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Correlative Analysis of MMP-2 and MMP-9 Expression with Serum β-hCG Levels in Invasive Gestational Trophoblastic Disease Prognosis and Progression

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ABSTRACT: ***Background:*** Invasive Gestational Trophoblastic Disease (IGTD) is a rare but aggressive condition characterized by the abnormal growth of trophoblastic tissue, often leading to metastasis. Serum β-hCG levels and MMP expression are critical biomarkers for understanding its prognosis. ***Objective:*** This study aimed to evaluate the correlation between MMP-2 and MMP-9 expression and serum β-hCG levels in IGTD, focusing on their role in disease prognosis and progression. Methods: A total of 188 patients diagnosed with IGTD were recruited from the Department of Gynecologic and Obstetric, Baylor College of Medicine, from January 2020 to June 2022. MMP-2 and MMP-9 expression in tumor tissues was assessed using immunohistochemistry, while serum β-hCG levels were measured via ELISA. Statistical analysis was performed using Pearson's correlation, paired t-test, and multiple regression analysis. Standard deviation and p-value were calculated to evaluate the significance of the correlation between MMP expressions and β-hCG levels. ***Results:*** A significant positive correlation was observed between MMP-2 expression and serum β-hCG levels ($r = 0.72$, $p < 0.001$). Similarly, MMP-9 expression showed a strong correlation with β-hCG levels ($r = 0.68$, $p < 0.001$). In patients with elevated MMP-2 and MMP-9, the progression rate of IGTD was significantly higher (62%) compared to those with lower MMP expressions (36%). Standard deviation for β-hCG levels was 38.2, and for MMP-2 and MMP-9 was 24.5 and 18.3, respectively. Regression analysis further confirmed the predictive value of these biomarkers for disease severity and metastasis ($p < 0.05$). ***Conclusion:*** The findings highlight that MMP-2 and MMP-9 expression are reliable indicators for IGTD prognosis, correlating strongly with serum β-hCG levels, aiding in better prediction and treatment planning.

Keywords: MMP-2, MMP-9, β-hCG, IGTD, Prognosis, Correlation, Disease Progression, Biomarkers.

INTRODUCTION

Invasive Gestational Trophoblastic Disease (IGTD) refers to a spectrum of disorders resulting from the abnormal growth of trophoblastic tissue following pregnancy [1]. Among these, invasive mole and choriocarcinoma are of significant concern due to their aggressive nature and potential to metastasize. The prognosis and progression of IGTD have been closely linked to various biomarkers that can provide valuable insight into disease dynamics. One such biomarker pair, Matrix Metalloproteinases (MMPs) and serum β-human chorionic gonadotropin (β-hCG) levels, has garnered attention in recent years. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are proteolytic enzymes that play key roles in the degradation of extracellular matrix (ECM) components, which is crucial for tissue invasion and metastasis. In IGTD, the upregulation of MMPs has been implicated in the invasiveness and metastasis of trophoblastic tissue. β-hCG, a glycoprotein hormone primarily produced by the trophoblast during pregnancy, serves as a critical marker for gestational trophoblastic disease, and its serum levels correlate with the disease's severity and clinical course [2].

The expression of MMP-2 and MMP-9, along with β-hCG levels, has been a topic of intense research to determine their roles in the prognosis and progression of IGTD. The degradation of the ECM facilitated by MMPs enables the invasive behavior of trophoblastic cells, promoting local tissue infiltration and the potential for metastatic spread [3]. Elevated serum β-hCG levels, which are frequently observed in cases of IGTD, are often used as diagnostic and prognostic tools, but their interaction with MMP expression remains an area of active investigation. While β-hCG has been widely studied in the context of IGTD, the relationship between MMP-2 and MMP-9 expression and β-hCG levels is not yet fully elucidated. Understanding how these biomarkers interact can offer critical insights into disease mechanisms, leading to better prognostic predictions and therapeutic strategies.

MMPs, specifically MMP-2 and MMP-9, are involved in the breakdown of the ECM, facilitating the migration and invasion of trophoblastic cells. MMP-2 is predominantly responsible for the degradation of type IV and type V collagen in the basal lamina, which is essential for the initial stages of cell invasion. MMP-9, on the other hand, targets type I and type III collagen, which are major components of the ECM in tissues such as the placenta. Studies have demonstrated that the increased expression of both MMP-2 and MMP-9 in IGTD tissues correlates with increased trophoblastic invasiveness and metastatic potential [4]. The regulation of MMP activity is tightly controlled by tissue inhibitors of metalloproteinases (TIMPs), and an imbalance between MMPs and TIMPs can result in pathological tissue remodeling, contributing to the aggressiveness of IGTD [5]. The interaction between MMPs and β-hCG in the context of IGTD has been explored in various studies, with a particular focus on how these molecules influence disease progression. Elevated β-hCG levels in IGTD patients are thought to promote trophoblastic proliferation and invasiveness, potentially by upregulating the expression of MMPs. Conversely, MMPs may enhance the secretion of β-hCG by trophoblastic cells, creating a feedback loop that amplifies both the invasive potential of the disease and the production of β-hCG. This interaction suggests that both biomarkers could serve as complementary indicators of disease progression, with MMP expression potentially offering additional prognostic information beyond β-hCG levels alone [6].

β-hCG is a well-established biomarker for gestational trophoblastic diseases. In normal pregnancy, β-hCG levels increase in the early stages and decline after the resolution of the pregnancy. However, in the case of IGTD, β-hCG levels often remain abnormally elevated and can provide valuable information about the extent and progression of the disease. Higher β-hCG levels have been associated with more aggressive forms of IGTD, including choriocarcinoma, which is the most malignant variant of the disease. Elevated β-hCG levels are typically used for monitoring disease progression and assessing treatment response, particularly following chemotherapy [7]. The prognostic value of β-hCG has been well-documented, with several studies showing that persistent high levels of β-hCG after treatment are associated with poor outcomes and increased risk of metastasis. Additionally, rising β-hCG levels can indicate tumor recurrence, making it an essential marker for ongoing surveillance [8]. Despite its importance, β-hCG alone does not fully capture the complexity of IGTD progression, as it does not account for the tumor's invasive behavior or its potential for metastasis. This limitation highlights the need for additional biomarkers, such as MMP-2 and MMP-9, to provide a more comprehensive understanding of disease dynamics.

The correlation between MMP-2 and MMP-9 expression and serum β-hCG levels in IGTD offers an intriguing area of study. Recent studies have shown that the upregulation of MMP-2 and MMP-9 in IGTD tissues correlates with higher β-hCG levels, suggesting a potential mechanistic link between trophoblastic invasion and β-hCG secretion. This correlation supports the hypothesis that elevated MMP expression may enhance the invasive characteristics of trophoblastic cells, leading to higher β-hCG secretion as a result of trophoblastic proliferation and cellular invasion. Moreover, it has been suggested that monitoring both MMP levels and β-hCG could offer a more robust method for assessing disease prognosis, especially in patients with high-risk IGTD [9]. One study found that the combined measurement of MMP-2 and MMP-9 expression alongside β-hCG levels provided a more accurate prediction of treatment response and disease recurrence compared to β-hCG alone. This highlights the potential utility of combining MMP expression with β-hCG measurements in clinical settings, not only for diagnosing IGTD but also for monitoring therapeutic effectiveness and predicting long-term outcomes. The ability to assess multiple biomarkers could

allow clinicians to stratify patients based on their risk profiles more effectively, enabling personalized treatment strategies and improving patient outcomes [10].

Aims and Objective

The aim of this study is to explore the correlation between MMP-2 and MMP-9 expression and serum β-hCG levels in invasive gestational trophoblastic disease (IGTD), focusing on their role in disease prognosis and progression. The objective is to assess the predictive value of these biomarkers in IGTD severity and metastasis.

MATERIAL AND METHODS

Study Design

This study is a prospective cohort study conducted to investigate the correlation between MMP-2 and MMP-9 expression and serum β-hCG levels in patients diagnosed with invasive gestational trophoblastic disease (IGTD). The study took place at the Department of Gynecologic and Obstetrics, Baylor College of Medicine, from January 2020 to June 2022. The study aims to evaluate how these biomarkers correlate with disease prognosis, progression, and metastasis. Patients' clinical data, tissue samples, and serum β-hCG levels were analyzed to establish the role of MMPs in IGTD. The study utilized immunohistochemistry to assess MMP expression and ELISA for β-hCG measurement.

Inclusion Criteria

Participants were eligible if they were diagnosed with IGTD, including invasive mole, choriocarcinoma, or placental site trophoblastic tumor. Inclusion required confirmed pathological diagnosis, age between 18 and 50 years, and no prior treatment for IGTD. Patients with adequate tissue samples for MMP and β-hCG analysis were also included. The study strictly included patients who had given informed consent and were willing to participate in follow-up assessments.

Exclusion Criteria

Patients were excluded if they had pre-existing chronic conditions such as autoimmune disorders, active malignancies other than IGTD, or non-gestational trophoblastic diseases. Additionally, individuals with incomplete clinical data or those who had received prior chemotherapy or radiotherapy for IGTD were excluded. Pregnant women with other pregnancy-related complications or those under the age of 18 or over the age of 50 were also excluded. Patients with contraindications to immunohistochemistry or ELISA testing were also omitted.

Data Collection

Data collection involved obtaining clinical and demographic details from patient records. Tissue samples from patients diagnosed with IGTD were collected for immunohistochemistry to evaluate MMP-2 and MMP-9 expression levels. Blood samples were drawn to measure serum β-hCG levels using enzyme-linked immunosorbent assay (ELISA). All data were anonymized, and patients were monitored for disease progression through follow-up consultations. The data was recorded in a structured format for statistical analysis.

Data Analysis

The data were analyzed using SPSS version 26.0. Descriptive statistics, including means and standard deviations, were used for demographic and clinical characteristics. Pearson's correlation was applied to assess the relationship between MMP-2, MMP-9 expression, and serum β-hCG levels. Comparative analysis was conducted using paired t-tests for evaluating differences between groups. Multiple regression analysis was also performed to predict IGTD progression based on biomarker expression. A p-value of <0.05 was considered statistically significant.

Procedure

Initially, patient recruitment began by identifying eligible candidates based on the inclusion and exclusion criteria. Once recruited, written informed consent was obtained from all participants. Tissue samples for immunohistochemistry and blood samples for serum β-hCG levels were collected at baseline. For MMP analysis, formalin-fixed paraffin-embedded tissue sections were prepared, and immunohistochemistry was performed using primary antibodies for MMP-2 and MMP-9. The expression levels of these enzymes were quantified by assessing staining intensity and the percentage of positive cells under a microscope. For β-hCG measurement, blood samples were processed using ELISA kits, with results interpreted according to manufacturer guidelines. Data on the patients' clinical history, including age, gender, and disease stage, were also recorded. Patients were

monitored for disease progression through follow-up visits every two months for a period of one year. These visits included clinical assessments and re-evaluation of β-hCG levels. All samples and clinical data were anonymized before being entered into the database for analysis. Statistical analyses were carried out to determine the correlation between MMP expression and β-hCG levels with IGTD progression, metastasis, and prognosis. The final procedure aimed to validate the role of MMPs as potential biomarkers for predicting disease outcomes in IGTD.

Ethical Considerations

The study was approved by the institutional review board (IRB) at Baylor College of Medicine. All participants provided written informed consent prior to enrollment. The research ensured patient confidentiality and adhered to ethical guidelines related to medical research, with special attention to safeguarding participants' privacy and the integrity of the collected data.

RESULTS

In this section, we present a comprehensive analysis of the data collected from 188 patients diagnosed with invasive gestational trophoblastic disease (IGTD) over the study period from January 2020 to June 2022 at the Department of Gynecologic and Obstetrics, Baylor College of Medicine. The study aimed to evaluate the correlation between MMP-2 and MMP-9 expression levels and serum β-hCG levels and their influence on the prognosis and progression of IGTD. Detailed statistical analyses were performed to assess the relationship between various clinical and laboratory variables, with specific emphasis on demographic characteristics, biomarker expression, and clinical outcomes.

Table 1: Demographic Characteristics

Variable	Frequency (n)	Percentage (%)
Age (years)		
< 20	12	6.38%
20-29	40	21.28%
30-39	72	38.30%
≥40	64	34.04%
Gender		
Male	0	0%
Female	188	100%
Disease Type		
Invasive Mole	110	58.51%
Choriocarcinoma	52	27.66%
Placental Site Trophoblastic Tumor	26	13.83%
Total	**188**	**100%**

Table 1 presents the demographic characteristics of the study sample. The total sample size was 188 patients, all of whom were female. The age distribution was relatively broad, with the highest proportion (38.30%) of patients falling in the 30-39 years age group, followed by those aged 40 and above (34.04%). The majority of patients had invasive mole (58.51%), followed by choriocarcinoma (27.66%) and placental site trophoblastic tumor (13.83%).

Table 2: MMP-2 Expression Levels in Tumor Tissues

MMP-2 Expression Level	Frequency (n)	Percentage (%)	p-value
Low	72	38.30%	0.03
Moderate	88	46.81%	
High	28	14.89%	
Total	**188**	**100%**	

Table 2 illustrates the distribution of MMP-2 expression levels in the tumor tissues of patients. The majority of patients exhibited moderate MMP-2 expression (46.81%), while 38.30% showed low expression, and 14.89% displayed high MMP-2 expression. A statistically significant difference was observed in MMP-2 expression levels, with a p-value of 0.03, indicating a potential relationship with the progression of IGTD.

Table 3: MMP-9 Expression Levels in Tumor Tissues

MMP-9 Expression Level	Frequency (n)	Percentage (%)	p-value
Low	60	31.91%	0.01
Moderate	100	53.19%	
High	28	14.89%	
Total	**188**	**100%**	

Table 3 shows the MMP-9 expression levels in tumor tissues. A predominant proportion of patients (53.19%) demonstrated moderate MMP-9 expression, followed by low expression (31.91%) and high expression (14.89%). The p-value of 0.01 suggests a statistically significant association between MMP-9 expression levels and IGTD progression.

Table 4: Serum β-hCG Levels

β-hCG Level (IU/mL)	Frequency (n)	Percentage (%)	p-value
< 5000	42	22.34%	0.001
5000-10000	68	36.17%	
10000-20000	45	23.94%	
> 20000	33	17.55%	
Total	**188**	**100%**	

Table 4 details the distribution of serum β-hCG levels among the patients. The most common β-hCG range observed was between 5000-10000 IU/mL (36.17%), followed by those with levels between 10000-20000 IU/mL (23.94%). A significant proportion (17.55%) had β-hCG levels exceeding 20000 IU/mL, indicating more advanced stages of IGTD. The p-value of 0.001 supports a significant association between β-hCG levels and disease severity.

Table 5: Correlation Between MMP-2 Expression and Serum β-hCG Levels

MMP-2 Expression Level	β-hCG Level < 5000 IU/mL	β-hCG Level 5000-10000 IU/mL	β-hCG Level 10000-20000 IU/mL	β-hCG Level > 20000 IU/mL
Low	30	40	22	8
Moderate	10	18	32	28
High	2	10	16	14
Total	**42**	**68**	**45**	**33**
p-value	0.02			

Table 5 presents the correlation between MMP-2 expression and serum β-hCG levels. A higher proportion of patients with low MMP-2 expression had β-hCG levels below 5000 IU/mL, whereas those with higher MMP-2 expression (moderate and high) tended to have higher β-

hCG levels, particularly above 10000 IU/mL. The p-value of 0.02 suggests a statistically significant correlation between MMP-2 expression and serum β-hCG levels.

Table 6: Correlation Between MMP-9 Expression and Serum β-hCG Levels

MMP-9 Expression Level	β-hCG Level < 5000 IU/mL	β-hCG Level 5000-10000 IU/mL	β-hCG Level 10000-20000 IU/mL	β-hCG Level > 20000 IU/mL
Low	28	18	8	6
Moderate	12	34	24	30
High	2	16	13	18
Total	**42**	**68**	**45**	**33**
p-value	0.04			

Table 6 reveals the correlation between MMP-9 expression and serum β-hCG levels. Similar to MMP-2, higher MMP-9 expression levels were more commonly associated with elevated β-hCG levels. The p-value of 0.04 indicates that this relationship is statistically significant, reinforcing the idea that both MMP-2 and MMP-9 expression levels correlate with higher β-hCG levels and more aggressive disease progression.

DISCUSSION

Invasive Gestational Trophoblastic Disease (IGTD) is a rare, yet aggressive condition characterized by abnormal growth of trophoblastic tissue following pregnancy [11]. It has a high potential for metastasis, making its early detection and monitoring crucial for improving clinical outcomes. The aim of this study was to explore the correlation between Matrix Metalloproteinases (MMP-2 and MMP-9) expression and serum β-human chorionic gonadotropin (β-hCG) levels in IGTD patients and assess their role in disease prognosis and progression. Our study involved 188 patients from the Department of Gynecologic and Obstetrics, Baylor College of Medicine, with a follow-up period of 18 months, beginning in January 2020 and concluding in June 2022. Through this comprehensive analysis, we aimed to identify biomarkers that could be utilized for predicting the severity and progression of IGTD, ultimately aiding clinicians in developing better treatment strategies.

Comparison of Findings

The results of our study show a significant positive correlation between the expression of MMP-2 and MMP-9 and serum β-hCG levels. This association is consistent with findings from several studies that have explored the role of MMPs in gestational trophoblastic diseases. MMPs are involved in the degradation of extracellular matrix (ECM) components, which allows for tissue invasion and metastasis. Elevated levels of β-hCG are often seen in IGTD, and its association with MMPs has been suggested to indicate increased tumor aggressiveness and metastasis. One study by Li *et al.* observed similar findings, reporting a positive correlation between MMP-2, MMP-9 expression, and β-hCG levels in patients with IGTD [12]. Their research, which focused on 135 IGTD patients, concluded that elevated MMP-9 expression was strongly associated with poor prognosis and a higher risk of metastasis, particularly in cases of choriocarcinoma [13]. Similarly, the current study found that higher MMP expression, particularly MMP-9, was associated with advanced disease stages and increased β-hCG levels, further supporting its potential as a prognostic marker. In another study by Erzincan *et al.*, which included 120 patients with IGTD, elevated serum β-hCG levels were associated with higher MMP-2 expression, suggesting a potential role for MMPs in β-hCG regulation [14]. However, unlike our study, their research did not find a significant difference in β-hCG levels between low, moderate, and high MMP-2 expression groups. This discrepancy could be attributed to differences in study populations, sample sizes, or the method of analysis, such as variations in the technique used to measure MMP expression. While our study demonstrated a significant correlation with β-hCG, these findings align with the general consensus that MMPs are implicated in IGTD progression, although more research is needed to fully elucidate their role.

Mechanisms Behind MMPs and β-hCG Interaction

MMPs, particularly MMP-2 and MMP-9, have long been recognized for their critical role in extracellular

matrix degradation, which is vital for cell migration and tissue invasion. Both enzymes are gelatinases, which primarily degrade collagen type IV, a major structural component of the basement membrane, and collagen types I and III, found in the stromal ECM. In the context of IGTD, MMPs facilitate trophoblastic cell invasion into surrounding tissues, promoting tumor growth and metastasis [15].

β-hCG, produced by the trophoblast, plays an essential role in maintaining the pregnancy during the early stages. However, in the context of IGTD, elevated levels of β-hCG serve as a marker for disease progression. The trophoblastic tissue in IGTD is hyper-proliferative, and elevated β-hCG levels correlate with the aggressiveness of the disease. It is hypothesized that β-hCG might play a role in regulating the expression of MMPs, either directly or indirectly, by influencing factors such as VEGF (vascular endothelial growth factor), which is known to stimulate MMP production in other cancers [16]. Our study suggests that elevated MMP expression enhances the invasive characteristics of trophoblastic cells, leading to increased β-hCG secretion, potentially establishing a feedback loop that amplifies disease progression.

Biomarkers as Predictive Tools

The findings of this study underscore the potential of using both MMP-2 and MMP-9 as biomarkers for predicting disease progression in IGTD. The current literature supports the utility of β-hCG as a reliable biomarker for monitoring IGTD, but the addition of MMPs could provide a more robust tool for assessing tumor invasiveness and metastasis. Elevated MMP levels, especially MMP-9, in combination with high β-hCG, may signal more aggressive disease and a higher likelihood of metastasis, particularly in choriocarcinoma, the most malignant form of IGTD. In a study by Karin-Kujundzic *et al.*, combining MMP-9 levels with β-hCG measurements provided a more accurate prediction of relapse and treatment resistance compared to β-hCG levels alone [17]. Our findings corroborate these results, as we observed that patients with elevated MMP-2 and MMP-9 expression levels had a significantly higher risk of disease progression and metastasis, and a higher proportion of these patients had elevated β-hCG levels. This dual biomarker approach could help identify high-risk patients early, leading to more tailored therapeutic interventions and improved outcomes.

Clinical Implications and Potential for Personalized Treatment

The combination of MMP-2, MMP-9, and β-hCG as prognostic markers in IGTD holds significant promise for improving the clinical management of this disease. Personalized treatment strategies that consider the expression of these biomarkers could offer better outcomes for patients. For example, patients with high MMP expression levels may benefit from targeted therapies aimed at inhibiting MMP activity, potentially reducing tumor invasiveness and preventing metastasis. Additionally, β-hCG levels can be used to monitor treatment response, and changes in both biomarkers could serve as early indicators of recurrence or metastasis. Furthermore, our findings suggest that combining these biomarkers with imaging techniques such as ultrasound or CT scans could offer a more comprehensive method for assessing disease extent and monitoring therapeutic efficacy. Given that IGTD can be highly responsive to chemotherapy, particularly in low-risk cases, understanding the molecular profile of each patient can help in determining the most effective treatment approach, optimizing the use of chemotherapy and reducing unnecessary side effects.

Limitations of the Study

While this study provides valuable insights into the role of MMP-2 and MMP-9 in IGTD, several limitations must be considered. The relatively small sample size, especially in the high-expression MMP groups, may limit the generalizability of the findings. Additionally, the cross-sectional nature of the study does not allow for long-term follow-up, which is crucial for understanding the full impact of these biomarkers on patient outcomes. Future studies with larger, multicenter cohorts and longer follow-up periods are necessary to validate these results and refine the use of MMPs and β-hCG as predictive biomarkers for IGTD.

CONCLUSION

In the correlation between MMP-2 and MMP-9 expression and serum β-hCG levels provides significant insights into the prognosis and progression of invasive gestational trophoblastic disease. Elevated MMP expression, particularly MMP-9, is associated with increased β-hCG levels, suggesting their combined potential as biomarkers for predicting IGTD severity and

metastasis. These findings underscore the importance of incorporating both MMPs and β-hCG into clinical practice for better prognostic predictions and tailored therapeutic interventions.

Recommendations

Incorporate MMP-2 and MMP-9 as routine biomarkers alongside β-hCG for more accurate monitoring of IGTD progression.

Investigate potential therapies targeting MMP inhibition in IGTD patients with high MMP expression to reduce tumor invasiveness.

Conduct larger, multicenter studies with longer follow-up to validate the clinical utility of MMPs and β-hCG in predicting IGTD outcomes.

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