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RESEARCH ARTICLE

Prognostic Value of Snail-1and Beta-Catenin Immunostaining in Astrocytoma.

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Abstract

Background: Astrocytoma is the commonest central nervous system tumor worldwide. Searching in molecular pathogenic processes of its progression is necessary to identify novel therapeutic targets to improve the clinical outcome of patients. Epithelial-mesenchymal transition (EMT) is the process by which cells lose their adhesive properties with surrounding cells and then they transformed into mesenchymal-like and motile phenotypes. Snail-1 is a zinc finger transcription factor that plays an essential role in EMT. Beta-catenin is a protein that binds to the cytoplasmic tail of E-cadherin. In the presence of mutations of Wnt-signal; it promotes transcription of several target genes involved in cell proliferation.

Aim of the work: To asses Snail-1 and Beta-catenin immune-expressions in astrocytoma of different grades in a trial to detect their prognostic values in that tumor. **Methods:** Immunohistochemical staining of Snail-1 and Beta-catenin was evaluated in 60 paraffin blocks of astrocytoma grades II, III and IV. The relationships between their expressions and clinicopathological parameters were analyzed. **Results:** The expressions of snail-1 and beta-catenin were significantly associated with astrocytoma grade (p=0.001 and 0.007 respectively) type of surgery, performance status, response to treatment (p <0.001) and progression of the tumor (p=0.048, 0.012 respectively). High Snail-1 and high Beta-catenin immune-expressions were inversely related to The 1, 2 and 3-year OS (P <0.001).

Conclusion: Snail-1 and beta-catenin expressions were frequently increased in astrocytoma and significantly associated with poor prognosis.

Introduction:-

Astrocytoma is the commonest central nervous system (CNS) tumors and represents 75% of them (**Lind-Landstrom et al., 2012).** In Egypt, it accounts for about 30% of CNS primary tumors **(Zalata et al., 2011).** The most important prognostic factor for astrocytoma is the histologic grade **(Chu et al., 2012).** The most common grading system for astrocytoma is the World Health Organization (WHO) classification that grades them into four grades (I-IV) based on cytological atypia, mitotic activity, vascular proliferation, and necrosis **(Louis et al. 2007).** Grades I and II are known as low-grade astrocytoma, while grades III and IV are known as high grade astrocytoma **(Henriksson et al., 2011).** High-grade astrocytoma is more highly invasive than low-grade astrocytoma **(Starkweather et al., 2011).** Searching in molecular pathogenic processes of tumor progression is necessary to identify novel prognostic and therapeutic targets for improving the clinical outcome of such patients **(Singh et al., 2010).** Epithelial-mesenchymal transition (EMT) is the process into which cells lose their adhesive properties with surroundings then transformed into mesenchymal-like and motile cells **(Ding et al., 2013),** it is a major modulator of metastasis in epithelial solid tumors; whereas in case of tumors of neuroepithelial type an EMT (-like) process still has been studied (**Kahlert et al., 2012).** EMT-like changes in astrocytoma are attributed to many markers

(Mikheeva et al., 2010). SNAL-1 which is a zinc finger transcription factor is one of them and it plays an essential role in EMT **(Peinado et al., 2007).** Beta-catenin is a protein that binds to the cytoplasmic tail of E-cadherin where it is participating in cell-cell adhesion and is degraded by interacting with the APC gene. Mutations in genes that encode the proteins involved in the Wnt-signaling and B-catenin degradation may lead to its persistence of in the cytoplasm and subsequent translocation to the nucleus, where it activate genes involved in cell proliferation and/or inhibition of apoptosis **(Fodde and Brabletz, 2007).**

In this study we aimed to asses Snail-1 and Beta-catenin immune-expressions in astrocytoma of different grades in a trial to detect their prognostic values in that tumor.

Patients and Methods:-

For this retrospective cohort study, formalin fixed paraffin embedded 60 cases of astrocytoma selected from archives of Pathology and Medical Oncology Departments Faculty of Medicine, Zagazig University and Pathology Department, Kasr El-Einy hospital during the period from January 2012 to January 2015. The clinical data of the patients were obtained from medical files. Written informed consent was taken from the patients before biopsy, stereotactic brain biopsies were performed in 10 cases and in other cases surgically removed tumor tissue was used.

The study follows guidelines of the local ethical committee.
Each tumor was re-evaluated by retrospective analysis of the medical records and the tissue slide file of the pathology department, sex, age, tumor size, histological subtype and grading the tumors according to WHO classification of brain tumors **(Louis et al., 2007).**

Patients were followed up till death or their most recent medical examination. The follow-up deadline was January 2015.

Immunohistochemical staining:-

Immunohistochemical staining was carried out using the streptavidin–biotin immunoperoxidase technique **(Hsu et al., 1981).** 3–5 μm thick sections cut from formalin fixed; paraffin-embedded blocks of all cases, mounted on positively charged slides, deparaffinized in xylene and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH6.0) for 20 min and then washed in phosphate buffer saline (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 6% H2O2 in methanol was carried out. The slides were then incubated overnight rabbit poly clonal antibodies; Snail-1 antibody (clone ab180714) dilution 1:200 Abcam, Cambridge, UK) and Beta- catenin (CTNNB): (Clone: 17C2, Dilution 1:100; Thermo Scientific/Lab Vision Corporation, Fermont,USA).at room temperature for 4 hours. The Strept ABC omplex/HRP Duet Kit (DAKO, Glostrup, Denmark) was used according to the manufacturer's instructions, and the results were visualized with diaminobenzidine. After rinsing in distilled water, sections were counterstained with hematoxylin, dehydrated, and cover glasses were applied. Sections of breast cancer were used as positive controls for Snail-1 and colorectal neoplasm was used as positive control for beta-catenin. For negative control omission of the primary antibodies and using a phosphate-buffered saline buffer was done. After 3 washes in phosphate-buffered saline, slides were incubated with normal goat serum IgG (1:100) for 30 minutes to prevent nonspecific binding.

Evaluation of immunohistochemical expression of SNAIL-1:-

Staining was evaluated according to the extent of stained cells and intensity of the stain. The extent was scored as: 0 (negative), 1 (up to 25% positive cells), 2 (26% to 50% positive cells), 3 (51% to 75% positive cells) and 4 (76% positive cells). The intensity of staining was scored as; 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive). The final scores was reached by multiplication of the intensity and the extent, scores; 0 (negative), + (1–4), ++ (5–8) and +++ (9–12) **(Zhang et al., 2010).** We consider the cutoffs value as 5 high expression if values were>5, low expression if values were ≤ 5.

Evaluation of immunohistochemical expression of Beta-catenin:-

Staining was evaluated according to the extent of stained cells and intensity of the stain. The staining intensity was classified as 0=negative, 1=weak, 2= moderate or 3= strong expression. The staining extent was classified as focal or diffuse and graded from 0 to 4 according to the percentage of positive cells β-catenin: 0% = 0; >0–10% = 1; >10–50% = 2; >50– 80% = 3; >80–100% = 4. Multiplication of the intensity and extent was taken as final result. The cutoffs value was 50% high expression if values were>50%). Low expression if values were ≤50% (**Schüle et al., 2012)**

Statistical analysis:-
All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).Strength of relationship between SNAIL-1 and Beta-catenin & clinicopathological features were determined by computing appropriate correlation coefficient (Kendall tau, biserial, point biserial and rank biserial), Survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used to calculate differences between the curves.. a *P*-value, 0.05 was considered statistically significant.

Results:-

Clinicopathological features:
Sixty patients, 35 males and 25 females were included in our study, their ages ranged from (25 – 80) years (Mean ± SD: 49.93 ± 16.13)**.**

These tumors were equally classified according to WHO classification system of CNS including 20 cases for each Grade. ECOG performance statuswas 1, 2,3 in 41.7%, 46.7%, and 11.7% of the patients, respectively. The most common histological subtype was anaplastic astrocytoma representing 33.3% (20/60) of the patients.

Table (1): Clinicopathological features of 60 patients with brain tumors.

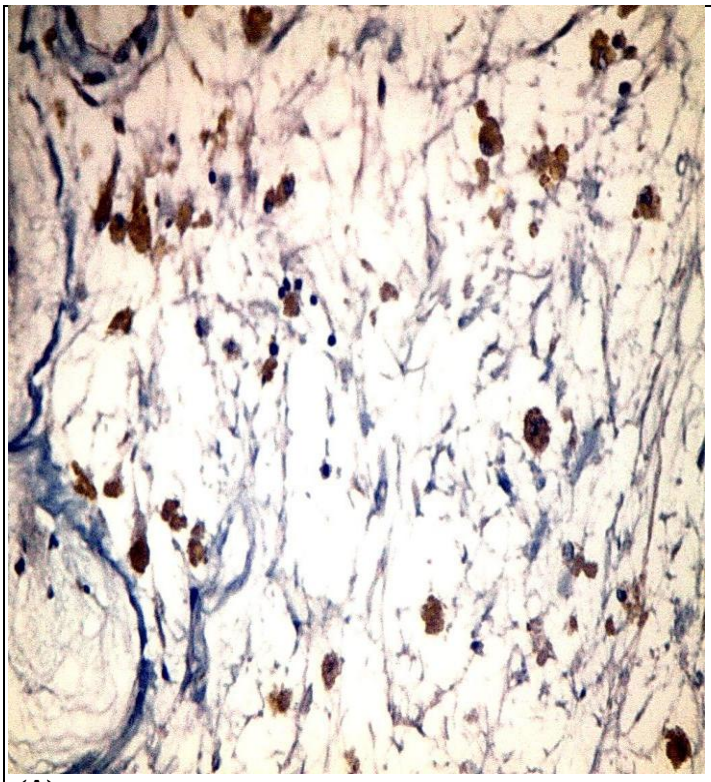
Characteristics	Number	Percentage (%)
Age (year)		
Mean ± SD	49.93 ± 16.13	
Median (Range)	48 (25 – 80)	
≤30 years	10	16.7%
31-40 years	14	23.3%
41-50 years	10	16.7%
51-60 years	12	20%
>60 years	14	23.3%
Sex		
Male	35	58.3%
Female	25	41.7%
Performance status		
ECOG 1	25	41.7%
ECOG 2	28	46.7%
ECOG 3	7	11.7%
Histology		
Fibrillary astrocytoma	14	23.3%
Gemistocytic astrocytoma	6	10%
Anaplastic astrocytoma	20	33.3%
Glioblastoma multiforme	18	30%
Gliosarcoma	2	3.3%
Grade		
Grade II	20	33.3%
Grade III	20	33.3%
Grade IV	20	33.3%

Continuous variables were expressed as mean ± SD & median (range); categorical variables were expressed as number (percentage).

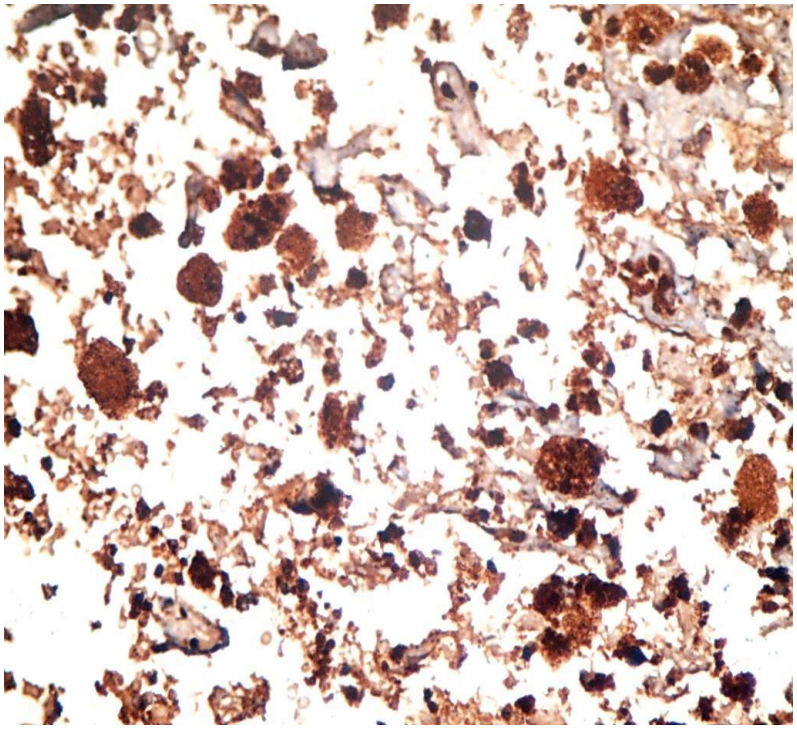
***Snail-1* and Beta catenin expression:-**
Snail-1 and Beta catenin *high* expressions were found in 41.7 % (25/60) and 43.3 % (26/60) astrocytoma patients, respectively (Figures 1and 2).
Patients with ≤ 60 years had significant higher levels than those who are > 60 years (78.6% vs. 21.4%, respectively, p =0.001) for Snail-1and (69.2% vs. 30.7%, respectively, p =0.002) for Beta catenin.

Also, the expression of Snail-1 was significantly correlated with grade (p= 0.001) and histological subtypes of astrocytoma (0.013).

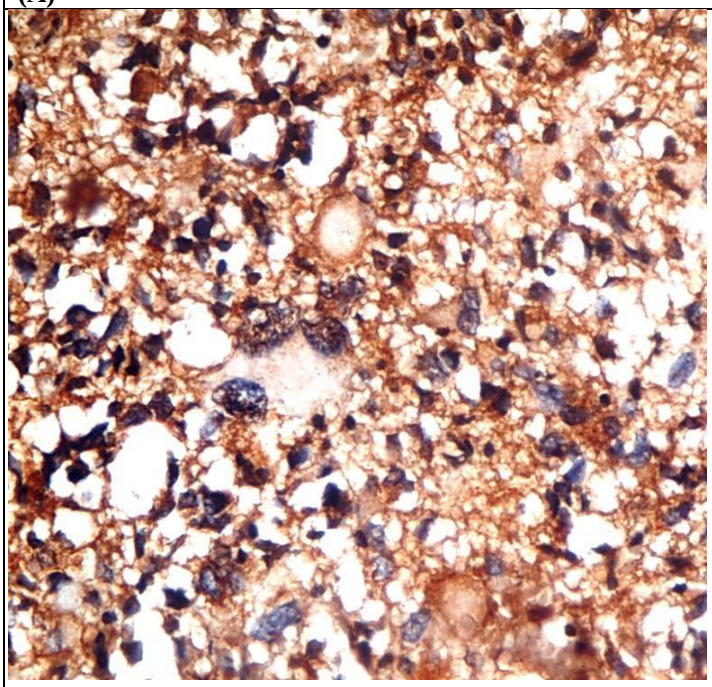
Also, the expression of Beta catenin was significantly correlated with grade and histololgic subtypes of astrocytoma (p= 0.007and 0.023 respectively)
Both Snail-1 and Beta catenin was expressed without significant differences between males and females.



(A)



(B)



(C)

Figure1. Immunohistochemical staining of Snail-1 in astrocytoma:

(A) Low Immunohistochemical expression in the cytoplasm of astrocytoma
Grade II X400.
(B) High Immunohistochemical expression in the cytoplasm of astrocytoma
Grade III X 400.
(C) High Immunohistochemical expression in the cytoplasm of astrocytoma
Grade IV X 400.
Note: High Snail-1 immunohistochemical expression (in the cytoplasm) inHigh gradeastrocytoma. Magnification: A, B& C the original magnification was ×400.

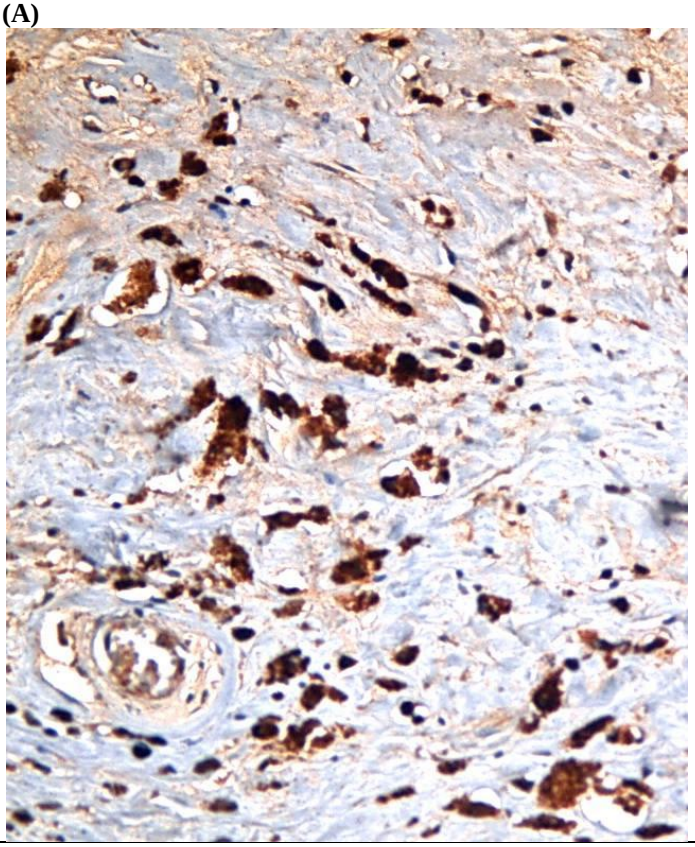


Figure 2. Immunohistochemical staining of Beta- catenin in astrocytoma
(A) Low Immunohistochemical expression in the cytoplasm of astrocytoma Grade II X400.
(B) High Immunohistochemical expression in the cytoplasm of astrocytoma Grade III X 400.
(C) High Immunohistochemical expression in the cytoplasm of astrocytoma Grade IV X 400.
Note: High Beta- catenin immunohistochemical expression (in the cytoplasm) inHigh gradeastrocytoma. Magnification: A, B& C the original magnification was ×400.

Table (2): correlation between SNAIL-1 and Beta-catenin immuno-expression and clinicopathological features

Characteristics	All (N=60)		Snail-1				p-value	Beta catenin (cytoplasmic)				p-value
			Low (N=25)		High (N=35)			Low (N=34)		High (N=26)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age (years)												
Mean ± SD	48.93	±16.13	41.48	±14.48	55.97	±14.60		44.47	±15.98	57.07	±13.53	
Median (Range)	48	(25-80)	35	(25-76)	58	(30-80)		40	(25-76)	59	(30-80)	
≤ 60years	46	(76.7%)	22	(78.6%)	24	(%)	<0.001•	28	(82.3%)	18	(69.2%)	0.002•
> 60 years	14	(23.3%)	3	(21.4%)	11	(%)		6	(17.6%)	8	(30.7%)	
Sex												
Male	35	(58.3%)	17	(48.6%)	18	(51.4%)	0.199‡	21	(60%)	14	(40%)	0.538‡
Female	25	(41.7%)	8	(32%)	17	(68%)		13	(52%)	12	(48%)	
Histology												
Fibrillary	14	(23.3%)	11	(78.6%)	3	(21.4%)	0.013‡	12	(85.7%)	2	(14.3%)	0.023‡
Gemistocytic	6	(10%)	3	(50%)	3	(50%)		5	(83.3%)	1	(16.7%)	
Anaplastic	20	(33.3%)	7	(35%)	13	(65%)		9	(45%)	11	(55%)	
Glioblastoma	18	(30%)	4	(22.2%)	14	(77.8%)		8	(44.4%)	10	(55.6%)	
Gliosarcoma	2	(3.3%)	0	(0%)	2	(100%)		0	(0%)	2	(100%)	
Grade												
Grade II	20	(33.3%)	14	(70%)	6	(30%)	0.001§	17	(85%)	3	(15%)	0.007§
Grade III	20	(33.3%)	7	(35%)	13	(65%)		9	(45%)	11	(55%)	
Grade IV	20	(33.3%)	4	(20%)	16	(80%)		8	(40%)	12	(60%)	
Snail-1												
Low	25	(41.7%)						25	(100%)	0	(0%)	<0.001‡
High	35	(58.3%)						9	(25.7%)	26	(74.3%)	
Beta catenin												
Low	34	(56.7%)	25	(73.5%)	9	(26.5%)	<0.001‡					
High	26	(43.3%)	0	(0%)	26	(100%)						

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range).

• Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; $p<0.05$ is significant.

Correlations of Snail-1 with Beta catenin immunoexpression:-

- Immunohistochemical expressions of Snail-1and Beta-catenin were significantly positively correlated with each other correlation coefficient r= +0.739 (p <0.001).
- Immunohistochemical expressions of Snail-1 and Beta catenin were significantly positively correlated with type of surgery, performance status, response to treatment (p <0.001) and progression of the tumor (p=0.048, 0.012 respectively; Tables 3).
- The sensitivity of combination of both markers as predictors for progression of astrocytoma, overall response to treatment and survival of the patients were 91.7, 50 and 66.7% respectively and the specificity was 45.5, 95.5, and 95.8% respectively.

Table (3): Correlation between SNAIL-1 and Beta-catenin immuno-expression, type of surgery, PS, response to treatment and outcome of treatment

Characteristics	All (N=60)		Snail-1				p-value	Beta catenin (cytoplasmic)				p-value
			Low (N=25)		High (N=35)			Low (N=34)		High (N=26)		
	No.	(%)	N	(%)	No.	(%)		N	(%)	N	(%)	
Surgery												
Bisopsy	10	(16.7%)	0	(0%)	10	(28.6%)	<0.001‡	0	(0%)	10	(38.5%)	
STE	27	(45%)	4	(16%)	23	(65.7%)		11	(32.4%)	16	(61.5%)	<0.001‡
GTE/NTE	23	(38.3%)	21	(84%)	2	(5.7%)		23	(67.6%)	0	(0%)	
Performance status												
ECOG 1	25	(41.7%)	22	(88%)	3	(8.6%)	<0.001§	24	(70.6%)	1	(3.8%)	
ECOG 2	28	(46.7%)	3	(12%)	25	(71.4%)		10	(29.4%)	18	(69.2%)	
ECOG 3	7	(11.7%)	0	(0%)	7	(20%)		0	(0%)	7	(26.9%)	<0.001§
Response to ttt												
NR	10	(16.7%)	0	(0%)	10	(28.6%)	0.003‡	0	(0%)	10	(38.5%)	
OAR	50	(83.3%)	25	(100%)	25	(71.4%)		34	(100%)	16	(61.5%)	<0.001‡
PD	5	(8.3%)	0	(0%)	5	(14.3%)	<0.001§	0	(0%)	5	(19.2%)	
SD	5	(8.3%)	0	(0%)	5	(14.3%)		0	(0%)	5	(19.2%)	<0.001§
PR	27	(45%)	4	(16%)	23	(65.7%)		11	(32.4%)	16	(61.5%)	
CR	23	(38.3%)	21	(84%)	2	(5.7%)		23	(67.6%)	0	(0%)	
Progression												
No	55	(91.7%)	25	(100%)	30	(85.7%)	0.048‡	34	(100%)	21	(80.8%)	0.012‡
Yes	5	(8.3%)	0	(0%)	5	(14.3%)		0	(0%)	5	(19.2%)	
Survival												
Alive	36	(60%)	24	(96%)	12	(34.3%)	<0.001‡	30	(88.2%)	6	(23.1%)	<0.001‡
Died	24	(40%)	1	(4%)	23	(65.7%)		4	(11.8%)	20	(76.9%)	

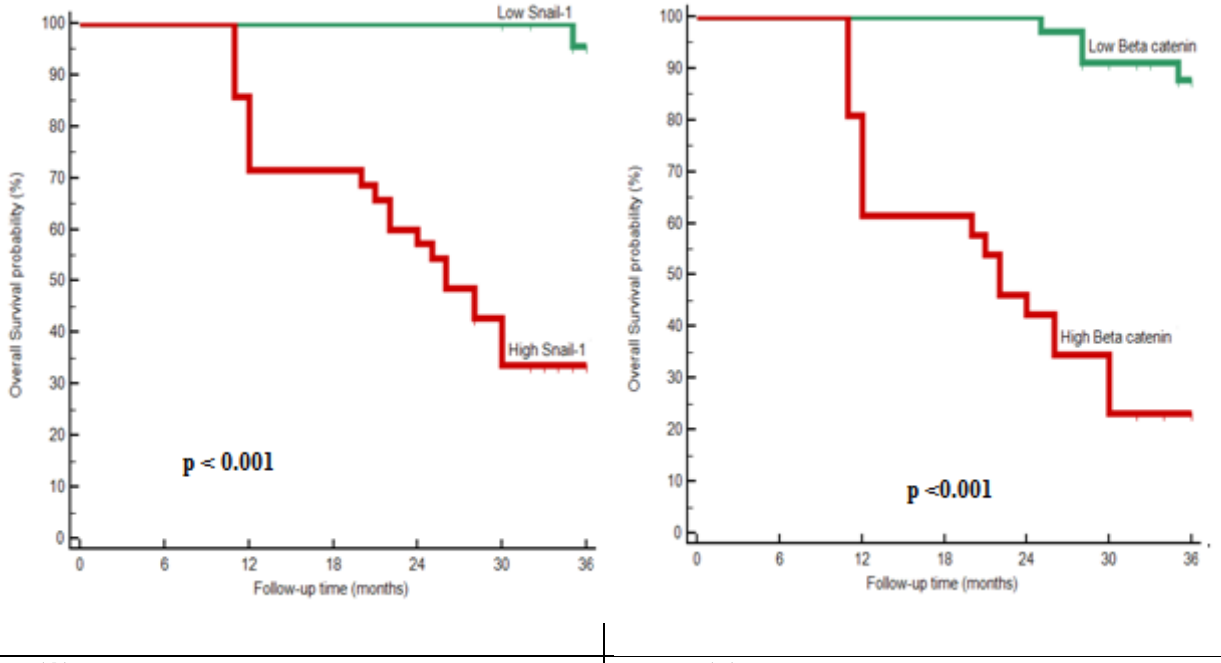
- Categorical variables were expressed as number (percentage).
- ‡ Chi-square test; § Chi-square test for trend; $p<0.05$ is significant.

Survival analysis:-

After a median follow up of 34.50 (11 – 36) months, the mean OS was 29.6 months while median OS was not reached.

For the 60 patients, the 1, 2, 3-years OS rates were 83.3%, 75% and 59.5%, respectively.

High immune-expressions of both Snail-1 and Beta catenin were inversely related to The 1, 2 and 3-year OS (P <0.001; Table 4; Figure 3).



(A) **(B)**

Figure (3) Kaplan-Meier plot of overall survival: (A) Stratified according to Snail-1, (B) stratified according to Beta catenin

Table (4): correlation between SNAIL-1 and Beta-catenin immuno-expression, clinicopathological features, response to treatment and survival of our patients

Characteristics	Survival						p-value	Overall survival				p-value†
	All (N=60)		Alive (N=36)		Died (N=24)			Median OS (month)	1 year OS (%)	2 years OS (%)	3 years OS (%)	
	No.	(%)	No.	(%)	No.	(%)						
All patients								NR	83.3%	75%	59.5%	
Age (years)												
Mean ± SD	48.93	±16.13	42.66	±13.28	60.83	±13.87	<0.001•					
Median (Range)	48	(25-80)	40	(25-76)	60	(30-80)						
≤30 years	10	(16.7%)	9	(90%)	1	(10%)	0.001‡	NR	100%	100%	90%	<0.001
31-40 years	14	(23.3%)	12	(85.7%)	2	(14.3%)		NR	100%	100%	85.7%	
41-50 years	10	(16.7%)	7	(70%)	3	(30%)		NR	90%	90%	68.6%	
51-60 years	12	(20%)	5	(41.7%)	7	(58.3%)		24	58.3%	50%	41.7%	
>60 years	14	(23.3%)	3	(21.4%)	11	(78.6%)		24	71.4%	42.9%	21.4%	
Sex												
Male	35	(58.3%)	22	(62.9%)	13	(37.1%)	0.593‡	NR	91.4%	77.1%	62.9%	0.576
Female	25	(41.7%)	14	(56%)	11	(44%)		NR	72%	72%	54.5%	
Histology												
Fibrillary	14	(23.3%)	12	(85.7%)	2	(14.3%)	0.002‡	NR	100%	100%	85.7%	<0.001
Gemistocytic	6	(10%)	5	(83.3%)	1	(16.7%)		NR	100%	100%	83.3%	
Anaplastic	20	(33.3%)	14	(70%)	6	(30%)		NR	85%	85%	68.2%	
Glioblastoma	18	(30%)	5	(27.8%)	13	(72.2%)		22	72.2%	44.4%	27.8%	
Gliosarcoma	2	(3.3%)	0	(0%)	2	(100%)		11	0%	0%	0%	
Grade												
Grade II	20	(33.3%)	17	(85%)	3	(15%)	<0.001§	NR	100%	100%	85%	<0.001
Grade III	20	(33.3%)	14	(70%)	6	(30%)		NR	85%	85%	68.2%	
Grade IV	20	(33.3%)	5	(25%)	15	(75%)		22	65%	40%	25%	
Snail-1												
Low	25	(41.7%)	24	(96%)	1	(4%)	<0.001‡	NR	100%	100%	95.7%	<0.001

High	35	(58.3%)	12	(34.3%)	23	(65.7%)		26	71.4%	57.1%	33.7%	
Beta catenin												
Low	34	(56.7%)	30	(88.2%)	4	(11.8%)	<0.001‡	NR	100%	100%	87.8%	<0.001
High	26	(43.3%)	6	(23.1%)	20	(76.9%)		22	61.5%	42.3%	23.1%	
Surgery												
Bisopsy	10	(16.7%)	0	(0%)	10	(100%)	<0.001‡	11.5	50%	20%	0%	<0.001
STE	27	(45%)	14	(51.9%)	13	(48.1%)		NR	92.6%	74.1%	51.6%	
GTE/NTE	23	(38.3%)	22	(95.7%)	1	(4.3%)		NR	100%	100%	95.2%	
Performance status												
ECOG 1	25	(41.7%)	24	(96%)	1	(4%)	<0.001§	NR	100%	100%	95.7%	<0.001
ECOG 2	28	(46.7%)	12	(42.9%)	16	(57.1%)		30	89.3%	71.4%	42.1%	
ECOG 3	7	(11.7%)	0	(0%)	7	(100%)		11	0%	0%	0%	
Response to ttt												
NR	10	(16.7%)	0	(0%)	10	(100%)	<0.001‡	11.5	0%	0%	0%	<0.001
OAR	50	(83.3%)	36	(72%)	14	(28%)		NR	100%	90%	71.4%	
PD	5	(8.3%)	0	(0%)	5	(100%)	<0.001§	11	0%	0%	0%	<0.001
SD	5	(8.3%)	0	(0%)	5	(100%)		12	0%	0%	0%	
PR	27	(45%)	14	(51.9%)	13	(48.1%)		NR	100%	81.5%	81.5%	
CR	23	(38.3%)	22	(95.7%)	1	(4.3%)		NR	100%	100%	95.2%	
Progression												
No	55	(91.7%)	36	(65.5%)	19	(34.5%)	0.008‡	NR	90.9%	81.8%	64.9%	<0.001
Yes	5	(8.3%)	0	(0%)	5	(100%)		11	0%	0%	0%	

Categorical variables were expressed as number (percentage); NR denote not reached yet; • Mann Whitney U test; ‡ Chi-square test; § Chi-square test for trend; † Log rank test; $p< 0.05$ is significant.
Hazard Ratio for mortality for high Snail-1 expression (95%confidence interval) : 25.098 (11.267-55.909).
Hazard Ratio for mortality for high Beta catenin expression (95%confidence interval) : 10.975 (4.631-26.010).

Discussion:-

In the current study Snail-1immunoreactivity was intensified significantly from low-grade astrocytoma to anaplastic astrocytoma and glioblastoma. So we proved that Snail-1 expression was correlated with tumor grade and used as a predictive factor of glioblastoma aggressiveness, also we found that tumors with low Snail-1 expression represent slow-growing and less-aggressive tumors than those with high Snail-1. These results are consistent with previous studies made by [**Myung et al., 2014, 2010 and Lim et al., 2010]** who proved that high grade astrocytoma showed higher expression of the Snail-1 protein than low-grade astrocytoma and ascertain a significant correlation between Snail-1 expression and various clinicopathological factors, also **Han et al., 2011** proved results near ours that Snail-1 was up-regulated in WHO grade IV astrocytoma. Glioblastoma, WHO grade IV, showed the highest protein expression as compared to other low-grade astrocytoma. We ascertained that the association between Snail-1 expression and the WHO grade of astrocytoma may be due to the fact that increased Snail-1 immune-expression enhanced the proliferation, viability and invasion ability by promoting EMT induction. Our results were taken together with previous results to verify the role of Snail-1 in EMT process regulation will provide new insights into the molecular mechanisms of astrocytoma progression.

In the correlation between Snail-1 distribution and patient survival in our study, we found a significant association between Snail-1 expression and survival of patients with various grades of astrocytoma, high immune-expressions of Snail-1 was inversely related to The 1, 2 and 3-year OS ($P <0.001$).

This is consistent with the results of [**Myung et al., 2010** and **Han et al., 2011],** they proved that WHO tumor grade and expressions of Snail-1 were significant prognostic factors affecting overall and disease progression-free survival rates. So Snail -1 was associated with WHO grade in astrocytoma and can be used as prognostic indicator. Immunohitochemical expression of Snail-1 associated with poor prognosis aggressive behavior, tumor recurrence and decrease over all survival of many cancers **(Cieply et al., 2013)** such as hepatocellular **(liu et al., 2013),** and colon carcinomas **(Jackstadt et al., 2013).**

Normally, Immunohistochemical staining for b-catenin shows a membranous pattern of staining in epithelial cells. However, if the Wnt signaling pathway is activated or the b-catenin degradation pathway is inactivated, b-catenin is seen to accumulate in the cytoplasm and/or the nucleus (**Campbell et al., 2002**). Mutations in genes that encode the proteins involved in the Wnt signaling and b-catenin degradation may be implicated in numerous human epithelial cancers, as the translocation of b-catenin away from E-cadherin may lead to reduced cell-cell adhesion that may promote cancer metastasis **(Iwaya et al., 2003).** Immunohistochemical staining for b-catenin provides an indication

of the integrity of the Wnt signaling and b catenin degradation pathways. Both cytoplasmic and nuclear accumulations of b-catenin are considered abnormal. Of course, a stronger staining for b-catenin may indicate an inability for rapid degradation and therefore be construed as abnormal even when limited to the cytoplasm (**Polakis, 2000).**

Our results showed that there was increased the cytoplasmic expression of β-catenin in astrocytoma and the expression was more in grade III and IV than grade II indicate destabilization and phosphorylation of β-catenin at the cell membrane, translocation to the cytoplasm.

Our results are similar to results of **Yang et al., 2012** who stated that beta-catenin is not expressed in normal astrocytes but only expressed in injured or neoplastic astrocytes, and that beta-catenin nuclear and cytoplasmic expression in high grade (grade III, IV) more than low grade astrocytoma (grade II), **Sareddy et al. 2009a** who proved that the expression levels of beta-catenin were progressively increased form low grade astrocytoma to higher grades and positively correlated with the histological grading of astrocytoma. Slightly different finding found by **Reszec et al., 2015** who proved that in beta-catenin positive diffuse and anaplastic astrocytoma cases and the staining was localized within the nucleus and in glioblastoma only next to the cellular membrane. This can be explained by the fact that beta-catenin nuclear expression is associated with the Wnt/beta-catenin pathway, leading to increased transcriptional activity of beta-catenin, and activation of many genes, associated with cellular proliferation and migration, while membranous staining is associated with the creation of gap junctions together with catenins and connexins, leading to cell-cell adhesion **(Giepmans, 2004).**

Gong et al. (2013) observed, that beta-catenin nuclear translocation, may be responsible for tumorigenesis and the progression of human astrocytoma, **Liu et al. (2011)** showed, that cytoplasmic nuclear beta-catenin status is an independent prognostic factor for astrocytoma patients, and the Wnt/beta-catenin pathway correlated with the progression of gliomas and might be a novel prognostic marker for gliomas**.** Our results were different from **Zhang et al. (2010**) observed that the distribution of beta-catenin was not correlated with astrocytoma grades.
Beta-catenin deregulation is important in the genesis of many tumors, as in breast cancer, cytoplasmic and nuclear localization of *β*-catenin was shown to predict a poor outcome **(L´opez-Knowles, et al., 2010).**

We found a significant association between beta-catenin expression and survival in various grades of astrocytoma, high immune-expressions of beta-catenin was inversely related to The 1, 2 and 3-year OS ($P < 0.001$). Patients with astrocytoma showing less expression of beta-catenin (astrocytoma grade II) tend to be associated with better prognosis than patients with high beta-catenin expression (astrocytoma grade III and IV).

Our results are inagreement with the results of Zhang et al. (2009) who found a significant correlation between levels of beta-catenin and 2-year patient survival, survival analysis showed that patients with astrocytoma showing less expression of beta-catenin tend to be associated with good prognosis whereas, astrocytoma patients with high beta-catenin expression associated with poor prognosis.

Schüle et al., 2012 stated that β-Catenin expression significantly positively correlated with higher WHO grade astrocytoma and found a significant association of increased β-catenin expression with patient overall survival.

Regarding the correlation between snail-1 and beta-catenin immunohistochemical expression in astrocytoma, we found significant positive correlation between them so we proved that the immunohistochemical determination of E-cadherin/β-catenin complex integrity loss and EMT marker Snail- might be a potential useful prognostic tool in astrocytoma patients that was like results of **Galván et al., 2014** who found similar results in pulmonary neuroendocrine tumors.

Regarding correlation between Snail-1with Beta catenin immune-expression they were significantly positively correlated with each other and that correlation can be a good prognostic tool for detection of prognosis and progression of astrocytoma patients.

Conclusion:-

According to our results and results of all previous studies regarding Wnt/B-catenin/Tcf signaling pathway insist that this pathway together with Snail-1expression are activated in human astrocytoma and involved in its malignant progression, which may be a potential therapeutic target for astrocytoma treatment.

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