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KI67 IMMUNOSTAINING'S DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE IN GESTATIONAL TROPHOBLASTIC DISORDERS: A RETROSPECTIVE STUDY.

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ABSTRACT:

BACKGROUND: Gestational trophoblastic diseases GTDs are rare gynecological disorders caused by abnormal trophoblastic progression. Subtypes include complete moles, partial moles, and hydropic abortions frequently share physical characteristics, challenging diagnosis. Ki67, a nuclear proliferation marker, has emerged as a viable tool for improving diagnostic accuracy and determining prognosis. **OBJECTIVE:** The aim of this study is to examine the diagnostic and prognostic impact of Ki67 immunostaining for classifying GTD subtypes and predicting high-risk patients, such as persistent trophoblastic disease PTD and choriocarcinoma. **METHODOLOGY:** A retrospective descriptive study was carried out from January to December 2023 in Lady Reading Hospital, Peshawar. In terms of 1,012 placental biopsy samples, 197 19.5% were diagnosed with GTDs, comprising 150 complete moles, 45 partial moles, and two unusual cases of choriocarcinoma and enhanced placental site reactivity. Fifty cases were chosen for Ki67 immunostaining. Staining intensity 0 to 3+ and extent 0 to 4+ were evaluated and classified, and statistical analyses were done to link findings to clinical outcomes. **RESULTS:** In 85% of cases complete moles revealed high 3+ Ki67 staining, with 70% showing a diffuse >50% extent. Partial moles showed moderate 2+ staining in 33.3% cases. However, hydropic abortions were primarily negative. All PTD and choriocarcinoma cases had significant and diffused Ki67 staining. **CONCLUSION:** Ki67 immunostaining is an effective technique for distinguishing GTD subtypes and detecting high-risk cases. Its integration into standard diagnostic techniques, combined with supporting markers, has the potential to improve GTD management, particularly in resource-constrained settings.

KEYWORDS: Gestational trophoblastic disease, Ki67 expression, Molar pregnancy, Complete hydatidiform mole, Immunohistochemistry.

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INTRODUCTION

Gestational trophoblastic diseases GTDs are an emerging but clinically significant group of gynecological disorders characterized by abnormal proliferation of placental trophoblastic tissue. These diseases include hydatidiform moles a whole or partial, invasive moles, placental-site trophoblastic malignancies, and choriocarcinoma[1]. GTDs distinguish themselves from other gynecological malignancies due to their prenatal origin, high chemosensitivity, and significant potential for improvement if detected and treated early. However, late detection or misdiagnosis can lead to life-threatening effects such as persistent trophoblastic disease PTD and metastatic choriocarcinoma[2].

GTDs account for less than 1% of gynecological malignancies, but their frequency varies immensely by geography and ethnicity. The United Kingdom recently reported the incidence of hydatidiform moles at 1-3 per 1,000 pregnancies. However, the ratio in Southeast Asia and Japan is significantly higher, at 2-10 per 1,000 pregnancies[3]. Current studies conducted in Pakistan indicate an even higher prevalence and promote such variables as early marriages, high parity, and socioeconomic challenges that constrain access to equity-based healthcare and follow-up[4]. Behind these variations, all GTDs have to be a straight challenge accurately differentiating their subtypes, which is critical for maintaining accurate prognosis and guiding clinical management[5].

Histopathological analysis is now considered a gold standard for diagnosing GTDs. However, the diagnostic process is more often complicated because of overlapping morphological features, which particularly occurs in early molar pregnancies. In such cases, partial moles may show hydropic abortions, while incomplete molar characteristics in the early phase may be misclassified as aborted [6]. Mostly showed misdiagnosed or may undermine its clinical outcomes, sometime complete moles often put a person at higher risk of disease progression to PTD or choriocarcinoma compared to partial moles or hydropic abortions[7,8].

To address such limitations in this study, immunohistochemistry markers like Ki67 have been highly put into follow-up as supplemental diagnostic techniques. On evidence-based analysis, Ki67 is an essential protein that is exclusively involved with cell proliferation. The cell structure may lie in active phases—G1, S, G2, if mitosis— to show its expression, however, quiescent G0 cells may not.[9]. Ki67 expression is crucial for evaluating trophoblast proliferative activity. In gestational trophoblastic diseases GTDs, Ki67 staining effectively distinguishes complete moles, which have high proliferative activity, from partial moles and hydropic abortions, which show moderate to minimal activity. This highlights its importance in clinical diagnostics and treatment.[10].

According to few studies the current prognostic expression of Ki67 is not updated, many studies likely reported that a higher Ki67 expression is related to more serious disease behavior and an increased risk of GTD progression to PTD or metastatic choriocarcinoma.[11,12]. Persistent trophoblastic disease has the potential for minor complications of complete moles, it may affect 15-20% of cases and is associated with a significant risk of cancer if early detection and prompt

treatment have failed. Ki67's capacity to predict such outcomes makes it an important biomarker for diagnosis, risk identification, and management planning[13]. The purpose of conducting this retrospective comparative study is to examine the diagnostic and prognostic use of Ki67 immunostaining in GTDs. Its emphasis is on its potential to differentiate its subtypes and predict disease progression. During these five-year retrospective studies, Ki67 expression patterns were identified in cases of hydatidiform moles complete and partial, hydropic abortions, and other trophoblastic disorders. By binding these findings with morphological assessments and clinical outcomes, this study intends to provide a brief view of Ki67's relevance in improving diagnostic accuracy and guiding prognosis in GTDs.

A combination of Ki67 immunostaining with routine diagnostic protocols has the potential to improve the early detection and management of GTDs, simultaneously reducing morbidity and mortality associated with these conditions. Gestational trophoblastic diseases GTDs a combination of few but clinically massive disorders caused by ultimate trophoblastic growth. Eaerly diagnosis of GTD subtypes, such as complete moles, partial moles, and hydropic abortions, is critical to prescribe suitable treatment and avoiding any edverse consequences including persistent trophoblastic disease PTD or choriocarcinoma. However, managing morphological features, especially in early-stage detection, can confuse the diagnostic process. The current gold standard, histopathological examination, may not be accurate but enough to consistently identify these subtypes. Ki67, a nuclear proliferation marker, has emerged as a useful tool in classical histology because of its capacity to measure cellular proliferation. Elevated Ki67 expression is associated to increased trophoblastic activity and cancer risk, making it a potential diagnostic and prognostic marker. This study assesses the effectiveness of Ki67 immunostaining in distinguishing GTD subtypes and identifying high-risk cases. This study intends to overcome diagnostic complications and improve patient outcomes by combining morphological and immunohistochemical insights, especially in resource-limited settings. The insights gained from this research could inform people about the development of standardized diagnostic and prognostic frameworks for GTDs, particularly in regions with high disease prevalence.

METHODOLOGY

This retrospective descriptive study was conducted at the Department of Pathology, Lady Reading Hospital, Peshawar, Pakistan from January to December 2023. The pathology reports of biopsy specimens acquired from LRH Gynecology Department and related obstetric care institutions were analyzed. Patients with molar pregnancies, hydropic abortions or associated trophoblastic neoplasms were included in the study. In order to contain interobserver variability, professional pathologists reevaluated hematoxylin and eosin H&E stained sections to confirm diagnosis. The study compared Ki67 expression patterns in entire moles, partial moles, hydropic abortions, and cases of persistent trophoblastic illness or choriocarcinoma. The ethical approval was taken from ethical review committee with Ref No. 617/LRH/MTI on dated 30th December 2022.

A total of 956 placental biopsies submitted during the study period were reviewed. Among these, 197 cases 19.5% were diagnosed as GTDs, including 150 complete moles, 45 partial moles, and 2 other rare conditions choriocarcinoma and exaggerated placental site reaction. For immunohistochemistry testing, 50 archive cases were chosen, including 20 complete moles, 15 partial moles, and 15 hydropic abortions. Cases were chosen according to the availability of well-preserved paraffin-embedded tissues. The

sample size for this study was calculated using statistical methods to ensure adequate representation of all GTD subtypes and significant comparisons between groups. The formula n was used to determine the prevalence of GTDs using available data and a 95% confidence level. Here, Z is the z-score 1.96 with 95% confidence, p is the expected proportion of GTDs in the population, and e is the margin of error 5%. Adjustments were made to stratified samples to ensure proportional representation of subtypes such complete moles, half moles, and hydropic abortions. The total calculated sample size was sufficient to retain 80% statistical power for detecting significant variations in Ki67 expression between subtypes.

Initial morphological study involved cutting 5 µm thick slices and staining them with hematoxylin and eosin. Ki67 immunostaining was performed using a monoclonal rabbit antibody and the HiDef detection system. The intensity of staining 0 to +++ and extent of staining 0 to 4+ were determined, and an additive rapid score was produced to divide cases into diagnostic categories.

- **Category 1:** Ki67-negative score 0–2.
- **Category 2:** Moderate positivity score 3–4.
- **Category 3:** Strong positivity score 5–6.

Data extraction from the histopathological and immunohistochemical examination were meticulously recorded and analyzed using SPSS version 22. For each case, the variation and result of Ki67 immunostaining were categorized and quantified. In addition, a quick score method combined staining intensity and extent, resulting in a comprehensive classification.

The data were analyzed via descriptive statistics to assess the incident ratio and distribution of GTD subtypes such as complete moles, partial moles, hydropic abortions, and cases of chronic trophoblastic diseases or choriocarcinoma. It varies from demographic trends and mean and standard deviation number of patient age were also determined across all subtypes. The connection between Ki67 immunostaining patterns and GTD subtypes was analyzed while using comparative statistical tests such as chi-squared tests. Furthermore, regression analysis was applied to clarify the relationship between high Ki67 values and clinical outcomes.

While obtaining such useful results, two experts independently evaluate all histopathological and immunohistochemical evaluations. Any kind of bias during the evaluation was solved via consensus. Data was interpreted through tables and figures to highlight main findings, such as the difference in Ki67 expression across all GTD subtypes. The main findings are also the frequency distribution of GTDs, Ki67's diagnostic process while distinguishing its subtypes, and its prognostic value in predicting high-risk cases.

The data of all required patients were kept confidential, and only the identified samples were analyzed. The study also followed the ethical code of conduct specified in the Declaration of Helsinki, including respect for human rights and privacy. The granted approval included the assessment of archival biopsy specimens, and the use of immunohistochemistry procedures indicated in the IRB protocol. During this retrospective study informed consent was obtained from the participants whose clinical data was used in the analysis. The participants had the right to withdraw from the study at any time. If retrospective consent was not obtained, then anonymized previous data was used. The consent form was converted into local languages, and the data collectors were trained to deal with complications. This holistic approach protected the participants' confidentiality and privacy.

RESULTS

This study focuses on the incidence, management, diagnostic characteristics, and prognostic importance of Ki67 immunostaining in gestational trophoblastic disorders GTDs. The findings of this study are based on 197 cases detected from 956 placental biopsy specimens collected within a five-year duration. Key findings include the distribution of GTD subtypes, the impact of this study on Ki67 staining across different categories, and its relationship with clinical outcomes including chronic trophoblastic disorder and choriocarcinoma. These findings emphasize Ki67's crucial component considering a diagnostic and prognostic marker, providing insights into its utility in GTD management.

Table 1. Frequency Distribution of GTD Subtypes.

G T D		**n**		**Percentage %**	
Hydaditiform mole	CM	150	195	76.9%	98.9%
	PM	45		23.0%	
Choriocarcinoma		1		0.50%	
Exaggerated placental site		1		0.50%	
Total		197		100%	

Only 197 19.5% samples were identified out of 1,012 placental samples within over five years, and its diagnoses with gestational trophoblastic disorders GTDs. Hydatidiform moles made up the majority of cases 98.9%, with 150 complete moles 76.9% and 45 partial moles 23%. Other diagnoses included choriocarcinoma 0.5% and excessive placental site reactivity 0.5%.

Table 2. Distribution of Non-Molar and Molar Gestations According to Age Range.

Age in years	**No. of cases n**	**Placenta % n**	**Hydatidiform mole % n**			**HA % n**	**CC % n**	**EPSR % n**
			Total	**CM**	**PM**			
≤15	1	-	0.51% 1	-	2.22% 1	-	-	-
16-20	103	8.23% 65	16.9% 33	16.0% 24	20.0% 9	19.23% 5	-	-
21-25	264	24.8% 196	30.2% 59	37.3% 56	28.8% 13	34.6% 9	-	-
26-30	353	36.6% 289	28.2% 55	23.3% 35	22.2% 10	26.9% 7	0.3% 1	100% 1
31-35	162	17.2% 136	11.7% 23	11.3% 17	13.3% 6	0.44% 3	-	-
36-40	64	6.46% 51	6.15% 12	6.66% 10	4.4% 2	0.14% 1	-	-
>40	9	0.50% 4	2.05% 4	2.00% 3	2.22% 1	0.14% 1	-	-
Total	956	100% 789	100% 195	100% 150	100% 45	100% 26	0.1% 1	100% 1
Mean ±SD	28.0 ± 5.63	28.3 ± 5.41	27.0 ± 6.16	27.2 ±6.11	29.3 ± 6.36	26.7 ± 7.06	27.0	30.0

Women aged 21-25 years reported the highest proportion as compared to male and considered to be age inappropriate for complete and partial moles, accounting for 37.3% and 28.8% of cases, respectively. Hydropic abortions were similarly most common in the same age group 34.6%. The average age of the patients was 28.3 years for non-molar gestations, 27 years for hydatidiform moles, and 26.7 years for hydropic abortions.

Table 3: Ki67 Staining Intensity and Extent in GTD Subtypes.

	Moles			**Total n%**
	Complete mole n%	**Hydropic n%**	**Partial mole n%**	
Intensity				
0	0 0.0	10 66.7	0 0.0	10 20.0
1+	0 0.0	5 33.3	7 46.7	12 24.0
2+	3 15.0	0 0.0	5 33.3	6 12.0
3+	17 85.0	0 0.0	3 20	22 44.0
Total	20 100	15 100	15 100	50 100
Extent				
0	0 0.0	10 66.7	0 0.0	10 20.0
1+	0 0.0	3 20.0	0 0.0	3 6.0
2+	0 0.0	2 13.3	8 53.3	10 20.0
3+	6 30.0	0 0.0	5 33.3	11 22.0
4+	14 70.0	0 0.0	2 13.3	16 32.0
Total	20 100	15 100	15 100	50 100

- Complete Moles: 85% showed strong 3+ Ki67 staining intensity, while 15% showed moderate 2+ intensity. Diffuse immunoreactivity >50% staining was found in 70% complete moles, with no evidence of negative immunostaining.
- Partial Moles: In 33.3% of cases the staining intensity was moderate 2+, whereas 20% was strong 3+. The amount of staining was mostly moderate 11-50%, with only 13.3% indicating diffuse positivity.
- Hydropic Abortions: The majority of cases 66.7% tested negative for Ki67 immunostaining, with the remaining 33.3% showing weak 1+ intensity. None of the cases had moderate or strong positive.

Table 4: Correlation of Ki67 Expression with Prognostic Outcomes

Leisions	**Category 1**	**Category 2**	**Category 3**
Complete Mole Uncomplicated	-	-	11/11 100%
Persistence trophoblastic disease	-	-	9/9 100%
Choriocarcinoma	-	-	1/1 100%

In cases of persistent trophoblastic disease PTD, 100% of entire moles complicated by PTD showed strong Ki67 immunostaining to a diffuse >50% extent. Similarly, only one case of choriocarcinoma demonstrated significant Ki67 positive, indicating a high proliferation index. When relatively easy complete moles were compared to those complicated by PTD, there was no significant difference in Ki67 staining structure, as both categories demonstrated consistently strong positivity. This shows that, while Ki67 is a reliable diagnostic marker, its prognostic value in delineating between simple and complex cases justifies additional investigation.

DISCUSSION

This study highlights the importance of Ki67 immunostaining as a diagnostic and prognostic marker for gestational trophoblastic diseases GTDs. The study examined the Ki67 expression across GTD categories such as complete moles, partial moles, hydropic abortions, and further its growth to the trophoblastic disease PTD, emphasizing Ki67's ability to increase diagnostic accuracy and predict disease outcomes

As an hughlighting the Ki67 staining intensity and extent to disticts significantly among GTD categories, such as complete moles having the highest positivity rate. In 85% of patients, complete moles had high Ki67 staining 3+, while 70% showed diffuse staining >50%. These conclusions are closely aligned with recent studies, which identified Ki67 as a critical marker for distinguishing between complete moles, partial moles, and hydropic abortions. The use of Ki67 in this regard is particularly important for resolving diagnostic challenges as suggested by overlapping with morphological features, especially in early-stage cases where histopathological features are not fully developed[15].

This study displayed the moderate activity of partial mole 2+ Ki67 staining in 33.3% of cases, while hydropic abortions were shown to be predominantly negative for Ki67 expression. These results closely align with findings by Buza and Hui 2022, who emphasized the value of Ki67 in differentiating molar pregnancies from their benign counterparts[16].

The prognostic relevance was shown based on Ki67 propagation and identifying high-risk cases. Persistent trophoblastic disease PTD and choriocarcinoma were strongly associated with high Ki67 expression. All PTD cases 100% and the single case of choriocarcinoma showed close link and strong association in Ki67 immunostaining. it also consistent with observations by Karl et al. 2019, this study reported that the elevation of Ki67 expression also correlates with increased disease aggressiveness and higher likelihood of malignant progression [17].

Interestingly, no significant difference in Ki67 expression was observed between unusual and less complicated complete moles, suggesting that while Ki67 is outermost in diagnosing high-risk subtypes, its ability to predict the progression of individual cases remains limited. This limitation may highlights the need for additional markers, such as p57 or genomic sequence, to complement Ki67 in prognostic evaluations [15].

The results impose the importance of histopathological examination with immunohistochemistry. In such cases where follow-up care may be difficult, it should especially be addressed in rural or underserved populations. The use of Ki67 as a strong marker for disease risk can guide clinicians in prioritizing patients for close monitoring or prophylactic treatments.

This study elaborates on a few limitations that occurred that must be considered when interpreting its findings for conducting future research in this domain. One known limitation is the sample size which was not sufficient for prompt analysis, and may not cover the whole

study of gestational trophoblastic disorders GTDs. These limitations are especially important for rare variants like placental-site trophoblastic cancers, which were not included in this study. Furthermore, the retrospective design of the study had unusual problems, since it depended on previous biopsy records which varied in quality and completeness.

One of the major problems throughout the study is the single use of Ki67 as a diagnostic and prognostic marker. While Ki67 was proficient in separating GTD subtypes and identifying high-risk cases, the exclusion of complementing markers such as p57 may have limited diagnostic findings for further research study. This study design also limits the application of the findings, as they may not be representative of other healthcare settings with varying diagnostic potential with population demographics. However, the lack of detailed follow-up data on patient outcomes, particularly for patients advancing to persistent trophoblastic disease PTD, limitless a complete assessment of Ki67's predictive usefulness over time.

While minimizing these limitations, future research would include broader, multicenter cohorts that can validate the findings across different populations and healthcare settings. Engaging some new indicators like p57 and using current molecular techniques like next-generation sequencing could give a more comprehensive diagnostic framework and improve prognosis categorization. Future Research using new techniques for sample collection, histopathological investigation, and immunohistochemistry staining can also improve accurate and useful results. Furthermore, efforts should be made to develop standardized tracking systems for Ki67 immunostaining to maintain its stability among laboratories and clinics. Broader insight into diagnostic technologies such as Ki67 in resource-constrained placement through proper training programs and resourceful development is crucial for reducing healthcare inequities.

Upcoming research in this domain should attempt to support the present study's limitations while evolving its novel ways to improve diagnostic and prognostic applicability. The combination of Ki67 with additional immunohistochemistry markers, such as p57, is a high-priority study since it has an especially great impact in distinguishing whole moles from partial moles and other imitators. Combining these indicators can increase diagnostic accuracy and reduce ambiguities, particularly in morphologically ambiguous circumstances. Next to these, modern molecular approaches, including next-generation sequencing NGS and genome-wide methylation studies, can reveal genetic and epigenetic changes that underscore the trophoblastic illness. These approaches may provide more information on the pathophysiology of GTDs to find new prognostic biomarkers for high-risk patients.

We need a quick focus on longitudinal study to fully grasp the significant follow-up with long-term data that are also required to understand the long-term effects of Ki67 expression in GTDs. Understanding the patient's condition over time can highlight the association between Ki67 levels and clinical outcomes, such as the risk of developing chronic trophoblastic illness or choriocarcinoma. The data would be extremely useful for improving risk classification models and adapting treatment regimens to patient profiles. Furthermore, identifying the use of Ki67 as a predictor of therapeutic response, particularly in chemotherapy for malignant GTDs, would be a known opportunity for translational research.

CONCLUSION

The study's core focus is the importance of Ki67 immunostaining utilizing the diagnosis and treatment of gestational trophoblastic disorders GTDs. The current study demonstrates the significant

differences in Ki67 expression across GTD variants, with complete moles showing the most proliferative activity, as seen by positive staining intensity and diffuse extent in 85% and 70% of cases, respectively. Partial moles highlight the considerable Ki67 reactivity, but hydropic abortions were initially immune-negative. This contrast emphasizes Ki67's diagnostic value in distinguishing morphologically similar conditions. Furthermore, the study confirmed Ki67's prognostic value, with all cases of severe trophoblastic disease PTD and choriocarcinoma showing robust and diffuse staining and indicating the ability to identify high-risk cases. However, Ki67's missing the distinguishing between simple and complex complete moles also underscores the need for additional markers and molecular tools. Overall, this study supports Ki67's importance as a diagnostic and prognostic marker, arranging for its use in standard clinical practice to improve GTD therapy.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

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