Ref: Ro J Neurol. 2024;23(4)
DOI: 10.37897/RJN.2024.4.12

Effect of hormonal contraceptive exposure on hormonal receptor and proliferative index expression in meningioma patients

Yunni Diansari[1], Asfitriani[2], Rizka Aprillia Syahputri[1], Achmad Junaidi[1], Dya Anggraeni[1]

[1]Neurology Department, Mohammad Hoesin General Hospital, Universitas Sriwijaya, Palembang, Indonesia
[2]Patology Anatomy Department, Mohammad Hoesin General Hospital, Universitas Sriwijaya, Palembang, Indonesia

Yunni Diansari **ORCID ID:** 0000-0002-1251-7344

ABSTRACT

Background and objectives. Hormonal contraceptives remain one of the most common birth control medications among females in the general population. The use of hormonal contraceptives is believed to be one of the risk factors for meningioma. Research on meningioma has provided substantial evidence regarding the existence of hormone receptors. Our study was designed to clarify whether the expression of hormonal receptors and proliferative index is influenced by the exposure to hormonal contraceptives.

Materials and methods. This cross-sectional study was conducted at Mohammad Hoesin General Hospital, Palembang, Indonesia, from 2018 to 2023. The inclusion criteria encompassed meningioma patients with a history of exposure to hormonal contraceptives. Patient data collection was obtained from medical records and detailed interviews (reproductive status and history of hormonal contraceptive use). Data regarding patients' characteristics, histology type, and WHO grade tumors were extracted from the patients' clinical files. Immunohistochemistry studies were performed to evaluate the progesterone receptor expression, estrogen receptor expression, and Ki-67 index.

Results. A total of 51 patients were reviewed in our study, with a mean age of 46.88 ± 9.62 years. According to the histopathological classification, 84.3% had WHO grade I, 13.7% had grade II, and 2% had grade III. Ninety-eight percent (98%) of subjects had PR positive with strong staining. Only 3.9% of patients had strong staining in ER expression. For the proliferative index, most patients were in the range of ≤4%. There was no significant difference among the type, duration, and status of hormonal contraceptive use with the expression of hormonal receptors and the Ki-67 index.

Conclusions. Our findings suggest that exposure to hormonal contraceptives did not influence the expression of PR, ER, and Ki-67. However, we discovered a trend of higher PR expression in patients with a history of progesterone-only hormonal contraceptives.

Keywords: hormonal receptor, hormonal contraceptive, proliferative index

Abbreviations (in alphabetical order):

CTBRUS – Central Brain Tumor Registry in the United States
ER – estrogen receptors
IGF – insulin like growth factor
NF-2 – neurofibromatosis 2
PR – progesterone receptor

INTRODUCTION

Meningioma is known as a slow-growing tumor. Globally, epidemiological data indicates that meningiomas account for 14.3% to 19% of all primary intracranial neoplasms, with some reports suggesting that their prevalence may reach as high as 30% [1-4]. The Central Brain Tumor Registry of the United States (CBTRUS) identifies meningioma as the predominant

Corresponding author:
Yunni Diansari
E-mail: yunnidiansari@fk.unsri.ac.id

Article History:
Received: 30 November 2024
Accepted: 26 December 2024

primary brain tumor in the United States, with glioma ranking as the second most common [5]. Epidemiological data on meningioma in Indonesia remain largely underreported. However, data from our institution indicate that meningioma accounts for 77.1% of primary brain tumors, followed by glioma at 13.1% [6]. The majority of meningioma cases occur in adults and the elderly, with a significantly higher prevalence among women, comprising approximately two-thirds of all cases [2,3].

Several risk factors for meningioma have been established, including age, female sex, cranial irradiation, genetic, and hormonal factors. Epidemiological and pathological evidence highlights a pronounced female predominance in meningioma incidence, underscoring the potential involvement of sex hormones in the tumor's pathogenesis and progression [3,4,7]. Numerous studies have explored the association between hormonal and reproductive factors and the risk of meningioma. This connection is supported by several observations, including the higher prevalence of meningioma in women, tumor growth during pregnancy and regression postpartum, links to breast cancer, and the presence of hormonal receptors in approximately two-thirds of meningioma cases [3,4,7-9].

Research on meningioma has provided significant insights into the existence of hormonal receptors. Molecular and immunohistochemical studies have shown that progesterone receptors (PR) and estrogen receptors (ER) are expressed in meningioma to varying degrees [7,8,10]. The proliferation of human meningioma cell lines has been documented following exposure to estrogen and progesterone [3]. The use of hormonal contraceptives is considered a potential factor linking exogenous hormones to the development of meningiomas. This hypothesis arises from the presence of progesterone and estrogen receptors in meningioma cells, which, when activated by their respective hormones, may stimulate cell proliferation [8].

Hormonal contraceptives remain one of the most common birth control medications among females in the general population. Data from the National Health Survey in 2013 showed that 84.5% of contraceptive users were in the age range of 30-39 years, the age group that is the highest user of hormonal contraceptives in Indonesia [11]. Several previous studies have demonstrated that hormonal contraceptive exposure influenced the grading of meningioma [8,10]. Whether hormonal contraceptive exposure influences hormonal receptor expression is still debated. A study by Supartoto showed that exposure to progesterone-containing contraceptives increased progesterone receptors in meningioma [10]. While a few studies have attempted to correlate the role of hormonal contraceptives with hormonal receptors and the Ki-67 proliferative index, conclusions regarding the correlation have varied [9,10,12,13].

Our study aimed to investigate whether the expression profiles of hormonal receptors are influenced by exposure to hormonal contraceptives. Additionally, we examined the presence of sex hormone receptors and their correlation with cellular proliferation markers, such as Ki-67. These aspects present intriguing avenues for further research to deepen our understanding of the role of hormonal contraceptives in the pathogenesis of meningiomas.

This study was approved by the Health Research Ethics Committee of Mohammad Hoesin General Hospital Palembang No. DP.04.03/D.XVIII.6.11/ETIK/89/2023.

MATERIALS AND METHODS

This cross-sectional study was conducted at Mohammad Hoesin General Hospital, Palembang, Indonesia, by collecting all patients histopathologically confirmed to have meningioma from 2018 to 2023. The inclusion criteria encompassed meningioma patients with a history of exposure to hormonal contraceptives. The study excluded cases with incomplete data or paraffin blocks unsuitable for immunohistochemistry analysis. Additional exclusion criteria were a history of hormone replacement therapy, a family history of malignancy, and a history of cranial irradiation. The sample size was determined retrospectively by reviewing all available records.

Patient data collection was obtained from medical records and detailed interviews. All participants underwent in-person or telephone interviews to document their history of exposure to hormonal contraceptives and reproductive status. Data pertaining to the patients' demographic characteristics, histological classification, and WHO tumor grading were meticulously retrieved from the clinical records. PR, ER, and Ki-67 status were recorded after reporting the hematoxylin-eosin and immunohistochemistry-stained tissue slides, respectively. Patient characteristics, including age, history of marriage, reproductive factors (e.g., age of menarche, number of parities, menopause status, age of first birth), and history of exposure to contraceptives (the status of hormonal contraceptive use, type of hormonal contraceptives, and duration of use) were obtained using a standardized questionnaire.

The status of hormonal contraceptive use was classified as: (1) “current use”, when the contraceptives were still being used until the diagnosis of meningioma or ended in the year before the diagnosis of meningioma; (2) “past use”, when the most recent use was more than 1 year before the diagnosis of meningioma. The type of contraceptives was classified as: (1) “progesterone only”, and (2) “combined

progesterone plus estrogen". The duration of use was classified as: (1) "less than 5 years", (2) "5 to 10 years", (3) "more than 10 years".

Pathological specimens (all meningioma samples) were reviewed by a neuropathologist who had no previous knowledge about these patients. The tumors were classified according to the 2016 WHO classification system. The histopathological grade of the meningioma was also divided into two groups: the benign group included those with grade I, and the non-benign group included those with grades II and III. Immunohistochemical staining for ER, PR, and Ki-67 was performed on subjects and samples that met the criteria.

The immunohistochemistry studies were performed to evaluate the PR, ER, and Ki-67 index expression. Immunohistochemistry staining was performed using the Ventana BenchMark (ASSY) Gx Stainer machine. The specimens were fixed in neutral buffered 10% formalin, embedded in paraffin, and sectioned into 5 mm thickness. The expression of estrogen receptors (ER) was assessed using a monoclonal antibody (clone Sp1, Rabbit) from the Ventana brand and PR was determined by a progesterone receptor polyclonal antibody (clone 1E2, Rabbit) from the Ventana brand. Both the estrogen and progesterone antibodies used breast carcinoma specimens as positive controls. All slides were examined for positively stained tumor cell nuclei. Positive smears were characterized by brown staining (weak to strong intensity) on the nuclei (Figures 1, 2, 3).

Ki-67 was used to detect the proliferative index of meningioma. The expression of Ki-67 was evaluated in all specimens using the ready-to-use Ki-67 monoclonal antibody (clone 30-9, Rabbit) from the Ventana brand. Tonsil tissue was used as a Ki-67 positive control in this study. For Ki-67 staining, the proliferative index was determined as the percentage of positively stained cells among 1,000 tumor cells counted within the most mitotically active regions of the tumor. The values of Ki-67 were classified into four groups: group I ≤4%, group II 4.1-7%, group III 7.1-11%, and group IV >11%.

SPSS for Windows was used for statistical analysis. Categorical and continuous variables were summarized as percentages and means ± standard deviations, respectively. Fisher's exact test, Kolmogorov-Smirnov test, and Kruskal-Wallis test were used to determine the association between the history of contraceptives use with PR, ER, and Ki-67 expression. The associations were considered statistically significant when the p-value was <0.05.

RESULTS

A total of 51 patients were reviewed in our study from January 2018 to December 2023. The demographic distribution, reproductive status, and history of exposure to hormonal contraceptives are shown in Table 1. The subjects ranged in age from 32 to 67 years with a mean age of 46.88 ± 9.62 years. The mean age at menarche in our study was 13.17 ± 1.07 years,

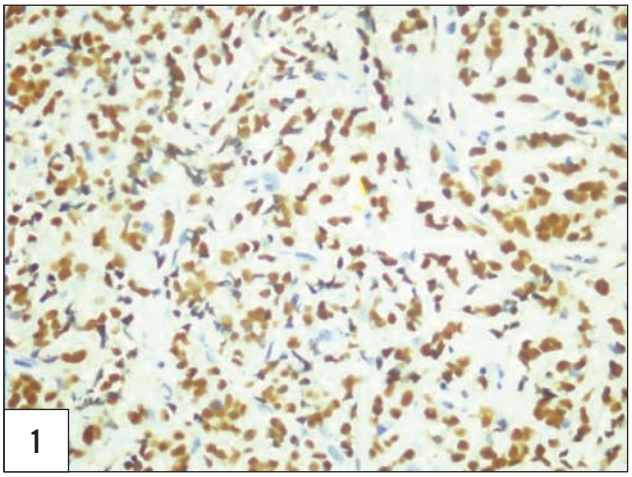

FIGURE 1. Immunohistochemistry stain positive of PR expression with strong intensity (400x)

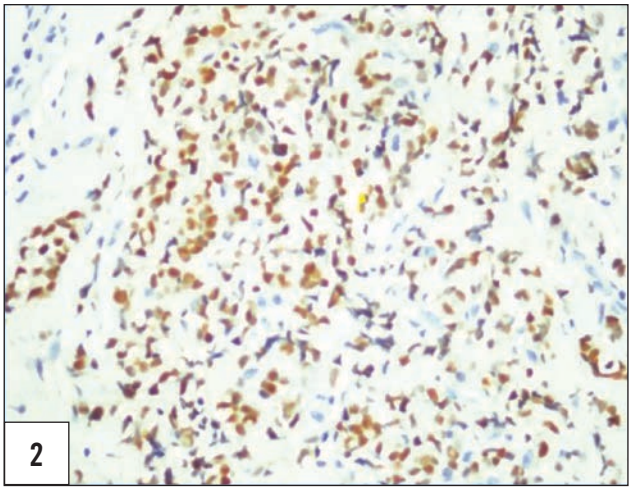

FIGURE 2. Immunohistochemistry stain positive of ER expression with strong intensity (400x)

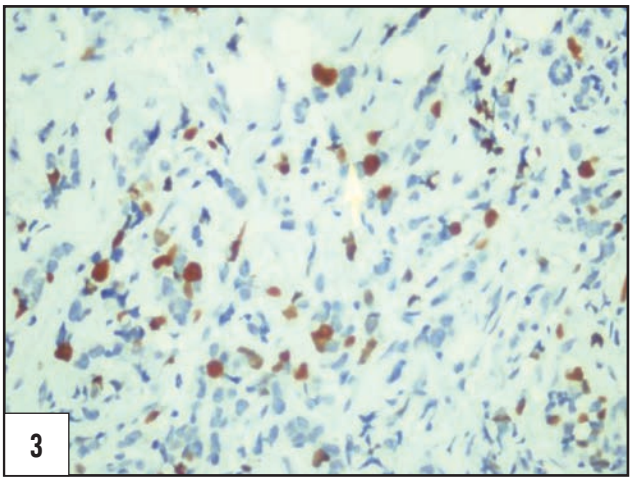

FIGURE 3. Immunohistochemistry stain of Ki-67 with high proliferative index (>11%)

TABLE 1. Demographic characteristics, reproductive status and history exposure of contraceptive

Characteristics	Frequency (n)	Percentage (%)
Demography		
Age (years)		
20-30	1	2
31-40	13	25.5
41-50	27	52.9
51-60	7	13.7
>60	3	5.9
Marital Status		
Marriage	5	100
Unmarriage		
Reproductive status		
Age of menarche (years)		
≤14	40	78.4
>14	11	21.6
Parity		
Nulliparaous	3	5.9
Multiparous	48	94.1
Aged of first use (years)		
≤20	30	58.8
>20	21	41.2
Menopause status		
Yes	12	23.5
No	39	76.5
History exposure		
Type of contraceptives		
Progesterone only	42	82.4
Combination	9	17.6
Duration of use (years)		
<5	6	11.8
5-10	7	13.7
>10	38	74.5
Contraceptive use status		
Past user	33	64.7
Current user	18	35.3

with the most prevalent parity being multiparous. Additionally, 76.5% of patients were of reproductive age. Based on the duration, most patients (74.5%) had used contraceptives for more than 10 years, and the combined contraceptive type was less frequent than progesterone-only.

According to the histopathological classification, 84.3% of meningioma patients had WHO grade I or benign. Moreover, 13.7% had WHO Grade II and 2% had Grade III, for a cumulative total of 15.7% in the non-benign group. The largest number (33.3%) of the meningothelial types were followed by transitional types (23.5%). Ninety-eight percent of the 51 subjects had PR positivity with strong staining, while ER was absent in 41.2%. There were only 3.9% of patients with strong staining in ER expression. Regarding the proliferative index, most patients were in the range of ≤4%. In contrast, only 3.9% of patients had a high proliferative index (Table 2).

Table 3 shows that the expression of PR was strong within the benign meningioma group and interestingly, no subject in the non-benign group revealed strong expression of ER. Among the PR positive cases, the majority exhibited strong staining while only one case was absent of PR expression (WHO grade III). Surprisingly, 87.5% of non-benign meningiomas had

TABLE 2. Histopathology and immunohistochemistry staining characteristics

Characteristics	Frequency (n)	Percentage (%)
Demography		
Histology type		
Metaplastic	7	13.7
Meningothelial	17	33.3
Choroid	4	7.8
Fibrous	3	5.9
Microcytic	1	2.0
Psammomatous	1	2.0
Atypical	3	5.9
Rhabdoid	1	2.0
En plaque	1	2.0
Angiomatous	1	2.0
Transitional	12	23.5
Histology grade		
WHO grade I	43	84.3
WHO grade II	7	13.7
WHO grade III	1	2.0
Estrogen Receptor (ER)		
Absent	21	41.2
Weak	18	35.3
Moderate	10	19.6
Strong	2	3.9
Progesterone Receptor (PR)		
Absent	1	2.0
Weak	0	0
Moderate	0	0
Strong	50	98.0
Ki-67 Index		
<4%	37	72.5
4.1-7%	8	15.7
7.1-11%	4	7.8
> 11%	2	3.9

TABLE 3. Association between hormonal receptor expression, proliferation index, and grading of meningioma

Immunohistochemistry staining	Benign (n=43)	Non-Benign (n=8)	P-value
Progesterone Receptor (PR)			
Absent	0 (0)	1 (12.5)	
Weak	0 (0)	0 (0)	0.157*
Moderate	0 (0)	0 (0)	
Strong	43 (100)	7 (87.5)	
Estrogen Receptor (ER)			
Absent	17 (39.5)	4 (50)	
Weak	16 (37.2)	2 (25)	1.000**
Moderate	8 (18.6)	2 (25)	
Strong	2 (4.7)	0 (0)	
Ki-67 Index			
0-4%	32 (74.5)	5 (62.5)	
4.1-7%	6 (13.9)	2 (25)	1.000**
7.1-11%	4 (9.3)	0 (0)	
>11%	1 (2.3)	1 (12.5)	

**Kolmogorov-Smirnov, ** Fisher's exact test*

TABLE 4. Association between exposure history of contraceptive hormonal, hormonal receptor expression, and proliferative index

Characteristics	Progesterone Receptor (PR)				P-value
	Absent (n = 1)	Weak (n = 0)	Moderate (n = 0)	Strong (n = 50)	
Type of contraceptives					
Progesterone only	1 (2.4)	0 (0)	0 (0)	41 (97.6)	
Combination	0 (0)	0 (0)	0 (0)	9 (100)	1.000**
Duration of use (years)					
<5	0 (0)	0 (0)	0 (0)	6 (100)	0.843**
5-10	0 (0)	0 (0)	0 (0)	7 (100)	
>10	1 (2.6)	0 (0)	0 (0)	37 (97.4)	
Contraceptive use status					
Past user	1(3.0)	0 (0)	0 (0)	32 (97.0)	1.000**
Current user	0(0)	0 (0)	0 (0)	18(100)	
	Estrogen Receptor (ER)				
	Absent (n = 21)	Weak (n = 18)	Moderate (n = 10)	Strong (n = 2)	
Type of contraceptives					
Progesterone only	18 (42.9)	15 (35.7)	8 (19)	1 (2.4)	
Combination	3 (33.3)	3 (33.3)	2 (22.2)	1 (11.1)	1.000**
Duration of use (years)					
<5	2 (33.3)	3 (50)	1 (16.7)	0 (0)	0.492**
5-10	1 (14.3)	4 (57.1)	2 (28.6)	0 (0)	
>10	18 (47.4)	11 (28.9)	7 (18.4)	2 (5.3)	
Contraceptive use status					
Past user	12 (36.4)	15 (45.5)	4 (12.1)	2 (6.1)	0.952***
Current user	9 (50.0)	3 (16.7)	6 (33.3)	0 (0)	
	Ki-67 Index				
	0-4% (n = 37)	4.1-7% (n = 8)	7.1-11% (n = 4)	>11% (n = 2)	
Type of contraceptives					
Progesterone only	32 (76.2)	7 (16.7)	2 (4.8)	1 (2.4)	0.689***
Combination	5 (55.6)	1 (11.1)	2 (22.2)	1 (11.1)	
Duration of use (years)					
<5	3 (50.0)	2 (33.3)	0 (0)	1 (16.7)	0.133**
5-10	7 (100)	0 (0)	0 (0)	0 (0)	
>10	27 (71.1)	6 (15.8)	4 (10.5)	1 (2.6)	
Contraceptive use status					
Past user	23 (69.7)	6 (18.2)	3 (9.1)	1 (3.0)	1.000**
Current user	14 (77.8)	2 (11.1)	1 (5.6)	1 (5.6)	

Fisher's exact test, **Kruskal-Wallis, * Kolmogorov-Smirnov*

strong expression of PR. We also performed immunohistochemistry for the Ki-67 index, and the majority of the benign group had Ki-67 expression <4%. There was one subject in both the benign and non-benign groups that expressed a high proliferative index with a Ki-67 level of >11%. The Ki-67 index was only significantly high in 1 of 8 subjects in the non-benign group. Statistical analysis revealed that there was no significant relationship between the grading of meningioma and the expression of hormonal receptors and the proliferative index.

Both types of hormonal contraceptives (progesterone-only and combination) had similarly strong expression of PR (Table 4). Conversely, the expression of ER was absent in 42.9% of subjects using progesterone-only contraceptives. In the combined contraceptive group, the expression of ER was predominantly absent. ER expression was only strong in 2 subjects with more than 10 years of contraceptive use. There was no significant relationship among the type, duration, and status of contraceptive use and the expression of hormonal receptors. However, quantitatively, it was found that there was an increase in the number of patients using progesterone-only contraceptives (89.1%) in cases of meningioma with a benign histopathological degree (data not shown). We also analyzed the association between Ki-67 expression with the type, duration, and status of contraceptive use, and the results showed no significant difference.

DISCUSSION

Sex hormones in both men and women play a crucial role in the etiopathogenesis and development of cancers in various organs, including the endometrium, breast, prostate, lung, and brain. Their contribution to cancer risk primarily involves influencing the rate and frequency of mitosis in epithelial cells within these organs [3,4]. In the case of meningioma, the predominance of the female sex is thought to suggest that hormonal factors contribute to the growth of meningioma, whether they are from the inside (endogenous hormone) or outside (exogenous hormone). The presence of hormonal receptors on meningioma tumor cells also raises the possibility of the relationship between meningiomas and sex steroids. Progesterone and estrogen jointly have the potential to stimulate the proliferation of meningioma cells. This is supported by findings that meningioma cell lines exhibit increased proliferation when exposed to these hormones, providing both molecular and physiological evidence for the role of sex steroid hormones in the development and growth of meningiomas [3,10,15].

Some researchers have described that the use of exogenous hormones increases the risk of meningioma [4,7,14,16-18]. Nevertheless, the role of estro-

gen and progesterone hormones is still not fully understood in meningiomas. Hormonal contraceptives containing progesterone and estrogen are widely utilized by women for birth control. Several studies have investigated the potential link between their use and the risk of meningiomas. However, the findings from these studies remain inconsistent and inconclusive to date [10,14-16,18,19].

Few studies have been conducted so far to investigate the role of ER, PR, and Ki-67 in meningiomas and their correlation with hormonal contraceptive exposure [10,15,19]. In our study, the mean age was 46.88 ± 9.62 years. The previous findings indicated that patients with meningiomas were more prevalent in the older age group while the prevalence of meningiomas gradually rises with age , peaking during the sixth and seventh decades [2,5,7,9]. This variation may be attributed to earlier exposure to oncogenes or substances that could trigger the development of meningiomas. Our study found that the initial use of contraceptive hormones was more common among younger individuals. In Indonesia, there has been an increasing trend in the use of hormonal contraceptives among younger women, with injectable contraceptives being the most commonly used method. In contrast, in the United States, contraceptive use is predominantly seen in women aged 40-49 years, with non-hormonal methods, such as sterilization, being the most frequently used [11,20]. This suggests a heightened and earlier exposure to exogenous hormones in Indonesian women compared to Americans. The early exposure to hormonal contraceptives might alter the function of suppressor genes such as Merlin, leading to oncogenesis [7,16].

Although there is a general agreement that the majority of meningiomas contain PR and lack ER, the biological function of these hormonal receptors and their molecular basis remain poorly understood [8]. The histopathological distribution of meningioma patients in this study was in line with other studies where the majority of patients had meningioma grade I [5,7,22,23]. PR has been found to be highly expressed in meningiomas, and this was also reflected in the present study, where the majority (98%) of the cases were PR positive. In this study, we found that 86% of PR positive cases were benign meningiomas, while 12.5% of non-benign meningiomas lacked PR expression. There were 58.8% of subjects that had ER, but only 3.9% showed strong expression of ER. Interestingly, strong ER expression was found exclusively in the benign group, whereas, the non-benign group showed 50% ER expression.

However, several previous studies have reported varying findings. The prevalence of progesterone receptor (PR) and estrogen receptor (ER) expression in meningioma patients has been shown to be influenced by various clinicopathologic factors, particularly tumor grade, as classified by the WHO. This could lead to inconsistent expression of hormone receptors across different tumor grades, and even within the same grade and among different individuals. For example, Agopiantz et al. reported 76.8% of grade I meningiomas expressed PR compared to 61.2% of grade II and 17.3% of grade III meningiomas. ER was found in 8.7%, 1.6%, and 6.8% of grade I, II, and III meningiomas, respectively [4]. A study by Deghan et al. showed that the level of expression varied from zero to 80% in grade I meningioma [7]. A study by Shanthi et al. found that none of their patients were ER-positive [21]. However, in the study by Mukhopadhyay et al., 3.66% of grade I meningiomas and all grade II cases were positive for ER [22]. The variation in the expression of PR and ER across studies may stem from several factors, including differences in methodological approaches, tumor biology, and the genetic constitution of the individuals included in the respective studies. However, the differences in the PR and ER expression regarding the WHO grades of the meningioma patients in our study were not significant. A similar finding was also reported by Portet et al. [8].

This present study reviewed the detailed history of exposure to hormonal contraceptives and the expression of hormonal receptors. Among individuals with strong PR expression, a higher prevalence (82.0%) was observed in those using progesterone-only hormonal contraceptives. Similarly, a greater prevalence (74%) was noted in users of long-term contraceptives (>10 years). Interestingly, a notable proportion (64%) of past users also exhibited strong PR expression. Although these findings did not reach statistical significance, the observed trend suggests a possible association between prolonged progesterone exposure and receptor activation in the development of meningiomas.

Similar results were described by several studies, indicating that the duration, type, and status of hormonal contraceptive use did not significantly correlate with PR expression [2,14,16]. This finding is not consistent with the study by Portet et al., which reported that meningiomas developed with cyproterone acetate (progestin agonist) were mostly PR positive with 97% positivity [8]. A study by Supartoto showed that the longer the exposure to exogenous progesterone injection, the lower the expression of PR [10]. Agopiantz et al. found that 89% of meningiomas that developed under hormonal treatment expressed progesterone receptors compared to 71% of meningiomas that developed without any form of hormonal treatment or exposure. This study also showed 6% of meningiomas developed under hormonal treatment expressed estrogen compared to 11% of meningiomas developed without any hormonal exposure [4].

These discrepancies in the results of these studies could be attributed to variations in the characteristics of the study subjects, differences in data collection methods (e.g., direct interviews versus medical record reviews), the techniques used for hormonal receptor analysis, or genetic factors. Tumor biology has been reported to be influenced by genetic composition, which is often determined by an individual's race and geographic location, further contributing to the variability in findings [23].

The precise mechanisms linking prolonged exposure to exogenous hormones and the development of meningiomas in females remain inadequately elucidated. Interestingly, meningiomas do not exhibit enlargement during the proliferative phase of the menstrual cycle, when estrogen levels are elevated. In contrast, these tumors have been observed to grow during the second and third trimesters of pregnancy, as well as during the luteal phase of the menstrual cycle, when circulating progestins are elevated. This suggests that progesterone may play a role in the growth of meningiomas [3,17]. There is evidence suggesting that long-term exposure to exogenous progesterone may alter *in vivo* progesterone levels and modulate the expression of progesterone receptors, potentially exerting a broader impact at the genetic level. Specifically, this alteration may influence the Merlin protein, encoded by the Neurofibromatosis 2 (NF-2) gene, which plays a critical role in regulating the growth of neuronal and meningeal tissues. Such changes could impair the tumor-suppressor function of Merlin, particularly within meningeal tissue, thereby promoting tissue overgrowth. Additionally, estrogens have been implicated in promoting genomic instability in cells. Estrogens also interact with insulin-like growth factor (IGF), which not only stimulates tumor growth but also inhibits apoptosis, further contributing to tumor progression [17,24].

Ki-67 is a nuclear non-histone protein expressed during the active phases of the cell cycle, including the G1, S, G2, and M phases, but it is absent in resting cells (G0 phase). Its expression serves as a marker of cell proliferation, indicating ongoing cell division [7,22]. As a marker of cell proliferation, it reflects the number of mitoses, while the value of Ki-67 aligns with the histology grading of meningioma based on several studies [22,23,25]. The relationship between Ki-67 expression, the grading of meningioma, and hormonal receptors has long been established. Benign tumors are typically associated with a low proliferative index, high PR positivity, and ER negativity. Meningiomas that have a higher proliferative index and PR negativity are more likely to be classified as Grade II or Grade III. Some literature suggests that benign meningiomas with high PR expression usually have a low proliferative index. However, whether exposure to hormonal contraceptives correlates with the proliferative index remains an area to be explored and established.

Our study found that the majority of benign group meningiomas had Ki-67 below 4%. Interestingly, 62.5% of the non-benign group had Ki-67 less than 4%. This result is consistent with the results of a previous study [7,25]. A study by Shahane revealed 85.33% of subjects with a Ki-67 index below 4% were largely grade I, 13.33% had Ki-67 in the range of 5-10% and were mainly grade II, and only 1 patient had a Ki-67 index of 14%, which was a grade III tumor [9]. A different result was found in a study by Telugu et al. that revealed a mean Ki-67 index in different grades of meningioma (grade I, 3.1%, grade II, 7%, and grade III, 14.2%) [23]. Another study showed that all grade I meningioma cases had a Ki-67 expression less than 4% [7]. This present series showed no significant difference in the mean value of Ki-67 between the benign group (1.39 ± 0.76) and the non-benign group (1.63 ± 1.06). Previous studies have shown contradictory results [7,12,13,22,23].

To our knowledge, no prior study has investigated the correlation between the proliferative index and a history of exposure to hormonal contraceptives. This present study found that the majority (72.45%) of subjects with Ki-67 below 4% had a history of progesterone-only contraceptives. Interestingly, Ki-67 indices above 7% were observed in 7.2% of patients with a history of progesterone-only contraceptives and 33.3% with a combined contraceptive. We also revealed that benign meningioma subjects with a low proliferative index mostly used progesterone-only contraceptives. However, statistical analysis showed no significant difference between the type, duration , and status of hormonal contraceptive use with the expression of the proliferative index.

Limitations

Our study's strengths include the use of comprehensive interviews to minimize recall bias. However, several limitations are evident. First, the study included a relatively small sample size due to the application of strict eligibility criteria. Second, the majority of our patients used hormonal contraceptives containing only progesterone. This uneven distribution of subjects across the variables may introduce bias into the study findings. Additionally, our study did not account for potential confounding factors such as genetic predispositions, comorbidities, or lifestyle factors (e.g., obesity, smoking). These factors could influence meningioma development and progression and should be considered in future studies. Our relatively small sample size and uneven distribution of progesterone-only versus combined contraceptive users may limit the generalizability of our findings.

Future studies should incorporate a larger, more diverse patient population to enhance generalizability. A longitudinal study design is recommended to better evaluate causal relationships. Incorporating a matched control group without hormonal contraceptive exposure would provide a comparative baseline to clarify the impact of exogenous hormones. Further subgroup analyses for PR expression trends across different contraceptive types and durations are needed to understand the underlying biological mechanisms.

CONCLUSION

Our findings suggest that exposure to hormonal contraceptives does not influence the expression of PR, ER, and Ki-67. However, we discovered that there is a higher trend of PR expression in patients with a history of progesterone-only hormonal contraceptives. Additionally, our study reveals that Ki-67 is not significantly different among different grades of meningioma. Further studies to investigate the impact of hormonal contraceptives on other biological markers at the molecular or genetic level are strongly needed to clarify the mechanisms.

Authors' contributions:
Conceptualization, Y.D., A., D.A.; methodology, Y.D. and R.S.; software, R.S.; validation, Y.D.; formal analysis, Y.D. and R.S.; investigation, D.A. and A.J.; resources, Y.D., D.A., and A.; data curation, Y.D. and D.A.; writing—original draft preparation, Y.D. and R.S.; writing—review and editing, Y.D. and R.S.; visualization, A. and Y.D.; supervision, Y.D. and A.; project administration, Y.D. and A.J.; funding acquisition, Y.D. and A.J. All authors have read and agreed to the published version of the manuscript.

Ethical clearance:
This study was approved by Health Research Ethics Committee of Mohammad Hoesin General Hospital Palembang, register under No .04.03/D.XVIII.6.11/ETIK/89/2023

Financial support:
This study was funded by Research Grant from Mohammad Hoesin General Hospital.

Conflict of interest:
The authors declare that they do not have any financial or personal relationships that might bias the content of this work.

REFERENCES

1. Greenberg MS. Handbook of neurosurgery. 9th ed. New York: Thieme, 2019:707-16.
2. Yang CC, Tsai CC, Chen SJ, Chiang MF, Lin JF, Hu CK, et al. Factors associated with recurrence of intracranial meningiomas after surgical resection: A retrospective single-center study. *Int J Gerontol.* 2018;12(1):57-61 doi: 10.1016/j.ijge.2017.02.002.
3. Ichwan S, Santoso F, Aman RA, Tandian D, Fachniadin A, Nugroho SW. Estrogen and progesterone in meningioma: Bridging the gap of knowledge. *Neurology Asia.* 2023;28(1):1– 11. doi: 10.54029/2023wxj.
4. Agopiantz M, Carnot M, Denis C, Martin E, Gauchotte G. Hormone receptor expression in meningiomas: A systematic review. *Cancers*. 2023;15(3):980. doi: 10.3390/cancers15030980.
5. Duong LM, McCarthy BJ, McLendon RE, Dolecek TA, Kruchko C, Douglas LL, et al. Descriptive epidemiology of malignant and nonmalignant primary spinal cord, spinal meninges, and cauda equina tumors, United States, 2004–2007. *Cancer*. 2012; 118(17):4220-7. doi: 10.1002/cncr.27390.
6. Diansari Y, Syafiera H, Anggraeni D, Asfitriani. Karakteristik tumor susunan saraf pusat di RSUP DR Mohammad Hoesin Palembang. *Jurnal Kedokteran dan Kesehatan*. 2022; 9(2):123-132. doi: 10.32539/JKK.V9I2.16938.
7. Dehghan A, Askari K, Basir HG, Jiriaei N. Investigating the expression of ER, PR, and Ki-67 in different grades of meningioma. *J Adv Zool* 2023;44(6):1219-1226. doi: 10.53555/jaz.v44iS6.2425.
8. Portet S, Banor T, Bousquet J, Simonneau A, Flores M, Ingrand P, et al. New insights into expression of hormonal receptors by meningiomas. *World Neurosurg.* 2020;140:87-96. doi: 10.1016/j.wneu.2020.04.168.
9. Shahane SN. Estrogen and progesterone receptor status in meningioma and its correlation with mib-1 labeling index. *Indian J Neurosci.* 2019;5(4):232-235. doi: 10.18231/j.ijn.2019.038.
10. Supartoto A, Sasongko MB, Respatika D, Mahayana IT, Pawiroranu S, Kusnanto H, et al. Relationships between neurofibromatosis-2, progesterone receptor expression, the use of exogenous progesterone, and risk of orbitocranial meningioma in females. *Front Oncol.* 2019;8:651. doi: 10.3389/fonc.2018.00651.
11. Riset Kesehatan Dasar. Tingkat pencapaian KB. Departemen Kesehatan Republik Indonesia tahun 2013. Jakarta.
12. Janah R, Rujito L, Wahyono DJ. Expressions of progesterone receptor of orbital meningiomas in Indonesia. *Asian Pac J Cancer.* 2022; 23 (12): 4137-43. doi: 10.31557/APJCP.2022.23.12.4137.
13. Rejeki DS, Agus S, Syafrita Y. Hubungan ekspresi reseptor progesteron dan Ki-67 labeling index. *Majalah Patologi.* 2019;28(3):7-13.
14. Hoisnard L, Laanani M, Passeri T, Duranteau L, Coste J, Zureik M, et al. Risk of intracranial meningioma with three potent progestogens: A population-based case-control study. *Eur J Neurol.* 2022;29(9)2801–2809. doi: 10.1111/ene.15423.
15. Nugroho SW, Istianggoro H, Santoso F, Tandian D, Aman RA, Eka Susanto E. Characteristics and correlation between progesterone receptors and the use of hormonal contraceptive in women with meningioma in dr. Cipto Mangunkusumo National General Hospital. *JKI.* 2022; 10(2):101-6. doi: 10.23886/ejki.10.161.101-6.
16. Wahyuhadi J, Heryani D, Basuki H. Risk of meningioma associated with exposure of hormonal contraception. A case control study. *Maj Obs Gin.* 2018;26(1):36-41. doi: 10.20473/mog.V26112018.36-41.
17. Hortobágyi T, Bencze J, Murnyák B, Kouhsari MC, Bognár L, Marko-Varga G. Pathophysiology of meningioma growth in pregnancy. *Open Med.* 2017;12(1):195-200. doi: 10.1515/med-2017-0029.
18. Samarut E, Lugat A, Amelot A, Scharbarg E, Hadjadj S, Primot C, et al. Meningiomas and cyproterone acetate: a retrospective, monocentric cohort of 388 patients treated by surgery or radiotherapy for intracranial meningioma. *J Neurooncol.* 2022;152(1):115-123. doi: 10.1007/s11060-020-03683-6.
19. Dustur S, Wahyuhadi J, Utomo B, Parenrengi MA, Bajamal AH, Dwiningsih SR. Relationship histopathology grading of meningioma with the use of medroxyprogesterone acetate (MPA) as a hormonal contraceptive. *Pharmacogn J.* 2022;14(6):938-41. doi: 10.5530/pj.2022.14.193.

20. Zaen NA. The relationship between age, education level, and residential area towards contraceptive use in married women in Indonesia. *JBK.* 2022;11(1):1–10. doi: 10.20473/jbk.v11i1.2022.1-10.
21. Shanthi V, Grandhi B, Rao NM, Rao BS, Reddy VM. Assessing the prognostic importance of ER, PR expression in meningiomas by comparing with proliferative rate using Ki-67. *Indian J Pathol Res Pract*. 2017;6(2):431-4. doi: 10.21088/ijprp.2278.148X.62(pt-II)17.16.
22. Mukhopadhyay M, Das C, Kumari M, Sen A, Mukhopadhyay B, Mukhopadhyay B. Spectrum of meningioma with special reference to prognostic utility of ER,PR and Ki-67 expression. *J Lab Physicians.* 2017;9:308-13. doi: 10.4103/JLP.JLP_158_16.
23. Telugu RB, Chowhan AK, Rukmangadha N, Patnayak R, Phaneendra BV, Prasad BCM, et al. Histopathological and immunohistochemical evaluation of meningiomas with reference to proliferative markers p53 and Ki-67. *J Clin Diagn Res.* 2016;10(1):15-9. doi: 10.7860/JCDR/2016/15661.7117.
24. Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis*. 2000:21;427433. doi: 10.1093/carcin/21.3.427.
25. Rajeshwari B, Shanmugam S, Niamath S, Ghosh M, Ghosh S. Hormone receptor status and KI67 proliferation index in meningiomas. *Ann Pathol Lab Med.* 2020;7(2):76-82. doi: 10.21276/apalm.2641.