation with the primary antibody (diluted according to the manufacturer’s recommendations: 30-60 minutes depending on the antibody).
5) Rinse in distilled water then TBS
6) Incubation with the secondary antibody (30 minutes).
7) Rinsing as above.
8) Place the developer (DAB:Di-amino-3.5 Benzidine or AEC: Amino-9 Ethyl-Carbazole) for 5 minutes.
9) Rinsing with normal water.
10)Do a counterstain with Hemalun of Mayer (aqueous solution).
11)Rinsing as above.
12)Assembly of the coverslips with aqueous adhesive (Paramount or Pertex or Eukit)
13)Optical microscope reading.
14)Molecular analyzes, in particular the 1p19q deletions are in progress and the results will be published later. Fig. 16 shows the different characteristics of the anti-IDH1 R132H antibody, clone H09:
Anti-IDH1 R132H / DIA-H09
Mouse monoclonal anti-brain tumor marker (Astrocytoma, Oligodendroglioma), Clone H09

Product Information
Catalog No.: DIA-H09 (100µg)
Clone: H09
Concentration: 0.2 mg/ml
Isootype: Mouse IgG2a
Specificity: Human IDH1 R132H point mutation
Immunogen: Synthetic peptide, amino acid sequence CKPIIIHGAYGD
Physical State: Lyophilized powder
Species Reactivity: Human
Positive Control: Oligodendroglioma, diffuse astrocytoma
Negative Control: Pilocytic astrocytoma, primary glioblastoma (ca. 95% of cases negative)
Visualization: Cytoplasmic

Reconstitution: DIA-H09 (100µg), reconstitute with sterile distilled water by gentle shaking for 10 minutes
Presentation: In PBS with 2% BSA, 0.05% NaNO3, pH 7.4. Antibody purified from culture supernatant
Applications: Immunohistochemistry
(Standard formalin-fixed paraffin sections)
Western blot
Dilutions: 1:20 Immunohistochemistry (IHC)
1:500 Western Blot
(General recommendation, validation of antibody performance/protocol is the responsibility of the end user. Positive/negative controls should be run simultaneously with patient specimen. Interpretation must be made by a qualified pathologist within the context of patient's clinical history and/or diagnostic tests.)
Control Antibody: DIA-W08, rat anti-hu IDH1wt, clone W08
(Staining protocols on www.dianova.com)

Reactivity
Antibody clone H09 reacts specifically with the isocitrater dehydrogenase 1 (IDH1) R132H point mutation in tissue sections from formalin-fixed brain tumor specimens. Heterozygous point mutations of IDH1 codon 132 are frequent in World Health Organization (WHO) grade II and III gliomas. IDH1 R132H mutations occur in approximately 70% of astrocytomas and oligodendrogliol tumors. The high frequency and distribution of the IDH1 R132H mutation among specific brain tumor entities allow the highly sensitive and specific discrimination of various tumors by immunohistochemistry, such as anaplastic astrocytoma from primary glioblastoma or diffuse astrocytoma WHO grade II from pilocytic astrocytoma or ependymoma. Noteworthy is the discrimination of the infiltrating edge of tumors with IDH1 mutation from reactive gliosis. This antibody is highly useful for tumor classification and in detecting single infiltrating tumor cells.

Instructions for Use
Immunohistochemical staining of standard formalin-fixed paraffin sections
Deparaffinize and rehydrate according to standard procedures. Heat induced epitope retrieval (HIER) is required. For immunohistochemical detection different techniques can be used: Indirect immunoenzyme labeling with a secondary antibody conjugate, biotin/streptavdin-based detection, soluble enzyme immune complex or polymer-based detection. To detect antibody, follow the instructions provided with the particular visualization system. The antibody is suited for immuno-histochemical staining using automated platforms. Use the antibody at 1:20 dilution for 30 min at RT.

Technical note
Diffuse astrocytoma WHO grade II may have low protein expression. At high dilution of the antibody single tumor cells in the infiltrated zone may not be stained.

European: For In Vitro Diagnostic Use / All other countries: For Research Use only

Storage and Stability
Store the lyophilized antibody at 2-8°C. For long time storage freeze at -20°C; thus the antibody is stable for at least one year. As reconstituted liquid store at 2-8°C short term (several weeks). For long term storage aliquot and freeze at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Safety Notes
The material contains 0.05% sodium azide as preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material. Avoid skin and eye contact, inhalation, and ingestion.
Figures
Immunohistochemistry of human IDH1 R132H in formalin-fixed paraffin-embedded brain tissue sections
(pictures courtesy of Prof. Dr. med. Andreas von Deimling, Department of Neuropathology, University Heidelberg / Clinical Co-operation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany)

A: Strong reaction of IDH1 mutation specific antibody clone H09 in tumor center of anaplastic oligoastrocytoma.
B: Infiltration zone of anaplastic astrocytoma with specific labelling of infiltrating glioma cells by antibody clone H09.
C: Identification of single tumor cells in white matter distant from tumor center with IDH1 mutation specific antibody clone H09.
D: Cortex infiltrated by oligodendroglioma with specific labelling of tumor cells by antibody clone H09.
E: Double staining of GFAP (glial fibrillary acidic protein, red) and clone H09 (brown) of oligodendroglioma infiltration zone demonstrating specific labeling of tumor cells but not of GFAP positive reactive astrocytes.
F: Strong reaction of IDH1 mutation specific antibody clone H09 with IDH1 R132H mutated diffuse astrocytoma (left) but not with wild type tumor (right).

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Symbols
Manufacturer | IVD | For In vitro Diagnostic Use | CE | Conformity with IVDD 98/79/EC

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Figure 1. Characteristics of anti-IDH1 antibody.
3. Results

3.1. Relative frequency of Central Nerve Tumors

During the period from 2004 to 2018, 34406 biopsies were recorded, including 64 cases of central nerve tumors, i.e a frequency of 0.18% (64/34406).

![Figure 2. Frequency of central nerve tumors.](image)

3.2. Relative Frequency of Different Histopathological Types of NC Tumors

| Histopathological Types     | Number | Frequency (%) |
|-----------------------------|--------|---------------|
| Meningiomas                 | 39     | 60.9          |
| Oligoastrocytomas           | 8      | 12.5          |
| Astrocytomas                | 5      | 7.8           |
| Oligodendrogliomas          | 3      | 4.6           |
| Glioblastomas               | 3      | 4.6           |
| Ependymomas                 | 3      | 4.6           |
| Retinoblastomas             | 2      | 3.1           |
| Choroid plexus papilloma    | 1      | 1.50          |
| Total                       | 64     | 100           |

The table above shows that meningioma comes first with 60.9% of cases followed by oligoastrocytomas.

3.2.1. Frequency of Different Histopathological Types of Meningiomas

| Histopathological Types     | Number | Frequency (%) |
|-----------------------------|--------|---------------|
| Meningothelial Meningioma   | 22     | 56.4          |
| Anaplastic Meningioma       | 5      | 12.8          |
| Psammomatous Meningioma     | 4      | 10.2          |
| Mixed Meningioma            | 3      | 7.6           |
| Fibroblastic Meningioma     | 2      | 5.1           |
| Papillary Meningioma        | 2      | 5.1           |
| Angiomatous Meningioma      | 1      | 2.5           |
| Total                       | 39     | 100           |

The table above shows that the meningothelial type meningioma is the most frequent with 56.4%.

3.2.2. Frequency of Different Histopathological Types of Astrocytomas

| Histopathological Types     | Number | Frequency (%) |
|-----------------------------|--------|---------------|
| Oligoastrocytoma            | 8      | 50            |
| Astrocytoma                 | 3      | 18.7          |
| Glioblastoma                | 3      | 18.7          |
| Diffuse fibrillary Astrocytoma | 2   | 12.5          |
| Total                       | 16     | 100           |

The table above shows a predominance of oligoastrocytomas with 50%.

3.3. Distribution of Tumors by Sex

The distribution of tumors by sex shows a predominance of woman with 67.1% (43/64) and men: 32.8% (21/64). The sex ratio is 0.48.

3.4. Distribution of Tumors According to Different Age Groups

| Age groups | Number | Frequency (%) |
|------------|--------|---------------|
| 1-10       | 4      | 6.20          |
| 11-20      | 4      | 6.20          |
| 21-30      | 1      | 1.50          |
| 31-40      | 7      | 10.90         |
| 41-50      | 16     | 25            |
| Sup. 50    | 32     | 50            |
| Total      | 64     | 100           |

The table above shows that the age group most affected by central nerve tumors was over 50 with 50%, followed by 41-50 with 25%. The youngest patient was 20 months old with retinoblastoma and the oldest with malignant meningioma was 75 years old.

3.5. Frequency of Different Histopathological Types of Gliomas

| Histopathological Types     | Number | Frequency (%) |
|-----------------------------|--------|---------------|
| Oligoastrocytoma            | 8      | 42.1          |
| Astrocytomas                | 5      | 26.3          |
| Glioblastomas               | 3      | 15.7          |
| Oligodendrogliomas          | 3      | 15.7          |
| Total                       | 19     | 100           |

The table above shows that gliomas constituted 29.6% of all central nerve tumors listed over the period of considered and the predominance of which was constituted by oligoastrocytomas with 42.1%.
3.6. Frequency of Different Histopathological Types of Gliomas by Sex

Table 6. Frequency of different histopathological types of gliomas by sex.

| Tumors                | Number | Feminine | Frequency (%) | Male | Frequency (%) |
|-----------------------|--------|----------|---------------|------|---------------|
| Oligoastrocytomas     | 7      | 6        | 31.5          | 1    | 5.2           |
| Oligodendrogliomas    | 3      | 1        | 5.2           | 2    | 10.5          |
| Astrocytomas          | 6      | 4        | 21.0          | 2    | 10.5          |
| Glioblastomas         | 3      | 2        | 10.5          | 1    | 5.2           |
| Total                 | 19     | 13       | 68.4          | 6    | 31.5          |

The frequency of different histopathological types of gliomas by sex, as shown in the table above, shows a predominance of women.

3.7. Immunohistochemical and Molecular Profile of Gliomas According to the World Health Organization Classification 2016

Table 7. Expression of IDH1 in gliomas.

| Gliomas                  | IDH 1      |
|--------------------------|------------|
| Polymorphic Glioblastoma | Negative   |
| Anaplastic Oligoastrocytoma | Negative   |
| grade I Oligodendroglioma | Negative   |
| Giant Cell Glioblastoma  | Negative   |
| Grade II Oligoastrocytoma| ++         |
| Grade II Oligoastrocytoma| Negative   |
| Grade II gemistocytic Astrocytoma | Negative   |
| Grade II Oligoastrocytoma | Negative   |
| Oligoastrocytoma         | Negative   |
| Diffuse gemistocytic Astrocytoma | Negative   |
| Grade II Oligoastrocytoma | +++       |
| Grade II Oligoastrocytoma | Negative   |
| Grade II gemistocytic Astrocytoma | Negative   |
| Grade II Oligodendroglioma | ++         |
| Anaplastic Astrocytoma   | Negative   |

This table illustrates the expression of IDH1 in gliomas. This expression is characterized by a strong positivity with the anti-IDH1 antibody in a case of grade II oligoastrocytoma in the 15 cases of glioma, i.e. 6.6% (Figure 3), low positivity in 2/15 (i.e. 13.3%) cases: 1 case of grade II oligodendroglioma and a grade II oligoastrocytoma case (Figure 4) and IDH1 negative in 12 cases.

This figure illustrates a strongly positive IDH1 oligoastrocytoma.

4. Discussion

4.1. Frequency Profile of Intracranial Tumors

In our study, the frequency of central nervous system tumors (SNC) in the aforementioned institutions was 0.18%, on a sample of 34,406 biopsies, during 14 years (2004-2018). Eyenga VC and coll. reported 6.3% and 5% respectively [8]. The annual incidence rate in France between 1980 and 2005 is 5.7 per 100,000 in men and 4.2 per 100,000 in women [9].

Compared to the overall number of recorded cases, our results are lower than western data [10], and approximate the data of the Maghreb countries [11].

The variations in frequencies can be explained by the absence in our country of a national cancer registry, consequently of a database; the disparity of the healthcare system, the size of the populations and accessibility to healthcare.

In our study, women represented 67.1% against 32.8% for men. Male predominance is reported in most of national series [12], maghrebian [13], Congolese [14], in our previous and international study [15]. This difference could be associated with the size of our sample. In our series, the age...
group over 50 is the most affected, our results are similar to most of the series reported in the literature [7].

Gliomas had a frequency of 29.6%, this result is contrary to that observed in other studies [15, 16].

The preliminary glioma analysis was carried out on 64 patients in the three aforementioned laboratories. The median age of our patients is 30 years with an age range of 20 months to 59 years. This average seems to be much lower compared to the world average given by the WHO which is 53 years old and the European average which is 50 years according to the WHO [7].

However, the average age that we obtained turns out to be close to that observed in Moroccan statistics, reported by the study of EL MADHI and coll. [15], which is 35 years old. This could correlate with a genetic predisposition and an environmental component. Indeed, if the TAO and coll study [17] affirms that brain tumors occur more commonly in humans, regardless of age and geographic location, it also gives a sex ratio M/F of 1.3. Our study, for its part, reports the opposite with a sex-ratio M/F of 0.48. This further supports the involvement of a genetic and environmental.

4.2. Immunohistochemical and Molecular Profile of Gliomas According to the WHO Classification 2016

One of the objectives of this study was to characterize from an immunohistochemical point of view, gliomas in the light of the WHO classification 2016. In this first part we were able to carry out the search for anti IDH1 antigens. In a subsequent study, the second part will be carried out which will consist in the practice of biomolecular analyzes. (codelletion 1p19q fusion KIAA1549-BRAF, ….)

The R132H mutation of IDH1 is present in 13.3% of grade II oligoastrocytoma and 6.6% oligodendrogliomas II. IDH1 mutations are common in grade II and III invasive gliomas [17, 18], associated with TP53 mutations in diffuse astrocytomas [19]. These mutations are involved in early gliomagenesis. However, the IDH1 mutations usually reported are between 50 and 70% of oligodendrogliomas [20, 22], against 7.4% in our series. Our results confirm that it is essential to determine IDH1 status in routine diagnosis and reinforces the hypothesis that the same tumor stem cell is at the origin of the different contingents of the same tumor but that the distinct oncogenic mechanisms are at the origin of infiltrating and non-infiltrating gliomas.

The expression of IDH1 is linked to the presence of an oligodendrogliagial contingent in an infiltrating glioma but not to that of the contingent «oligo-like» in non-infiltrating tumors.

The analysis of survival data is consistent with the literature. In our serie, the cases for which the expression of IDH1is positive, have a better prognosis. Non-infiltrating tumors (Pilocytic astrocytomas) have a better prognosis than invasive gliomas. Among the latter, les oligodendrogliomas have a favorable evolution than oligo-astrocytomas and glioblastomas. In oligodendrogliomas, the presence of an IDH mutation correlates with better recurrence-free survival, which is consistent with known.

The many negative anti-IDH1 cases, with a poor prognosis, could be explained by the fact that patients consult late, when the tumor has already progressed sufficiently in grade.

5. Conclusion

This study revealed a frequency of 0.18% of central nerve tumors, over a period of 14 years, a lower frequency compared to other studies, both national and international.

Meningioma comes first, followed by oligoastrocytomas. Meningothelial-type meningioma is the most common.

A predominance of diffuse gemistocytic astrocytomas and glioblastomas was noted.

The distribution of our series by sex showed a predominance of women.

They were more concerned with the age groups above 50 years and the female sex.

The immuno-molecular study of the different tumor cell contingents of non-infiltrating gliomas in comparison with oligodendrogliomas, indicates that morphologically identical contingents have a different molecular profile. The oncogenesis of non-infiltrating gliomas is quite non different from that of invasive gliomas (oligodendrogliomas). Infiltrating and non- infiltrating gliomas, therefore do not derive from the same tumor stem cell.

We note here a predominance of negative cases for anti-IDH1 antibodies.

The immunohistochemical study is useful for the differential diagnosis between non invasive pilocytic astrocytoma and oligodendroglioma using anti-IDH1 antibodies.

Our results encourage us to continue this study by expanding our sampling and exploring all the biomolecular analyzes that are in progress.

Conflicts of Interest

The authors declare no conflict of Interest.

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