

# Pathological implication of progesterone receptor expression in patients diagnosed with meningioma

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## ABSTRACT

**Objectives.** To assess the progesterone receptor expression in meningioma patients and to characterize the histopathological and demographic features of them.

**Materials and methods.** A cross sectional study conducted during a period of six months (from April to October 2023) included 50 formalin fixed, paraffin embedded tissue blocks diagnosed histopathologically as meningioma.

Progesterone receptor was detected using immunohistochemistry in the other section.

**Outcomes.** The most common location of meningioma was cerebrum (56%) and the most common histopathological type was meningothelial. Regarding progesterone receptor status, a negative score was presented in 44% of cases. There is a significant association between the progesterone receptor status and both grades of the histopathological type of meningioma.

**Conclusion.** High progesterone receptor positivity was linked to low grade tumors. Meningioma grade and many subtypes can be identified with the use of progesterone receptor expression, particularly in cases that are borderline.

**Keywords:** meningioma, progesterone, receptor, histopathological, expression

## BACKGROUND

Meningiomas are the most common primary tumors of the central nervous system (CNS). They are believed to originate from meningothelial (arachnoid) cells and are typically benign, slowly developing neoplasms [1,2]. They account for about 37.6% of primary CNS tumors; and approximately 50% of all benign brain tumors [3]. Meningioma is the second tumor to attack the central nervous system in Iraq, after astrocytoma. Meningiomas account for around 15.3-20.5% of all CNS tumors, with a 0.4% incidence in women and 0.1% in males, with a peak incidence around the age of 45 [4]. The World Health Organization (WHO) has categorized them into three classes. Most of them (82–92%) are considered benign, and they are categorized as Grade 1. Meningiomas can become malignant in about 1-3% of cases, with a 32–64% 5-year survival rate. Because of their benign nature and location within the central nervous system, they can result in major issues that need to be treated by a doctor [5]. Ionizing radiation to the skull is thought to be a risk factor for meningioma formation; there is no obvious dose-response

association and a six-to ten-fold relative risk after a varied latency time. Moreover, epidemiological factors such a history of head trauma, cigarette smoking, and mobile phone use have not consistently been demonstrated to be linked to a noticeably higher risk of meningioma [6]. Progesterone, estrogen, androgen glucocorticosteroids, dopamine, prolactin, prostaglandin, and growth factors are all present in varying amounts in meningiomas [7]. The obvious female predominance (female/male ratio:2:1), the documented fast growth during pregnancy, and the women who take oral contraceptives or hormone replacement treatment are some of the clinical data that points to the possibility that sex steroids contribute to the growth of meningiomas [8, 9]. Progesterone receptors (PR) are expressed in healthy meninges and are expressed in 33–89% of meningiomas. Three isoforms were identified using immunoblot analysis: Particularly when it comes to PR-B expression, PR-A has a somewhat suppressive effect, PR-B has a higher affinity and a more prominent activating activity, and PR-78 [10]. PR expression prevalence in meningioma patients has been found to be influenced by many clinicopathologic pa-

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rameters, most notably tumor grade, as defined by the WHO [11]. It has been shown that PR-negative status is correlated with a worse meningioma prognosis. Furthermore, there appears to be a correlation between level of PR expression and NF2 mutations, a crucial early genetic event in development of meningiomas [12]. The aim of study is to assess the progesterone receptor expression in meningioma patients and to characterize the histopathological and demographic features of them.

## PATIENTS AND METHODS

**Study design, setting, and time:** This was a cross sectional study conducted in the Department of histopathology and forensic medicine at Al-Mustansirya Medical College and Medical City and Teaching lab of Al-Yarmouk Teaching Hospital and Medical City during a period of six months from (April to October 2023).

**Tissue sample:** The study included 50 formalin fixed, paraffin embedded tissue blocks that were collected retrospectively depending on the archived files of patients diagnosed histopathologically as meningioma. From every block, two 5 µm sections were extracted. Hematoxylin and eosin stain (H&E stain) was used to stain the first for histopathological revision. The histopathological type was appraised, and the meningioma was graded using the current WHO (2016) grading scheme. Progesterone receptor was detected using immunohistochemistry in the other section.

**Material used:** Reagents used in the study were distilled water, xylene, ethanol, rinse buffer, target retrieval solution, secondary detection system (PolyExcell HRP/DAB detection system (PathnSitu)), primary antibody, mounting media, and hematoxylin.

**Instrument used:** Binocular light microscope, tissue paper, microscopic camera, positive charged slides, glass jars, elastic jars, flask 200 ml, graduated cylinder, filter paper, water bath, microwave oven, oven, pipettes and tips (fine and course tips), PAP pen, timer, cover slip, slide holders, plastic slide holder, and humid chamber.

**Immunohistochemical staining procedure:** The specimens were paraffin embedded, formalin-fixed tissue blocks. Four micrometer-thick tissues were extracted from these blocks, deparaffinized, and stained using hematoxylin and eosin staining technique.

## METHOD

**1. Deparaffinization:** The procedure involved first incubating the sections in an oven at 60 degrees Celsius for an hour, then changing the xylene twice for five minutes each, and finally rehydrating the tissue in ethanol at varying concentrations (100%, 70%, and 50%) for an additional five minutes each.

## 2. H & E staining method:

- ✓ Step 1: Deparaffinization (5 minutes in xylene, changes).
- ✓ Step 2: Rehydration: Graded alcohol (5 minutes, 3 changes).
- ✓ Step 3: Nuclear stain: hematoxylin (3-10 minutes).
- ✓ Step 4: Wash well in running tap.
- ✓ Step 5: Differentiation (1% acid alcohol for 5-10 second, 2 dips).
- ✓ Step 6: Wash well in running tap (Until regain their blue).
- ✓ Step 7: Counter stain: eosin (2-5 minutes).
- ✓ Step 8: Dehydration (Graded alcohol 3-5).
- ✓ Step 9: Clearing by xylene (For 5 minutes).
- ✓ Step 10: Mount with DPX.

## 3. Immunohistochemical staining protocol:

3-steps polymeric detection system immunostaining method was used and included the following steps:

- a. Cut and mount 3 to 4-micron formalin-fixed paraffin –embedded tissue on positive charged slide.
- b. Deparaffinization was done by incubating the sections in an oven at 60c for 2 hours followed by two changes of xylene then rehydrate tissue in decreasing concentration of alcohol (100%, 70%, 50%).
- c. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as Immuno DNA Retriever with citrate, PH 9 and incubate at 95c for 30 minutes in water bath.
- d. Wash with 3 changes of IHC wash buffer each for 5 minutes.
- e. Place slides in polyDetector peroxidase Blocker for 5 minutes.
- f. Wash with 2changes of IHC wash buffer each for 5 minutes.
- g. Cover tissue with the primary Antibody (p53) and incubate for 45 minutes which is already diluted.
- h. Wash with 2 changes of IHC wash buffer each for 5 minutes.
- i. Cover tissue with polyDetector plus link and incubate for 15 minutes.
- j. Wash with 2 changes wash buffer each for 5 minutes.
- k. Cover tissue with polydetector HRP Label and incubate for 15 minutes.
- l. Wash with 2 changes of IHC wash buffer each for 5 minutes.
- m. One drop of polydetector DAB Chromogen should be added to one millilitre of polyDetector DAB buffer to prepare DAB.
- n. After preparing the DAB substrate-chromogen solution, cover the tissue and let it sit for 10 minutes.

- o. Rinse with 3 changes of IHC wash buffer each for 5 minutes.
- p. Counterstain with Mayer's hematoxyline for 2 minutes and then dehydrate.
- q. Coverslip.

**Quality control:** In parallel After every immunostaining set, sections designated as positive controls were processed. With every run, positive controls from breast cancer sections known to demonstrate ER and PR, respectively, were used.

**Negative control slides:** sections untreated with primary antibody (ER, PR).

**Scoring:** A golden brown color at the location of a particular cellular antigen localization (nucleus) indicates a positive stain. The percentage of positive nuclei among 100 was used to describe the quantitative assessment of PR. The following score was used:

- 0= Negative
- 1= Positivity < 15% of nuclei.
- 2= Positivity between 15 - 50% of the nuclei.
- 3= Positivity > 50 % of nuclei.

**Statistical analysis:** Version 26 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. The information was displayed as ranges, means, and standard deviations. Percentages and frequencies are used to present categorical data. The progesterone receptor status was correlated with specific information using the chi square test; in cases where the predicted frequency was less than five, the Fisher exact test was utilized instead. P-values less than 0.05 were regarded as significant at this level.

## RESULTS

In this study, mean age was  $56.84 \pm 9.9$  years; 66% of them were aged  $\geq 40$  years; 62% were females; meningioma was graded I in 58%; the most common location of meningioma was cerebrum (56%) and the most common histopathological type was meningothelial (36%).

Regarding progesterone receptor status, negative score was presented in 44% of cases while positive score (3) was presented in 22%. (Table 1).

Table 2 showed that 79.3% of patients with grade I meningioma and 83.3% of patients with meningothelial type of meningioma were showed positive progesterone receptor status with a significant association ( $P < 0.05$ ) between progesterone receptor status and both grades of histopathological type of meningioma. No significant associations ( $P \geq 0.05$ ) between progesterone receptor status with age, gender, and location of meningioma.

## DISCUSSION

Numerous studies have been conducted thus far to determine the function of the progesterone receptor in

**TABLE 1.** Distribution of study samples by certain characteristics

Variable	No. (n= 50)	Percentage (%)
<b>Age (Year)</b>		
< 40	<b>17</b>	<b>34.0</b>
$\geq 40$	33	66.0
<b>Gender</b>		
Male	19	38.0
Female	<b>31</b>	<b>62.0</b>
<b>Meningioma grade</b>		
I	<b>29</b>	<b>58.0</b>
II	13	26.0
III	<b>8</b>	<b>16.0</b>
<b>Meningioma location</b>		
Cerebral	<b>28</b>	<b>56.0</b>
Olfactory Groove	9	18.0
Orbital	<b>7</b>	<b>14.0</b>
Parasagittal	3	6.0
Spinal	<b>2</b>	<b>4.0</b>
Sphenoid	1	2.0
<b>Histopathological type of meningioma</b>		
Meningothelial	18	36.0
Angiomatous	<b>10</b>	<b>20.0</b>
Atypical	9	18.0
Anaplastic	<b>7</b>	<b>14.0</b>
Fibroblastic	3	6.0
Transitional	<b>3</b>	<b>6.0</b>
<b>Progesterone receptor status</b>		
Negative	<b>22</b>	<b>44.0</b>
+1	8	16.0
+2	<b>9</b>	<b>18.0</b>
+3	11	22.0

meningiomas and to evaluate their significance as predictive indicators for meningioma behavior due to the fact that it may help invention of progesterone receptor inhibitors that can be of therapeutic benefit to the patients [11,13].

In this study, it had been discovered that meningiomas have high expression levels of progesterone receptors. This was also evident in the fact that progesterone receptor positivity was present in 56% of the cases. This result is approaching to that obtained by Mnango L et al study in 2021 when 54.5% of cases were progesterone receptor positive [13]. Higher results were found in studies conducted by Mukherjee S et al in India (82.9%) [14] and Fakhrjou A et al in Iran (65%) [15]. The variations in the techniques used, the biology of the tumor, and the genetic constitution of the subjects involved in the various research could all be contributing factors to the variations in progesterone receptor expression observed across the studies. Research has indicated that formalin-fixed paraffin-embedded tis-

**TABLE 2.** Association between progesterone receptor status and certain characteristics

Variable	Progesterone receptor status		Total (%) n= 50	P - value
	Negative (%) n= 22	Positive (%) n= 28		
Age (Year)				
< 40	7 (41.2)	10 (58.8)	17 (34.0)	0.772
≥ 40	15 (45.5)	18 (54.5)	33 (66.0)	
Gender				
Male	8 (42.1)	11 (57.9)	19 (38.0)	0.832
Female	14 (45.2)	17 (54.8)	31 (62.0)	
Grade of meningioma				
I	6 (20.7)	23 (79.3)	29 (58.0)	0.001
II	9 (69.2)	4 (30.8)	13 (26.0)	
III	7 (87.5)	1 (12.5)	8 (16.0)	
Meningioma location				
Cerebral	11 (39.3)	17 (60.7)	28 (56.0)	0.831
Olfactory Groove	5 (55.6)	4 (44.4)	9 (18.0)	
Orbital	3 (42.9)	4 (57.1)	7 (14.0)	
Others	3 (42.9)	3 (50.0)	6 (12.0)	
Histopathological type of meningioma				
Meningothelial	3 (16.7)	15 (83.3)	18 (36.0)	0.002
Angiomatous	3 (30.0)	7 (70.0)	10 (20.0)	
Atypical	5 (55.6)	4 (44.4)	9 (18.0)	
Anaplastic	7 (100.0)	0 (0)	7 (14.0)	
Others	4 (66.7)	2 (33.3)	6 (12.0)	

sue blocks may become negative for immunohistochemical staining if they are delayedly fixed or stored for an extended period of time [16]. Consequently, the amount of immunohistochemistry antibodies, including progesterone receptors, is increased by promptly fixing the specimens and storing the tissue blocks for the best amount of time [13].

In this study, 79.3% of patients with grade I meningioma and 83.3% of patients with meningothelial type of meningioma were showed positive progesterone receptor status with a significant association ( $P < 0.05$ ) between progesterone receptor status and both of grade histopathological type of meningioma; while no significant associations between progesterone recep-

tor status with age, gender, and location of meningioma.

Regarding grade, similar findings observed in Mukhopadhyay M et al in 2017 [11] and Iplikcioglu AC et al in 2014 studies [17] when they discovered a markedly increased frequency of instances with low PR expression in atypical WHO II tumors and high expression in benign WHO I tumors. Although the exact cause of the association between progesterone receptor and meningioma grading is unknown, it is likely because progesterone receptor-deficient tumor cells undergo a higher rate of mitosis and cellular turnover, while progesterone receptor-positive tumor cells experience an increase in angiogenesis [18].

Concerning histopathological type, agreement achieved with Poniman J study in 2020 as more expression of progesterone receptor in meningiothelial type of meningioma [19]. Small levels of progesterone are usually expressed in non-neoplastic meningiothelial cells; these levels rise in cases of cell proliferation, such as meningioma, and fall in cases of cell differentiation alterations [20].

About age, it appears to be contradiction in the relationship between age and progesterone receptor expression in meningiomas. While some researches had shown a favorable correlation between progesterone receptor expression and age, other studies like ours have found no such correlation.

## CONCLUSION

High progesterone receptor positivity was linked to low grade tumors. Meningioma grade and many subtypes can be identified with the use of progesterone receptor expression, particularly in cases that are borderline.

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