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# IDH1 mutation in Balinese glioma patients and its relationship with clinicopathological parameters



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## ABSTRACT

**Purpose:** *Isocitrate dehydrogenase 1* (IDH1) mutation plays an essential role in the carcinogenesis of gliomas. The most recent WHO classification of central nervous system tumors has added molecular genetic and histological features of the tumor, including IDH mutation in glial tumors. This study aimed to determine the prevalence of IDH1 mutations in Balinese glioma patients and its association with clinicopathological features.

**Patients and methods:** This was a cross-sectional study involving 30 glioma patients at Sanglah General Hospital from January 2018 until June 2019. DNA extractions were carried out from the FFPE of tumor tissue, followed by amplification of codon 132 in exon 4 of the IDH1 gene by allele-specific PCR. The relationship between IDH1 mutation status and clinicopathological features was tested with  $\chi^2$  with 0.05 significance.

**Results:** The age range of patients is 14-81 years, with an average age of 43.4 years ( $SD \pm 17.9$  years). Most patients with astrocytic tumors (25/30; 83.3%), others were oligodendroglial

tumor (2/30; 6.7%) and oligoastrocytic tumor (3/30; 10%). Most patients were found with grade IV glioblastoma (18/30; 60%). Genotyping analysis showed IDH1 mutations in the majority of glioma patients (90%). Most cases (92.6%) of mutant IDH1 showed heterozygous mutations (GA genotype), while the rest showed homozygous mutations (AA genotype). There was no significant relationship between IDH1 status and age ( $p=0.09$ ), sex ( $p=0.63$ ) and histologic type (0.14), but there was significant relationship between IDH1 mutation status and tumor grade ( $p=0.01$ ). All of the IDH1 mutations were found in diffuse glioma (grade II and III gliomas and grade IV glioblastoma).

**Conclusion:** Most of the diffuse glioma (grade II-IV glioma) patients in Bali had IDH1 mutation, and IDH1 mutation status has significant relationship with tumor grade. Further research about genetic abnormalities to improve therapeutic efficacy against IDH-mutated gliomas is needed.

**Keywords:** brain tumor, clinicopathology, glioma, IDH1

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## INTRODUCTION

Molecular research in central nervous tumors has improved our understanding of carcinogenesis, tumor biology, and tumor classification. According to the latest WHO classification of central nervous system tumors, the central nervous system tumor classification is based on tumor cell origin (histogenesis) and molecular features. In glioma, histogenesis of tumor determined by light microscopic features based on evaluation of hematoxylin and eosin stain section and immunohistochemical expression of lineage associated protein and ultrastructure features. Molecular genetic of glioma determined by IDH (*isocitrate dehydrogenase*) mutation, ATRX loss, p53 mutation, and 1p-19q co-deletion.<sup>1-4</sup> As we know, IDH is an important enzyme involved in the metabolic process, such as lipogenesis, Krebs cycle, redox regulation, and glutamine metabolism. It consists of three isoforms, IDH1, IDH2, and

IDH3, located in the cytoplasm and peroxisomes (IDH1) and mitochondrial matrix (IDH2 and IDH3).<sup>5</sup> The mutation of IDH 1 is much more commonly identified in gliomas and mostly occurs in the R132H locus. Other mutations were found in another locus of the IDH1 gene and IDH2 gene.<sup>6</sup>

In addition to determining the tumor classification, the status of the IDH1 mutation is also related to the prognosis and therapeutic response.<sup>3</sup> Previous study showed that IDH1 mutated tumor has a better prognosis than IDH wild type tumors.<sup>7</sup> It was also correlated with better response rate to temozolomide and prolonged patient survival time.<sup>8,9</sup> Also, IDH1 mutated gliomas have less infiltration of immune cells such as monocytes, microglia, neutrophils, and macrophages than wild-type gliomas, since these infiltrations are associated with poor prognosis in various cancer types.<sup>9</sup>

The most common mutation of IDH1 in glioma leads to a specific amino acid change from arginine to histidine at codon 132 (c.395G>A, p.R132H).<sup>10</sup>

IDH1 mutation is found frequently in grade II and III gliomas and grade IV secondary glioblastoma. In Bali, the molecular study of central nervous system tumors is limited. This study aimed to determine the prevalence of IDH1 mutations in glioma patients in Bali and their relationship to the clinicopathological features.

## MATERIAL AND METHODS

### Selection of patients

A cross-sectional study was conducted on all glioma patients at Sanglah General Hospital Denpasar from January 2018 until June 2019, with total of 30 subjects were recruited. After obtaining ethical clearance approval from the Medical Research Ethics Commission, Faculty of Medicine, Universitas Udayana (No: 1196/UN14.2.2.VII.14/LP/2019), the research was carried out.

### Molecular analysis

DNA extraction is carried out from the FFPE tumor tissue from glioma patients using QIAamp® DNA FFPE Tissue kit as manufacture instruction. Primers for PCR amplification were as described by Loussouarn et al. (2012).<sup>10</sup> Briefly, there were two forward primers with variations in their 3' nucleotides such that each was specific for the wild-type (R132; GGTAACCTATCATCATAGGTCG) or the mutated variant (132H;

GGGTAAACCTATCATCATAGGTCG), and one common reverse primer (AS; CACATACAAGTTGGAAATTTCTGG). The sequence-specific forward and reverse primers were then combined in 'Primer mix R' (primers R132 and AS) and 'Primer mix H' (primers 132H and AS). The amplification conditions were optimized for the MyGo Mini (IT-IS Life Science, UK). PCR amplifications were performed using the SensiFAST SYBR (Bioline, UK). The reaction mixture contained 10 µl of the supplied 2X master mix, 0.8 µl of each primer (10 µM each), and 8.4 µl of the template (50 ng genomic DNA). The cycling conditions were as follows: denaturation for 10 min at 95°C, amplification for 45 cycles, with denaturation for 30 sec at 95°C, annealing for 30 sec at 62°C and extension for 30 sec at 70°C. The specific 117-bp (for R132) or 118-bp (for 132H) PCR products were separated with 2% agarose gel and visualized with Gel Green (Biotium, USA). IDH1 mutation and wild type cases will be compared with clinicopathological features.

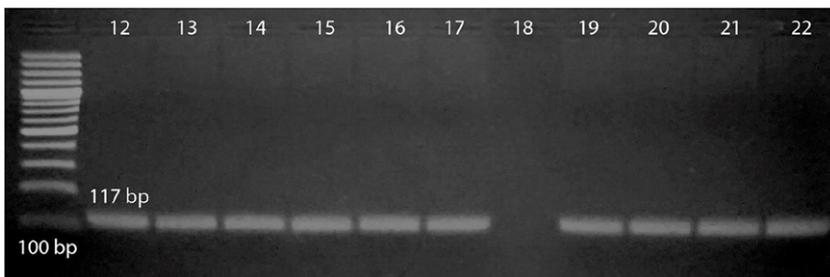
### Statistical analysis

Statistical analysis was done with SPSS version 22 for windows (IBM SPSS Statistics). The relationship between IDH1 status mutation and clinicopathological features (sex, age, histologic tumor type and tumor grade) was tested with  $\chi^2$ , with the threshold for statistical significance was  $p=0.05$ .

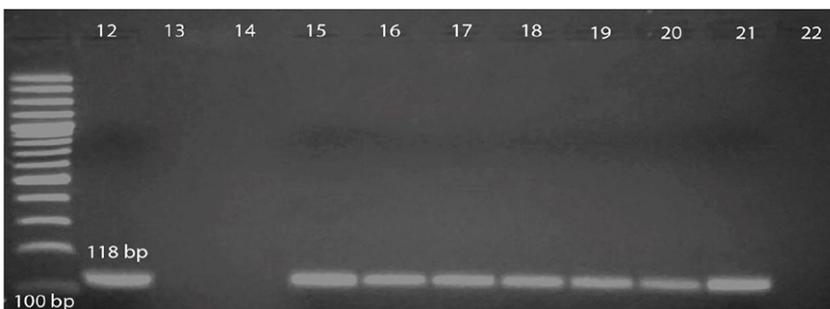
## RESULTS

From 1 January 2018 to 30 June 2019, there were 30 glioma patients in Sanglah General Hospital. Although the total sample was small, most patients were high grade (23/30; 76.7%). Patients' age range is 14-81 years, with an average age of 43.4 years ( $SD \pm 17.95$  years). The prevalence of male patients is slightly more frequent than in female patients (16:14; 1.1: 1). Most common tumor type was astrocytic tumors (25/30; 83.3%), others were oligodendroglial tumor (2/30; 6.7%) and oligoastrocytic tumor (3/30; 10%). Most patients were found with grade IV glioblastoma (18/30; 60%). The patient's age tends to increase in higher grade glioma; the mean age of grade I, II, III and IV gliomas was 23 years, 32.2 years, 48.0 and 47.1, respectively.

Genotyping analysis showed that the majority of glioma patients in Bali had IDH1 mutations (90%). Only 3 cases (3.3%) showed wild-type IDH. Most cases (25/27; 92.6%) of mutant IDH1 showed heterozygous mutations (GA genotype) while the rest showed homozygous mutations (AA genotype). (Figure 1 & 2)



**Figure 1.** Gel Electropherogram of wild type IDH1. The amplicon size was 117 bp. All samples carried allele G as wild type except sample no 18.



**Figure 2.** Gel Electropherogram of mutant type IDH1. The amplicon size was 118 bp. All samples carried allele A as mutant type except sample no. 13,14, and 22.

There was no significant relationship between IDH1 status and age ( $p=0.09$ ), sex ( $p=0.63$ ) and histologic tumor type ( $p=0.14$ ), but there was significant relationship between IDH1 mutation status and tumor grade ( $p=0.01$ ). All of the IDH1 mutations were found in diffuse glioma (grade II and III gliomas and grade IV glioblastoma), but no mutation was found in localized, grade I, and glioma. (Table 1)

## DISCUSSION

The mutation of IDH1 is common molecular change identified in gliomas. The first study by Parson *et al.* (2008), who sequencing more than 20,000 protein-coding genes in glioblastoma, found that 12% of glioblastoma cases exhibited IDH1 mutation, which was mainly seen in secondary glioblastoma multiforme.<sup>11</sup> A study by Yan *et al.* (2009), who conducted IDH1 and IDH2 sequencing of central nervous system tumors in a significant number of cases, found that IDH1 mutations in 132 amino acid were found in more than 70% of cases of grade II and grade III gliomas and secondary glioblastoma.<sup>6</sup> Other mutations in the IDH coding gene were found in other than 132 amino acids of IDH1 and mutations in IDH2.

This study found 90% of glioma patients had IDH1 mutation. The prevalence of IDH mutation in glioma patients in Bali is higher compared to other studies. The first large study in America showed IDH1 mutation in more than 70% of patients with grade II and III glioma and secondary glioblastoma.<sup>6</sup> Study by Mukase *et al.* (2011) showed IDH1 mutation was found in 29% of Japanese glioma patients,<sup>12</sup> while IDH1 mutation detected

in 58.7% patients in Chinese patients with grade II and III gliomas and 50% patients with secondary glioblastoma.<sup>13</sup> Another study by Yusoff *et al.* (2016) found that IDH1 mutation was detected in 35% of Malay glioma patients.<sup>14</sup> Different results could be due to different epidemiology or other methods of IDH1 mutation detection. Several methods that are often used are PCR with specific probes or immunohistochemistry. At the same time, the gold standard for detection of IDH mutations is sequencing to detect R132H IDH1 mutation and other than R132H IDH1 mutation.<sup>10</sup> This study used allele-specific PCR that detects IDH1 mutation specifically in R132H mutation. This study also found that most mutant IDH1 had heterozygous mutations (GA genotype), and few cases showed homozygous mutations (AA genotype). A study by Mukase *et al.* (2011) showed all detected mutations of Japanese glioma patients were heterozygous.<sup>12</sup>

Additionally, this study showed that most of the glioma patients were high grade; (5/30 (16.7%) grade III gliomas and 18/30 (60%) grade IV glioblastomas). The high-grade tumor (grade III and IV gliomas) occurred in more aged patients than low-grade tumors (grade I and II gliomas). According to the previous study, this result found that increased age tends to be associated with enriched grade.<sup>13</sup> There was significant association between tumor grade and IDH1 mutation status. IDH1 mutation was observed in grade II and III gliomas and grade IV glioblastoma but was not observed in grade I glioma. This result is consistent with other studies which showed IDH mutation observed in grade II and III gliomas and secondary, grade IV, glioblastomas.<sup>6</sup>

The mutation of IDH1 occurs at the early stage of gliomagenesis. Secondary genetic abnormalities are needed for malignant transformation, such as loss of ATP-dependent helicase ATRX, X-linked helicase II (ATRX), chromosomal region, or mutations of tumor protein 53 (TP53).<sup>4</sup> These alterations associate with the histological classification of tumors. In oligodendrogliomas have 1p/19q co-deletion in addition to IDH mutant, this alteration is ATRX loss and p53 mutation. Based on histologic and molecular features, glioblastoma can divide into two groups, de novo primary glioblastoma and secondary glioblastoma, which occurred as a progression of lower-grade glioma. The previous study revealed IDH1 mutation occurred in secondary glioblastoma but not in primary glioblastoma. In this study, we found that IDH1 mutation occurred in grade II and III gliomas and most of grade IV glioblastomas. It seems that most glioblastoma patients in Bali are secondary glioblastomas. No previous study has been done

**Table 1. IDH genotype in various clinicopathological features in glioma patients**

Clinicopathological features	Wild type IDH1 (n=3)	Mutant IDH1 (n=27)	P
Age			
< 50	3 (100%)	13 (48.1%)	0.09
≥50	0 (0%)	14 (51.9%)	
Sex			
Male	2 (66.7%)	14 (51.9%)	0.63
Female	1 (33.3%)	13 (48.1%)	
Histologic type			
Astrocytic tumor	2 (66.7%)	23 (85.2%)	0.14
Oligodendroglial tumor	1 (33.3%)	1 (3.7%)	
Oligoastrocytic tumor	0 (0%)	3 (11.1%)	
Grade			
I	1 (33.3%)	0 (0%)	0.01
II	1 (33.3%)	5 (18.5%)	
III	1 (33.3%)	4 (14.8%)	
IV	0 (0%)	18 (66.7%)	

to differentiate between primary and secondary glioblastoma in Balinese patients.

In addition to gene mutations that play a role in glioma pathogenesis, epigenetics also affect tumor development. The previous study showed several silencing genes such as lactate dehydrogenase A, VEGFA, and CA9 with IDH mutants in glioma cells are associated with hypermethylation mechanisms.<sup>5</sup>

Future research is needed to determine its relationship between IDH mutation status and tumor recurrent and patient survival and to examine other genetic abnormalities and epigenetic dysregulation. These molecular mechanisms are required to improve therapeutic efficacy against IDH-mutated gliomas.

## CONCLUSION

Most glioma patients in Bali had IDH1 mutation in grade IV (glioblastoma). Further study is required to determine the relationship between IDH mutation status and patient prognosis and study other genetic abnormalities and epigenetic dysregulation in glioma.

## CONFLICT OF INTEREST

The author declares no conflict of interest related to the material presented in this article.

## FUNDING

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## ETHICS CONSIDERATION

The Ethics Committee, Faculty of Medicine, Universitas Udayana, Bali, Indonesia, has approved this study with letter number 1196/UN14.2.2.VII.14/LP/2019.

## AUTHOR CONTRIBUTION:

Ni Putu Sriwidyani arranged the study design, analysis and data interpretation and drafted the manuscript. Ida Ayu Ika Wahyuniari revised the manuscript critically. Herman Saputra arranged the study design. I Gusti Kamasan Nyoman Arijana performed the laboratory testing.

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