Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

Immunohistochemical Study of P53 and Ki-67 in Astrocytoma

Liqaa Mohammed Muslim [1], Mais Mohammed Salim M. Hasan [2], Zainab Nassir Dosh [3], Kaswer Musa JaafarAltoraihi [4]

[1–4] Faculty of Medicine, University of Kufa, Iraq
Email: [1]ahmadhejajo@gmail.com, [2] maysm.mhasan@uokufa.edu.iq
[3] Zainabn.dosh@uokufa.edu.iq, [4] Qaswar.alturaihi@uokufa.edu.iq

Abstract:
Background: The most common primary CNS neoplasms are astrocytomas, derived from an immortalized astrocyte, accounting for 4-5 % of all cancer related death. Ki-67 and P53 are two cellular proteins playing roles in pathogenesis and evolution of astrocytomas.Ki-67 is an antigen represents a nuclear non histone protein, produced by cells in the proliferative phases. P53 gene produces a protein functions as a tumor suppressor gene. Both biomarkers were considered to be of prognostic value.
Aims: The paper aims theassessment of p53 and ki-67 immunoexpression in astrocytomas,relation of these markers with histologic grade of astrocytomas, andto use p53 and Ki-67 as a prognostic tool.
Materials and Methods: Forty-five patients with astrocytoma were conducted in the study which performed in Department of Pathology, Faculty of Medicine, university of Kufa in the period from January 2018 through April 2019.Cases were collected from "neurosurgical hospital in Baghdad, and laboratory of histopathology in Al-sadder Medical city in Al-Najaf and from some private laboratories of the same governorate", ages ranging from 2-65 years with a mean of 29.04 years.EnVision method (polymer based immunohistochemistry) was used for evaluation of p53 and ki-67.
Results: Grade II patients predominant the studied sample and constitute (55.6%), whereas patients of grade I constitute (22.2%), grades IV and III compose (13.3%) and (8.9%) respectively.P53 was detected in (33.34%) of the cases and was "significantly positively correlated" with grade IV, gender not with age.Ki-67 LI was (>5%) in (31.11%) of patients and was "significantly positively correlated" with tumor grade, age of the patient but not with gender, both biomarkers were "positively correlated with each other, and with grade of astrocytoma".
Conclusions and recommendations:p53 and ki-67 over expression have an important role in pathogenesis of astrocytoma, as their positivity associated with higher tumor grade, so merging these biomarkers with histopathological grade in a prognostic index will accurately predict clinical outcome and the effectiveness of anti cancer therapy.
Keywords: Immunohistochemical study, p53, Ki-67, astrocytoma

INTRODUCTION

Astrocytoma is the most prevalent primary intra-axial CNS tumors deriving from an immortalized astrocyte [1]. CNS neoplasms are 5th common tumor in adults and 2nd of children ranking in Iraq [2]. Astrocytic tumors are the predominant CNS neoplasms in Iraq [3-4].In USA,there are 12000 new cases/year [1]. Astrocytic tumors accounting for 4-5 % of cancer related death (about 20.000 deaths / year in North America) [5].They were divided into two categories; (1)Fibrillarastrocytic neoplasms (low grade astrocytoma, anaplastic astrocytoma, and glioblastomamultiform) [1], (2) A diverse group of tumor types that differ from fibrillarastrocytic series because of their distinctive clincopathological features. These include: juvenile pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and subependyal

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

giant cell astrocytoma [1]. The former group is with diffuse zone of infiltration while the latter one is with narrow zone of infiltration [1].Many grading systems based on histopathological characteristics have been established, like Baily and Cushing scheme, Kernohan scores I-IV, (WHO) I-IV, and DaumasDuport grading system 1-4 [6-7].

Classification and grading of astrocytic tumors

In 1993 a new WHO classification had been approved reflects that a progress has been achieved in brain tumor classification [8].WHO grading schema 2000 is a four- tiered system. Nuclear atypia, mitotic activity, and vascular proliferation are the parameters used in grading astrocytic tumors [9].

Immunohistochemistry

IHC technique applied in this study is Polymer-Based Immunohistochemistry (EnVision)

P53

Is a tumor suppressor gene, mutations of which represent the most common genetic alteration in human tumors [10]. Its gene product is a nuclear protein known to be engaged in control of cell cycle, apoptosis, and genomic stability.The altered product of mutant gene has a much longer half life and so can be visualized by immunohistochemical techniques [10].The biology of astrocytoma progression is a complex topic, the allelic loss and mutations involving the p53 gene (TP53) as early genomic destabilizing events, play a role in the evolution of most low grade fibrillarastrocytic neoplasms [11].

Ki-67

It represents a nuclear non histone protein antigen. Monoclonal antibodies have been manufactured to detect formalin resistant epitopes (MIB-1). In the purpose of prognostication and treatment planning; quantifiable methods for the determination of proliferative potentials are now in wide employ as adjuncts to conventional morphologic assessment and to correlate between the histologic grading and labeling proliferative index of the tumor, also we employ MIB-1 in the evaluation of histological low-grade or borderline fibrillarastrocytomas [11], in our study we focus on the correlation of both markers with grade of astrocytoma and correlation of markers to each other.

Ki-67/ MIB-1 labeling index in astrocytoma

Recent WHO classification has limited prediction for prognosis and diagnosis of astrocytomas, so in need for additional parameters [19].The applicable value of tumor reproductive activity have been investigated, specifically Ki-67/MIB-1 labeling index (LI) [12].Almost all studies reveal elevating values of Ki-67/MIB-1 LI in grade increment. Furthermore, in many researches, positive association between MIB-1 LI and survival are detected, in spite that intended cut-off values vary substantially among data [13].The researches naming MIB-1 LI as an important prognostic factor in human astrocytomas [14],due to the wide range of values among various grades, MIB-1 LI may not be used alone as a diagnostic factor but could be used after merging with an established criteria of histological malignancy [15]. It might be specifically useful in cases of low-grade astrocytoma [16].Ki-67/MIB-1 labeling fractions in excess of 5–7.5% reported from certain studies as powerful predictors of relatively shortened survival, but not all researchers indicate their utility as independent prognostic parameters [11].

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

P53 and Ki-67 in relation to prognosis of astrocytoma

Many researchers have reported that the grade is the most significant statistic parameter detecting patient survival, and a significant correlation is identified between its increment and worst prognosis [17]. Furthermore, studies have found excellent association between quantitative increase in detection of MIB1 and p53, with shorter interval for recurrence and reduced patient survival [18].Western reports results differ regarding benefit of p53 as a prognostic factor in glial neoplasms, some expressing its important role, and others failed to conclude it [19]. Other studies have shown a inverse correlation between Ki-67 LI and survival. A suggestion has been made that combination of Ki-67 LI, tumor type, and prognosis makes Ki-67 immunoexpression of value in the histological diagnosis of astrocytic tumors [20].

Materials and methods

This study performed in Pathology department, Faculty of Medicine, Kufa University, in time from January 2018 to April 2019.

Sampling of cases:

(a) Study group: Forty five cases (20 female and 25 male patients) with astrocytoma (as confirmed by H&E-stained slides) were engaged in the study. They were sampled from "neurosurgical hospital in Baghdad, laboratory of histopathology in Al-sadder Medical City in Al-Najaf and some private laboratories of the same governorate".

(b) Comparative group: Twenty five cases from normal brain autopsy (forensic medicine cases).

(c) Positive control slides: Parallel processed with each set of immunostaining,taken from breast and bladder cancer sections known to express Ki-67 and p53, respectively.

(d) Negative controls: Sections without primary antibody (Ki-67 and/or p53).

Staining methods: Samples were formalin-fixed, paraffin embedded tissue blocks, 5 micrometer-thickness, were obtained and stained with hematoxylin eosin and immunohistochemical Envision method. According to WHO grading system [9], the grades of the studied cases was as the following:

- 10 cases pilocytic astrocytoma (grade I)
- 24 cases fibrillar astrocytoma (grade II)
- 5 cases anaplastic astrocytoma (grade III)
- 6 cases glioblastoma multiform (grade IV)

Equipment and materials

A-The following were used throughout the research:

Water bath, microwave oven, humidity chamber, hot plate, light microscopy, pap pen, micropipettes with tips, pastures, timer, cotton swabs, gloves, positively charged slides, cover slides, staining jars, callipered cylinders, calibrated test tube, tissue papers,buffer solution, xylene, hematoxylin, distilled water, ethanol of different concentrations and paramount.

B- Primary antibody

1- P53: Monoclonal Mouse Anti-Human p53 Protein, 11 ml, Ready-To-Use, DAKO, Clone DO-7, Code N1581, LOT 00005848, Dako North America, Inc. 6392 Via Real Carpentaria, CA 93013 USA.

2- Ki-67: Monoclonal Mouse Anti-Human ki-67 antigen clone MIB-1, code M7240.

IHC method used in the study was the Envision technique

Scoring system:

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

A: P53 Scoring systemaccording to Sophia KA et al. [21]at objective 40.
B: The labeling index of p53 [22]**:** For statistical analysis, another parameter "the labeling index (LI)" was used, according to ROC curve and in accordance with newly published reports on p53 expression; cases were categorized into two groups; as the table below:

Table1: P53 Labeling index

Low p53 labeling index	P53 less than 20% (the percentage of stained cells are less than 20%)
High p53 labeling index	P53 equal or more than 20% (the percentage of stained cells are equal or more than 20%)

C: Ki-67 labeling index (in astrocytoma)

Table 2: Ki-67 labeling index

Histopathological grade of astrocytoma	Range of ki-67 labeling index according to the histopathological grade of astrocytoma
Low grade astrocytoma	Nuclear staining in <5% of the malignant cells.
Anaplastic astrocytoma	Nuclear staining between 5-10% of the malignant cells.
Glioblastomamultiform	Nuclear staining in > 10% of the malignant cells.

D: Ki-67 labeling index cut off point
According to prognostic purposes, another parameter was used; a cutoff point was used to assess astrocytoma into low and high grade categories [(11)]; as in the following table**:**

Table 3: Ki-67 labeling index cut off point

L.I cut off point	Staining pattern
Low	Nuclear staining in <5% of the malignant cells.
high	Nuclear staining in >5% of the malignant cells

Statistical analysis
Using SPSS software version 18, significant differences between non-parametric variables were evaluated by Chi square test and in parametric variables by independent t-test. Pearson Correlation to evaluate the relations between parameters. P values ≤ 0.05 were considered statistically significant and P values ≤ 0.01 as highly significant.

RESULTS
Clincopathological analysis
Age distribution
Forty five cases of astrocytoma were conducted in this study, ages from 2 to 65 years, and mean of 29.04 years.
Evaluation of their ages revealed 7 (15.5%) were in first decade of life, 14 (31.11%) in second decade, 5 (11.11%) in third decade, 7 (15.5%) in forth decade, 3 (6.6%) in fifth, 5 (11.11%) in sixth, 4 (8.8%) in seventh decade of life.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

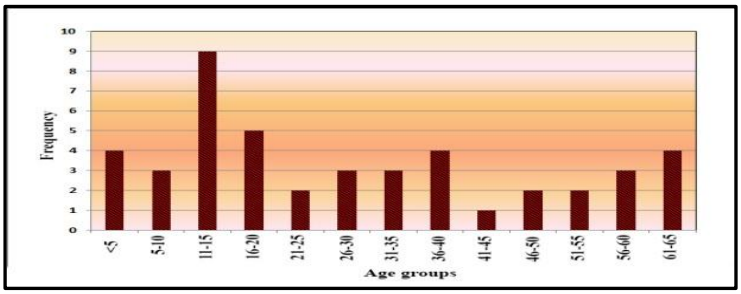

Figure1: frequencies of patient age groups

Gender distribution

Out of 45 included patients, 25 (55.6%) were male and 20 (44.4%) female, (figure 2), with a male: female equal (1.25:1)

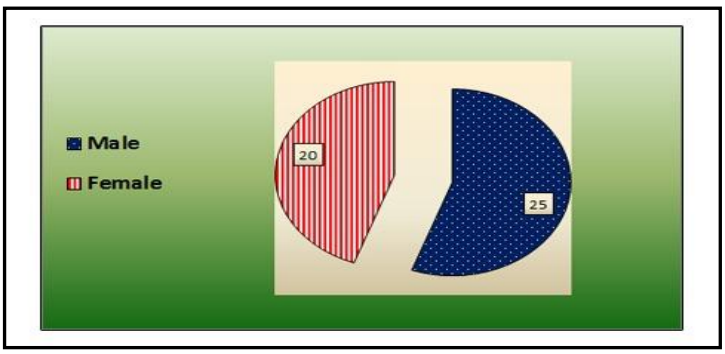

Figure 2: gender composition of studied patients

Grade distribution

Patients of grade I forming 22.2%(10 cases)of whole sample, grade IIwere predominant 55.6%(25 cases), grades IV and III forming 13.3%(6 cases) and 8.9%(4 cases) respectively, as in figure (3).

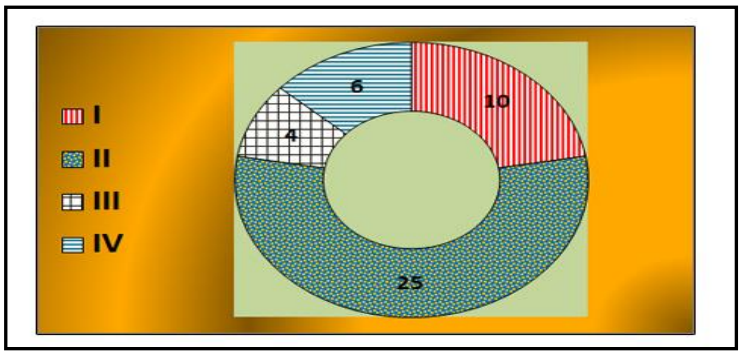

Figure 3: Grade distribution of studied patients

Base line characteristics of studied patients in regards to grade, age and gender

In table (4), younger ages were observed within grade I while older ages were reported in other grades. All patients in grade III are male.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

Table (4) Base line Characteristics of studied patients in regards to Grade, age and gender

Grade	no	AGE(Years)		GENDER			
		MEAN±SD	RANGE	Male		Female	
				NO	%	NO	%
I	10	11.2±9.647	2.0 - 33.0	7	70	3	30
II	25	29.28±16.298	10.0 - 65.0	10	40	15	60
III	4	44.25±20.613	17.0 - 63.0	4	100	0	0
IV	6	47.67±23.526	5.0 - 65.0	4	66.67	2	33.33
Total	45	29.04±19.91	2-65	25	55.56%	20	44.44%

Age distribution in relation to grade of astrocytoma

Figure (4) shows significant positive correlation between grade and age of patients (r= 0.574, p= 0.000)**.**

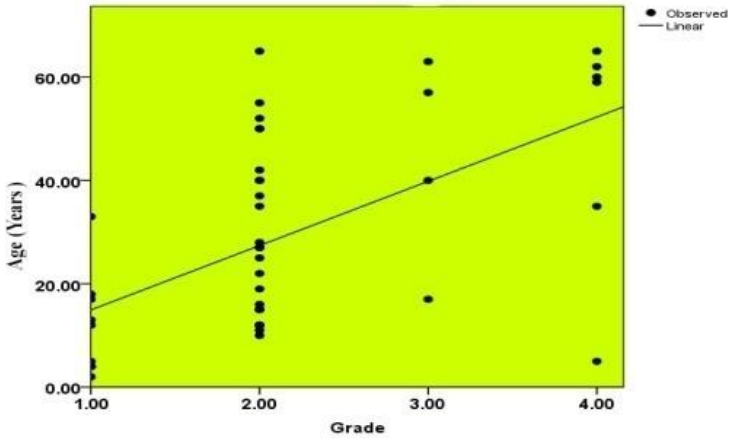

Figure (4): Correlation between Age and grade of patients

Gender distribution in relation to grade of astrocytoma

Table (5) and Figure (5) shows gender distribution in relation to grade of patients, all patients of grade III are male, also male predominates grade I and IV, while female predominates grade II.

Table (5): Gender distribution in relation to grade of patients

Grade	NO	GENDER			
		Male		Female	
		NO	%	NO	%
I	10	7	70	3	30
II	25	10	40	15	60
III	4	4	100	0	0
IV	6	4	66.67	2	33.33
Total	45	25	55.56%	20	44.44%

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

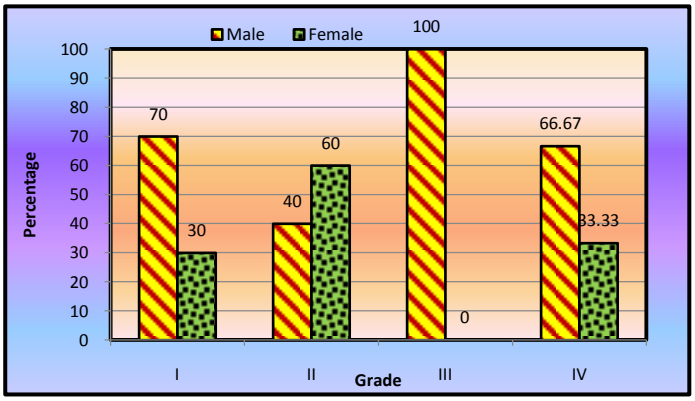

Figure (5): Gender distribution in relation to grade of tumor

p53immunohistochemical study

A:p53 score and grade of astrocytoma

Comparison of P53 score according to grade of patients was shown in table (6). Negative P53 score was predominant (P≤0.01) in grade I, II and III in comparison with grade IV, on the other hand strong P53 score was more frequent (P≤0.01) in grade IV than other grades. Also, positive correlation (r= 0.429, p= 0.002) between grade o and P53score was significantly observed, as shown in figure (6).

Table (6): Comparison of P53 score according to grade of patients

Grade	NO	P53 score							
		Negative		Weak		Moderate		Strong	
		NO	%	NO	%	NO	%	NO	%
I	10	9	90	0	0	0	0	1	10
II	25	18	72	3	12	3	12	1	4
III	4	3	75	1	25	0	0	0	0
IV	6	0	0	2	40	1	20	3	60
Total	45	30	66.67%	6	13.33%	4	8.89%	5	11.11%
P value		0.000							

X^2test, P≤0.01, df=3

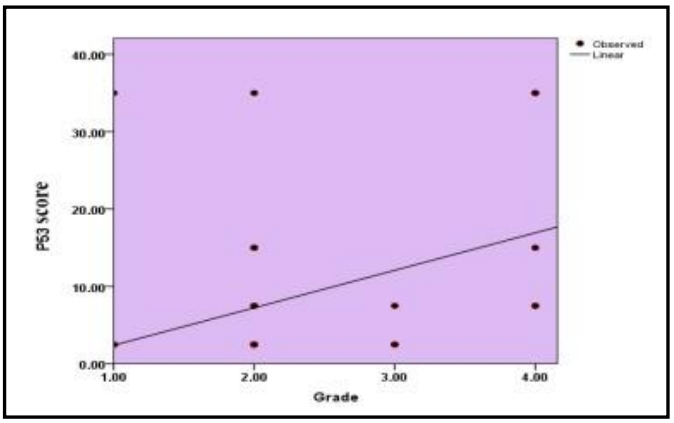

Figure (6): Correlation between Grade and P53 score

B:p53 labeling index and grade of astrocytoma

Table (7) showed significant differences (P≤0.01) between low and high P53 labeling index in regard to grade, low P53 LI was more frequent in patient of grade I, II, and III than high P53 labeling index.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

Table (7) Comparison of P53 labeling index in regard to grade of tumor

Grade	NO	(LOW) P53 <20%		(HIGH) P53>20%	
		NO	%	NO	%
I	10	9	90	1	10
II	25	24	96	1	4
III	4	4	100	0	0
IV	6	3	50	3	50
TOTAL		40	88.89%	5	11.11%
P value		0.000			

X^2test , P≤0.01, df=1

C: p53 and age of the patients

Table (8) shows Mean age (±SD) of studied patient in regard to P53 labeling Index, No significant differences (P>0.05) were observed between mean age that have more or less 20% P53 labeling Index.

Table (8): Age of studied patients in regard to P53 labeling Index

P53 labeling Index	NO	Mean age ±SD	P value
<20%	40	28.55±3.156	0.643
>20%	5	33.00±9.612	

Independent T-Test, P≤0.05

D: p53 and gender of the patients

Table (9) shows gender frequency (%) of studied patients in regard to P53 labeling Index, significant differences (P≤0.01) were noticed between these groups.

Table (9) Gender distribution in regard to P53 labeling Index

P53 labeling Index	Male		Female		P value
	No	%	No	%	
<20	21	52.5	19	47.5	0.000
>20	4	80	1	20	

Ki-67immunohistochemical study

A: Ki-67 labeling index and grade of astrocytoma

Comparison of Ki-67 labeling index according to grade was shown in table (10). Figure (7) shows significant positive correlation (r= 0.724, p= 0.000) between grade and Ki-67 LI.

Table (10) Comparison of Ki-67 indexaccording to the grade

Grade	NO	Ki-67 index					
		<5%		5-10%		>10%	
		NO	%	NO	%	NO	%
I	10	10	100	0	0	0	0
II	25	21	84	1	4	3	12
III	4	0	0	0	0	4	100
IV	6	0	0	2	33.33	4	66.67
Total	45	31	68.89%	3	6.67%	11	24.44%
P value	0.000						

X^2 test, P≤0.01, df=2

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

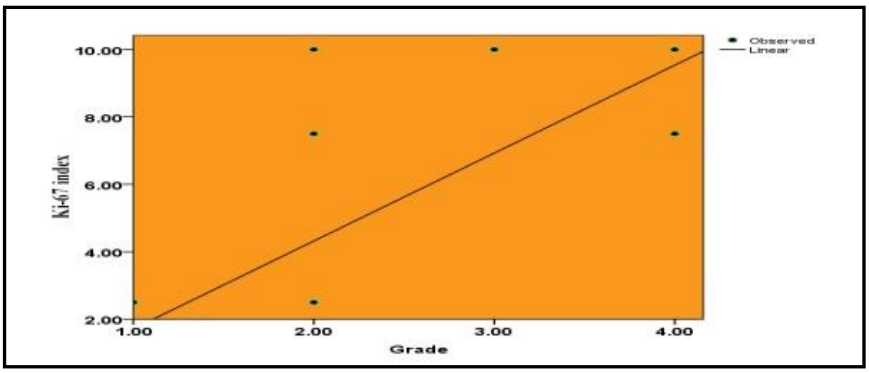

Figure (7): Relation between Grade and Ki-67 index

B: Ki-67 labeling index cut-off point in relation to grade of astrocytoma
Table (11) shows significant positive correlation (r= 0.724, p= 0.000) between grade and Ki-67 LI cut off point, in which (100%) of grade cases had ki-67 LI <5% ,and 84% of grade II cases had ki-67 LI <5%,on the other hand all cases of grade III (100%) and all cases of grade IV (100%) had ki-67(LI>5%).

Table (11) Comparison of Ki-67 labeling index (cut-off point) according to grade of tumor

Grade	NO	Ki-67 <5%		Ki-67>5%	
		NO	%	NO	%
I	10	10	100	0	0
II	25	21	84	4	16
III	4	0	0	4	100
IV	6	0	0	6	100
TOTAL		31	68.89%	5	31.11%
P value		0.000			

X^2 test, P≤0.01, df=2

C: Ki-67 labeling index and patient age

Table (12) revealsMean age (±SD) of studied patient in regard to Ki-67 labeling index. Patient of <5%Ki-67 indexwere younger thanthose of >5% Ki-67 index. Significant differences (P≤0.01) were observed between mean ages of both groups.

Table (12) Age of studied patients in regard to Ki-67 labeling index

Ki-67 labeling index	M		F		P value
	No	%	No	%	
<5%	16	51.61	15	48.39	0.849
>5%	9	54.28	5	45.72	

Independent T-Test, P≤0.01

D: Ki-67 labeling index and gender

Table (13) shows gender frequency (%) of studied patient in regard to Ki-67 index, No significant differences (P>0.05) were recorded between patient groups that have more or less 5% Ki-67 labeling index.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6507 – 6525
Received 15 December 2020; Accepted 05 January 2021.

Table (13) Gender distribution in regard to Ki-67 labeling index

Ki-67 labeling index	NO	Mean age ±SD	P value
<5%	31	23.23±16.35	0.003
>5%	14	41.93±21.58	

Correlation between p53 and ki-67 labeling indices and the grade

Assessment of P53 andKi-67 labeling indices in relation to grade of tumorare shown in table (14), both P53 LI and Ki-67 LI shows significant differences (P≤0.01) in relation to grade of tumor.

Table (14) P53 labeling Indices and Ki-67 labeling indices in relation to grade

Grade	Ki-67<5%		Ki-67>5%		P53<20%		P53>20%		Total	P value
	NO	%	NO	%	NO	%	NO	%	NO	
I	10	100	0	0	9	90	1	10	10	0.000
II	21	84	4	16	24	96	1	4	25	
III	0	0	4	100	4	100	0	0	4	
IV	0	0	6	100	3	50	3	50	6	

Correlation between p53 and ki-67 labeling indices

Relation between P53 and Ki-67 labeling indices was shown in table (15), 64.44% of patients revealed low P53<20% and low Ki-67<5%indices but24.44% of them showed lowP53<20%and highKi-67>5%indices, on the other hand 4.44% patients possess high P53>20% and low Ki-67<5% indices, meanwhile 6.67% have high P53>20% and high Ki-67>5% indices. The total percentage of these crossing labeling indices showed significant differences (P≤0.01).

Table (15) Relation between P53 and Ki-67 labeling indices

	Ki-67<5%		Ki-67>5%		Total		P value
	No	%	No	%	No	%	
P53<20%	29	64.44	11	24.44	40	88.89	0.000
P53>20%	2	4.44	3	6.67	5	11.11	
Total	31	68.89	14	31.11	45	100	

DISCUSSION

P53 expression

In this study we used two established parameters for presenting and analyzing p53 immunostaining results (p53 extent and LI). In our study, p53 positive immunostaining cases was observed in 15 of 45 cases (33.34%), which is more than another Iraqi study(AL-Munem et al) [23] (18.4%). Our result is near to a study in Brazil done by Isolan et al [24], (34.04%). And our result is lower than other studies;Haapasalo et al [25] in Finland (37.25%). Nozaki et al and Tada et al [26]in Japan found (39.68%),Mee-Yon Cho et al [27],(54%), NayakAnupma et al [28] (46%)and Ellison et al [29](65.85%).

This difference due to many factors:

1- Type of primary antibody and its sensitivity.
2- Fixation and pretreatment.
3- Type of visualization system.

4- The type of chromogen used.
5- The cases selection.
In this study, both p53 extent and LI showed high expression (+++ve) in 3 cases of glioblastomamultiform, mild +ve and moderate ++ve p53 extent and low LI in also 3 samples of glioblastomamultiform. 1 case of pilocytic astrocytoma (grade I), and 1 case of fibrillar astrocytoma (grade II) showed strong p53 extent and high labeling index, 1 case of anaplastic astrocytoma (grade III) showed mild p53 positivity(+ve) and low labeling index, while the remaining cases showed negative p53 immunostaining. These results are near to AL-Munem et al [23].Broniscer et al [30] observed that p53 over expression was more common after malignant transformation, which agree with our results.

P53 scoring and labeling index in relation to grade of astrocytoma

In our study there is significant positive correlation (p=0.002) between grade and p53 score, in regard to p53 labeling index, significant differences ($P \leq 0.01$) between low and high P53 labeling index in regard to grade. This mean that p53 over expression is significantly associated with grade IV (glioblastomamultiform), this results agree with AL-Munem et al [23],Isolan GR et al [24], Barbareschi et al [31], Lee et al [32], Haapasalo et al [25], Pollack et al [33],Ellison et al [29] in UK and Zubair Ahmed [34] in Pakistan. Our study differs from OS Abdelaziz et al [35] in Egypt and Korkolopouloul et al [36].

Pilocytic astrocytoma (grade I)

Our study revealed that p53 was negative in 90% of pilocytic astrocytoma and strong positive with high p53 labeling index in 1 case (10%), this result is near to AL-Munem et al [23].Also Schiffer D et al [37] in Italy, Haapasalo et al [25], and Ishii et al [38] in Japan resulted that p53 mutation is not essential in tumorigenesis of pilocyticastrocytomas, making this entity genetically distinct from grade II-IV. Also NayakAnupma et al [28] suggested the role of other genes in evolution of pilocytic astrocytoma especially neurofibromatosis type 1 (NF1) gene,Lang FF et al [39],Patt S et al [40], and Burger PC et al [41].We found 1 of 10 cases pilocytic astrocytoma (10%) to be strong positive (+++ve) with high labeling index. Isolan GR et al [24] found 1 of 4 cases positive for p53 (25%). The explanation for this result may be that:
1-Pilocytic astrocytoma rarely undergoes transition to a higher grade. So the positive case may be one of the rarely mutated cases that undergo a progression to a higher grade.
2-p53 accumulation (nuclear over expression) not necessarily meanTP53 (p53 gene mutation),because binding of p53 to disordered cellular proteins (like MDM2,70kDa heat shock protein and large T antigen) result in inhibition of p53 function and abnormal accumulation to be detected immunohistochemical.
3- No concordance between immunohistochemical expression and histology thus implying that histological types could over or under estimate the actual behavior of astrocytoma. Our results are different from Bodey B et al [42].

Grade II and Grade III Astrocytoma

Our results revealed that 7 of 25 cases (28%) of grade II was p53 positive, and 1 of 4 cases (25%) anaplastic astrocytoma grade III was positive. And only 1 case of grade II (diffuse fibrillar) showed high p53 labeling index. This result is near to Isolan GR [24] and NayakAnupma et al [28]. Zubair Ahmed [34] found equal percentage between grade II and III. But our results are different from Haapasalo et al [25]who found lower positivity in grade II(19%) compared to grade III (56%). Also Nozaki et al [26], Ellison et al [29], andMee-Yon

Cho et al [27],so most of the researches showed that p53 positivity and labeling index increased with increasing grade of astrocytoma.
The explanation of our results that differ in this regard (grade II P53 labeling index more than grade III) could be due to:
1- Small number of anaplastic astrocytoma (grade III) (4 cases) compared to higher number of diffuse fibrillar (grade II) (25 cases).
2- Presence of other genetic abnormalities in anaplastic astrocytoma (grade III) like (loss of heterozygosity at 19q and 1oq, p16/INK4a loss, RB1 loss, PTEN/MMAC1loss)**.**
3- Tumor heterogeneity.
4- A small percentage of tumors do not show concordance between histological type and immunohistochemistry.
5- Ono et al [44] estimated that one fourth of their astrocytomas were p53 immunoreactivity without an associated gene mutation.

Glioblastomamultiform (grade IV)

Our study revealed that all grade IV cases was p53 positive (100%), 3 of 6 (50%) had low p53 LI and the other 3 (50%) had high p53 LI, so there is strong positive correlation between p53 and grade IV,also significant positive correlation between p53 score and grade,our study consistent with Isolan G.R et al [24], Zubair Ahmed [34] and AL-Munem et al [23],but different from Tada et al [45], Ellison et al [29]and Haapasalo et al [25]. This counterintuitive phenomenon is explainable if we accept dual pathways of progression to glioblastoma: one pathway where low-grade astrocytoma progresses to grade IV by duplication of cells with mutated p53 and another as glioblastoma arise de novo with different genetic changes like EGFR, and PTEN/MMAC1 mutations). We can explain this difference by:
- Small number of grade III cases (4) and of grade IV (6) cases.
- Could be all our glioblastoma cases of secondary type with p53 mutation.
- Different genetic pathways for anaplastic astrocytoma grade III like (loss of heterozygosity for 19q)
- Nuclear accumulation of p53 due to binding to abnormal proteins like Mdm2 (194) (due toMDM2 gene amplification) which occur in 10% of high grade astrocytomas, or binding to other disordered cellular proteins.

P53 in relation to age and gender of studied patients

We found no significant positive correlation between p53 score and age of studied patients ($p<0.05$), while there was significant positive correlation with gender ($p<0.01$), in which high p53 score was more frequent in males. Our results agree with Haapasalo et al [25], OS Abdelaziz et al [35], and Isolan et al [24], but both OS Abdelaziz et al [35] andIsolanet al [24] disagree with our study regarding association between p53 and gender. The explanation of this is that high grade astrocytoma (grade IV) was more frequent in male patients; as a result they have high p53 score and labeling index.

Ki-67 expression

Ki-67 LI in relation to grade of astrocytoma
Positive significant relation between ki-67 LI and grade of astrocytoma ($p<0.01$),our study agree with the Iraqi study done by MQChallop [46], also agree with Abdelaziz et al [35],Mahzouni et al [47], Atik et al[48],Kayaselcuk et al [49],Liwei et al andXiuzhen etal [50],Huang et al [51],Rodriquez-Pereira et al [52], Scott et al [53],Ellison et al [29],

Zubair[50], Ahmed [34], Korkolopouloulet al [36],and Isolan et al [24],we did not found a research that disagree with our findings.

Grade IPilocytic astrocytoma

All cases of pilocyticastrocytomas (100%) had low Ki-67 LI (<5%) this result agree with MQ Challop [46]) and Zubair Ahmed [34], we did not found a study that disagree with our results.

Grade II astrocytoma

Our study revealed that (84%) of grade II had low Ki-67 LI (<5%), (4%) had Ki-67 between (5-10%) and (12%) had high Ki-67 (>10%).This result agree with most previous studies done by Karamitopoulou et al [54],Khalid et al [55], Wakimoto et al [17], Di et al [56], Hsu et al [15], Abdelaziz et al [35], and Isolan et al[24]. Regarding our case that have a Ki-67 between (5-10%) agree with M Q Challop [46] andSallinen et al [16] and Enstrom et al [13]. The three cases (12%) that show high ki-67 LI (>10%), revealed absence of concordance between histological grade and ki-67 labeling index. The issue highlighted through our results is that all histological low grade astrocytoma could not be eventually as biologically indolent,this subset of cases with biologically aggressive behavior require close clinical monitoring and serial neuro imaging to examine early recurrence and progressive transformation. This explanation is supported by Yue et al [57].

Grade III astrocytoma and glioblastomamultiform

All grade III astrocytoma (100%) had high Ki-67 LI (>10%). 2 cases of grade IV (33.33%) had Ki-67 (5-10%) and 4 cases (66.67%) had Ki-67(>10%). With no significant difference between them, so it is less beneficial in differentiating between grade III and grade IV when used alone without other parameters like (histopathological typing, and micro vessel density). Our results agree with Abdelaziz et al [35], Karamitopoulou et al [54], Hsu et al [15],Ralte et al [58] and Torp et al [14],but disagree with Die et al [56], and Enstrome et al [13].

Ki-67 LI in relation to age and gender of studied patients

Cases evaluation of ki-67 LI<5% were younger than those of >5%, with highly significant differences ($p<0.01$) between the mean age of both groups. This result is supported by MQChallop [46],Abdelaziz et al [35], and Rodriquez-Pereira et al [52], but differ from Isolan et al [24] this difference could be due to different type of antibody used or sample size.Regarding the relation between gender and ki-67 labeling there is no significant difference($p>0.05\%$), this result agree with MQ Challop [46], Abdelaziz et al [35], Mahzouni et al [47], Liwei et al [50], but differ from Yonghua et al [51], the explanation of this difference may be due to:

- Environmental, racial and geographical differences.
- Difference in sample size
- Different type of antibody used.

Co expression of p53 and Ki-67 labeling indices in grade of astrocytoma

Our study showed the immunoexpression of P53 and Ki-67 labeling indices regarding grade of tumor; Ki-67<5% predominates in grade I and II while Ki-67>5% predominates in grade III and IV, on the other hand p53<20% predominates grade I , II and III, meanwhile grade IV have equal percent of p53<20% and>20%. Both P53 LI and Ki-67 LI show significant differences ($P\leq0.01$) in relation to grade of tumor. Haapasalo et al [25] also

reported that. Jaros et al [19] found that all grades of p53 positive astrocytomas had a higher Ki-67 LI than p53-negative astrocytoma.

Correlation of P53 and Ki-67 labeling indices to each other

P53 and Ki-67 labeling indices revealed64.44% of patients with low P53<20% and low Ki-67<5% indices but 24.44%of them showed low P53<20% and high Ki-67>5% indices, in contrary to 4.44%patients possess high P53>20% and low Ki-67<5% indices, meanwhile 6.67%have high P53>20% and high Ki-67>5% indices. The total percentage of these crossing labeling indices showed significant differences ($P \leq 0.01$).This result agree with Abdelaziz et al [35] and Ellison et al [29].

CONCLUSIONS

1- P53 protein over expression was noticed in 33.34% of astrocytoma cases and "significantly correlated" with grade IV.
2- P53 score is "significantly positively correlated" with male gender, but not correlated with age of the patient.
3- Ki-67 LI is "significantly positively correlated" with increasing grade of astrocytoma and patients age, but not with gender.
4- Both p53 and Ki-67 labeling indices are "significantly positively correlated" in relation to grade of astrocytoma and in relation to each other.

RECOMMENDATIONS

- Follow up of the studied patients (especially those that are of low grade which show disconcordance with immunohistochemical evaluation) to know the relation of these 2 markers with survival, recurrence, and malignant transformation.
- Further concurrent genetic DNA analysis to assess the role of other gene mutations in the pathogenesis of astrocytoma like (NF1, EGFR, PTEN/MMAC1 and MDM2 amplification), and to assess TP53 mutation in p53 positive tumors in order to know the cause of p53 accumulation whether due to gene mutation or another mechanism.
- Larger study including higher number of patients with introducing other angiogenic marker (like VEGF and MVD).
- Study the role of p53 protein in therapeutic strategies.

REFERENCES

[1] Fuller GN, and Burger PC. Giloma pathology. In: Willkins RH. Neurosurgery. 2nd ed.Vol.(1) New York, MC Growhil Inc. 1996; 735-797
[2] Iraqi cancer registry 2005.
[3] Iraqi cancer registry 1999
[4] Iraqi cancer registry 2000
[5] Mahely MS, Natarjan N. National survey of patterns of care for brain tumors patients. J Neurosurg 1989; 71:826-836.
[6] Cavenee WK, Binger DD, and Newcomb EW: Diffuse astrocytoma. Pathology and Genetics: Tumors of the nervous system. Lyon, France: International agency for cancer research; 1997; 2-9.
[7] Tatter BS, Wilson CB, and harsh GR. Neororepithelial tumors of the adult brain.In: Yeoman's Neurological Surgery. 4th ed. Philadelphia, PA: WB Saunders Company; 1996; 2612-2648.

[8] Kleihues P, Burger PC, and Scheithauer BW. Histological typing to tumors of CNS.Geneva. WHO. 1993; 1-51.
[9] Prayson R.A, Banett GH. Current concept in the diagnosis of glioma: Astrocytoma, 2005; http://www.uscap.org/site~194th/shorth40-1.htm
[10] Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature, 2000; 408, p.307-310.
[11] Louis DN, Ohagi H, Wiestler OD, Cavenne WK, ed. WHO classification of tumours of the central nervous system, Lyon: IARC; 2007; 25-49.
[12] Gerdes J, Li L, Schlueter C, Duchrow M, Wohlenberg C, Gerlach C,Stahmer I, Kloth S, Brandt E, Flad HD: Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 1991; 138:867-873
[13] Enestrom. S, Vavruch L, Frønlund B, et al: Ki-67 antigen expression as a prognostic factor in primary and recurrent astrocytomas. Neurochir 1998; 44:25-30
[14] Torp SH: Diagnostic and prognostic role of Ki67 immunostaining; in human astrocytomas using four different antibodies. ClinNeouropathol 2002; 21:252-257
[15] Hsu DW, Louis DN, Efird JT, et al: Use of MIB-1 (Ki-67) immunoreactivity in differentiating grade II and grade III gliomas. J NeouropatholExpNeurol 1997; 56:857-865
[16] Sallinen PK, Haapasalo HK, Visakorpi T, et al: Prognostication of astrocytoma patient survival by Ki-67 (MIB-1), PCNA, and S-phase fraction using archival paraffin-embedded samples. J Pathol 1994;174: 275-282
[17] Wakimoto H, Aoyagi M, Nakayama T, et al: Prognostic significance of Ki-67 labeling indices obtained using MIB-1 monoclonal antibody in patients with supratentorialastrocytomas. Cancer 1996;77:373-380
[18] Kleinhues P, Cavence WK.World health organizationclassification of tumors. Pathology & genetics. Tumors of the nervous system. IARC Press Lyon, 2000.
[19] Jaros E, Perry RH, Adam L, et al. Prognostic implicationsof p53 protein, epidermal growth factor receptor and Ki67 labeling in brain tumors. Br J Cancer, 1992; 66, 373-85
[20] Pardo FS, Hsu DW, Zeheb R, et al. Mutant, wild type or overall p53 expression: freedom from clinical progression in tumors of astrocytic lineage. Br J Cancer, 2004; 91, 1678-86.
[21] Sophia K Apple, J Randolph Hecht, and W David. IHC evaluation of K-ras,P53 and HER-2/ neu expression in hyperplasic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. Human Pathology, 1999; Vol. 30, No.2:123-130
[22] RiouxLeclerq N, Turlin B, Bansard JY, et al. Value of immunohistochemical Ki67 and P53 determinations as predictive factors of outcome in renal cell carcinoma. Urology 2000; 55:501-50.
[23] Al Munem S.S p53 Expression in various grades of astrocytoma Thesis, scientific council of pathology , Iraqi commission for medical specialization . Baghdad 2009
[24] Isolan GR, RibasFilhoJM,Isolan PM, et al. Astrocytic neoplasms and correlation with mutate p53 and ki67 proteins. ArqNeuropsquiatr2005; 36 (4):997 – 1004.
[25] Haapasalo H ,Isola J , Sallinen P, Kalimo H, Helin H, Rantala I, Aberrant p53 expression in astrocytic neoplasms of the brain : association with proliferation A J pathol 1993 ; 142 :1347- 51
[26] Nozaki M, Tada M, Matsumoto R, Sawamura Y, Abe H, Iggo RD. Rare occurrence of inactivating p53 gene mutations in primary on – astrocytic tumors of the central nervous system :Reappraisal by yeast functional assay. ActaNeouropathol .1998; 95, 291-296.

[27] Cho MY, Jung SH, Kim Ts. P53 protein over expression in astrocytic neoplasms Yonsei medical journal 1995;36:521 – 526.
[28] AnupmaN, Angel Mercy R, Chand SM, et al. p53 protein alterations in adult astrocytic tumors and oligodendroglioma. Neurology India 2004;52:228-232.
[29] Ellison DW, StreatPV, Bateman AC. prognostic indicators in a range of astrocytic tumors an immunohistochemical study with ki – 67 and p53 antibodies. J Neurosurgery psychiatry 1995; 59:413 – 419.
[30] Broniscer A, Baker SJ, West AN, Fraser MM,Proko E, Kocak M, et al. Clinical and molecular characteristics of malignant transformation of low – grade glioma in children . J ClinOncol 2007; 25: 682-9.
[31] Barbareschi M, Iuzzolino P, Pennello A, Allegranza A, arrigoni G , et al . p53 expression in CNS neoplasms . J Clinpathol1992;45:583 – 586.
[32] Lee SS, Cho KR, Yan CH, et al over expression of mutant p53 in human anaplastic astrocytoma, and glioblastomamultiforme. Korean J pathol 1994 ; 28:376-380
[33] Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, Yates AJ,Boyett JM, Finlay JL,SpostoR. Expression of P53 and prognosis in children with malignant gliomas . NEngl J Med 2002;346 (6):420-7.
[34] ZubairA,Huma A. SheemaH. Gliomas: Correlation of Histologic Grade Ki 67 and p53 Expression with Patient Survival. Asian Pacific Journal of Cancer Prevention,2010;Vol11: 1637 – 1639.
[35] AbdelazizOS, EL – SabaaBM,AbdelazizA. Characterization of cancer stem cells in patients with brain astrocytomas: a clincopathological and immunohistochemicalstudy. Bull Alex Fac Med 2010; 46 (4): 357 – 363.
[36] Korkolopouloul P, Christodoulou P, Kouzelis K, Hadjiyannaki M, Prifits A, Staoulis G, Seretis A, Thomas-Tsagli E..MDM2&p53 expression in gliomas: amultivariate survival analysis including proliferation markers&epidermal growth factor receptor. Br j cancer 1997; 75 (9):1269-78.
[37] Schiffer D, Cavalla P, Di Sapio A, Giordana MT, Mauro A, Mauro A. Mutations and immunohistochemistry of p53 and proliferation markers in astrocytic tumors of childhood . J Clinoncol2007;25: 682 – 9.
[38] Ishii N, Sawamura Y, Tada M, Daub DM, JanzerRC., Meagher – Villemure M, de Tribolet N, Van Meir EG. Absence of p53 gene mutations in a tumor panel representative of pilocytic astrocytoma diversity using a p53 functional assay. Int,J. Cancer 1998; 76, 797-800.
[39] Lang FF, Miller DC, pisharody S, Koslow M, NewcombEW. High frequency of p53 protein accumulation without p53 gene mutation in human juvenile pilocytic, low grade and anaplastic astrocytomas. Oncogene.1994; 9, 949-954.
[40] Patt S, Gries H, Giraldo M, Cervos Navarro J, Martin H,Janisch W, Brockmoller J. p53 gene mutations in human astrocytic brain tumors including pilocyticastrocytomas . Hum. Pathol. 1996; 27, 586 – 589.
[41] Burger PC,ScheithauerBW, Paulus W, et al. Pilocyticastrocytoma. In: Lauhuep,Caveen WK, (Ed) pathology and genetics of tumor of the nervous system. Lyon: IARC press 2000:45.
[42] Bodeyb. et al. Immunohischemical detection of p53 expression in various childhood astrocytomas: significance in tumor progression.Anticanc Res 1997; 17 "(2A):1187-94.
[43] Reifenberger G,ReifenbergerJ, Ichimura K., Meltzer PS, Collins VP. Amplification of multiple genes from chromosomal region 12q13-14in human malignant gliomas: preliminary mapping of the amplicon shows preferential involvement of CDK4, SAS, and MDM2 Cancer Res .1994; 54, 4299-4303.

[44] Ono Y, Tamiya T, Ichikawa T, Matsumoto K,FurutaT, et al Accumulation of wild type p53 in astrocytomas is associated with increased p21 expression.ActaNeouropathol1997;94:21-7.
[45] Tada M, Iggo RD,Waridel F, Nozaki, M, Matsumoto R, Sawamura Y, Shinohe Y, Ikeda J, Abe H, Reappraisal of p53 mutations in human malignant astrocytic neoplasms by p53 functional assay :comparison with conventional structural analyses .Mol .Carcinog .1997; 18 , 171 – 176
[46] Challop MK, Ali HH, Qasim BJ, Mohhammed AM.Immunohistochemical Expression of Ki – 67, PCNA and CD34 in Astrocytomas: A Clincopathologicalstudy, Oman Medical Journal 2012; Vol27, No 5: 368-374.
[47] Mahzouni P, Mokhtari M, AmirmansourB. Differentiation between reactive gliosis and astrocytic by MiB-1/Ki67 immunostaining. JRMS 2007; 12 (5):241-245.
[48] AtikE,Searslan Y, Bakaris S, Savas N. The importance of proliferative activity and angiogenesis in hemangioma and glioma. Turk Neurosurg. 2005; 15 (4):176-181
[49] Kayaselcuk F, Zorludemir S, Gmurdulu D, Zeren H, Erman T. PCNA and Ki -67 in central nervous system tumors:correlation with the histological type and grade . J Clinoncol2002;57:115-121.
[50] Liwei Y, Xiuhen W. Expression and clinical significance of PTEN and Ki – 67in humanastrocytoma. Jiangsu Medical Journal 2010; 36 (5):73- 75.
[51] Hunag Y. Expression and significance of hypoxia inducible factor – Ia, Ki – 67 and micro vessel density in diffuse infiltrating astrocytoma. ClinExp Med. 2010;23:51-56.
[52] Rodriguez – Pereira C , Suarez – Penaranda JM , Vazquez – Salvado M, Sobrido MJ, Abrades M , Barros F , et al . Value of MIB – 1 labeling index (LI)ingliomas and its correlation with other prognostic factors. Aclinicopathologicstudy. J NeurosurgSci 2000 Dec; 44 (4):203 – 210.
[53] Scott IS, Morris Ls, Rushbrook SM, Bird K,VowlerSL, Burnet NG, et al Immunohistochomas estimation of cell cycle entry and phase distribution in astrocytomas: applications in diagnostic neuropathology. NeouropatholApplNeurobiol 2005 Oct; 31 (5):455-466.
[54] KaramitopoulouE,Perentes E, DiamantisI, et al: ki – 67 immunoreactivity in human central nervous system tumors: a study with MIB 1 monoclonal antibody on archival material. ActaNeouropathol 1994; 87 :47-54.
[55] Khalid H, Shibata S,Kishikawa M, et al: Immunohistochemicalanalysis of progesterone receptor and Ki – 67 labeling indexingastrocytictumors. Cancer 1997; 80:2133- 2140.
[56] Di NishizakiT, Harada K, et al: proliferative potentials of glioma cells and vascular components determined with monoclonal antibody MIB -1. JExpclincanc Res.1997; 16:389 – 394.
[57] YueWY, Yu SH, Zhao SG, Chen ZP. Molecular markers relating to malignant progression in Grade II astrocytoma. J Neurosurg2009; 110: 709- 14.
[58] RalteAM, Sharma MC,KarakAK, et al:Clincopathological features, MIB – 1 LABELING index and apoptotic index in recurrent astrocytic tumors. PatholoncolRes. 2001; 7: 267 – 278.
[59] Bekchanov D; Kawakita H; Mukhamediev M; Khushvaktov S; Juraev M. Sorption of cobalt (II) and chromium (III) ions to nitrogen- and sulfur-containing polyampholyte on the basis of polyvinylchloride /Polymers for Advanced Technologies 2021 https://doi.org/10.1002/pat.5209
[60] Davron, B., Mukhtar, M., Nurbek, K., Suyun, X., Murod, J. Synthesis of a New Granulated Polyampholyte and its Sorption Properties. *International Journal of Technology*. Volume 11(4), pp. 794-803. ., (2020) https://doi.org/10.14716/ijtech.v11i4.4024

[61] Mukhamediev, M.G., Bekchanov, D.Z. New Anion Exchanger Based on Polyvinyl Chloride and Its Application in Industrial Water Treatment. Russ J ApplChem 92, 1499–1505 (2019). https://doi.org/10.1134/S1070427219110053
[62] Mukhamediev, M.G., Auelbekov, S.A., Sharipova, Z.T. et al. Polymer complexes of gossypol and their antiviral activity. Pharm Chem J 20, 276–278 (1986). https://doi.org/10.1007/BF00758817
[63] Ikramova, M.E., Mukhamediev, M.G., Musaev, U.N. Complexation of hydrazine- and phenylhydrazine-modified nitron fibers with iodine/ Plasticheskie Massy: SintezSvojstvaPererabotkaPrimenenie, (12), стр. 41–45 (2004)
[64] Gafurova, D.A., Khakimzhanov, B.S., Mukhamediev, M.G., Musaev, U.N. Sorption of Cr(VI) on the anion-exchange fibrous material based on nitron. Russian Journal of Applied Chemistry, 75(1), стр. 71–74, (2002)
[65] Rustamov, M.K., Gafurova, D.A., Karimov, M.M. et al. Application of ion-exchange materials with high specific surface area for solving environmental problems. Russ J Gen Chem 84, 2545–2551 (2014). https://doi.org/10.1134/S1070363214130106
[66] Bazarova D. Some problems of counteracting crimes related to laundering of illegal proceeds in Uzbekistan Journal of Advanced Research in Dynamical and Control Systems. Volume 11, Issue 7, 2019, Pages 873-885
[67] Ismailova, Z., Choriev, R., Ibragimova, G., Abdurakhmanova, S., &Abdiev, N. (2020). Competent model of Practice-oriented education of students of the construction profile. Journal of Critical Reviews. Innovare Academics Sciences Pvt. Ltd. https://doi.org/10.31838/jcr.07.04.85
[68] Ismailova, Z., Choriev, R., Musurmanova, A., &Aripjanova, M. (2020). Methods of training of teachers of university on advanced training courses. Journal of Critical Reviews. Innovare Academics Sciences Pvt. Ltd. https://doi.org/10.31838/jcr.07.05.85
[69] Ismailova, Z., Choriev, R., Salomova, R., &Jumanazarova, Z. (2020). Use of economic and geographical methods of agricultural development. Journal of Critical Reviews. Innovare Academics Sciences Pvt. Ltd. https://doi.org/10.31838/jcr.07.05.84
[70] Isakov, A., Tukhtamishev, B., &Choriev, R. (2020). Method for calculating and evaluating the total energy capacity of cotton fiber. IOP Conference Series: Earth and Environmental Science, 614(1), 012006
[71] Davirov, A., Tursunov, O., Kodirov, D., Baratov, D., &Tursunov, A. (2020). Criteria for the existence of established modes of power systems. IOP Conference Series: Earth and Environmental Science, 2020, 614(1), 012039
[72] Obidov, B., Choriev, R., Vokhidov, O., &Rajabov, M. (2020). Experimental studies of horizontal flow effects in the presence of cavitation on erosion-free dampers. IOP Conference Series: Materials Science and Engineering, 883(1), 012051
[73] Khasanov, B., Choriev, R., Vatin, N., &Mirzaev, T. (2020). The extraction of the water-air phase through a single filtration hole. IOP Conference Series: Materials Science and Engineering, 2020, 883(1), 012206
[74] Shokhrud F. Fayziev The problem of social stigma during a pandemic caused by COVID-19 International Journal of Advanced Science and Technology Vol. 29, No. 7, (2020), pp. 660-664 http://sersc.org/journals/index.php/IJAST/article/view/13965/7188
[75] FayziyevShokhrudFarmonovich Medical law and features of legal relations arising in the provision of medical services. International journal of pharmaceutical research Volume 11, Issue 3, July - Sept, 2019 P. 1197-1200 doi:10.31838/ijpr/2019.11.03.088 http://www.ijpronline.com/ViewArticleDetail.aspx?ID=11016
[76] Bryanskaya Elena, FayzievShokhrud, Altunina Anna, MatiukhaAlena Topical Issues of an Expert Report in the Process of Proving in a Criminal Examination. International

Journal of Engineering and Advanced Technology (IJEAT) ISSN: 2249 – 8958, Volume-9 Issue-1, October 2019 5345-5349 DOI: 10.35940/ijeat.A2946.109119 https://www.ijeat.org/wp-content/uploads/papers/v9i1/A2946109119.pdf [77]

[78] FayzievShokhrud (2019) Legal Aspects of Transplantology in the Republic of Uzbekistan. Systematic Reviews in Pharmacy, ISSN: 0976-2779, Vol: 10, Issue: 2, Page: 44-47 doi:10.5530/srp.2019.2.08 http://www.sysrevpharm.org//fulltext/196-1575419211.pdf?1586863081 [79]

[80] Tulaganova, G.Some issues of observance of international legal norms of fight against legalization of criminal incomes in the Republic of UzbekistanJournal of Advanced Research in Dynamical and Control Systems 12(2 Special Issue), c. 143-155