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Original article

Significance of Expression of VEGF, BCL-2, p63 and β-hCG in Gestational Trophoblastic Diseases

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Abstract:

Background: A diverse range of conditions linked to an abnormal trophoblast growth are collectively known as gestational trophoblastic disease (GTD), which are classified into: tumor-like lesions, molar pregnancies and gestational trophoblastic neoplasms. **Aim of the work:** to study Immunohistochemical expression of VEGF, BCL-2, p63 and serum β-hCG in hydropic abortion, molar pregnancy & choriocarcinoma to elucidate their role in pathogenesis and diagnosis in these entities. **Material and Methods:** This retrospective Immunohistochemical study of VEGF, BCL-2, p63 & serum β-hCG- was carried upon 65 selected cases of gestational trophoblastic diseases. **Results:** VEGF was increased gradually from hydropic abortion, molar pregnancy to choriocarcinoma (P=0.000). While BCL-2 and p63 were downregulated markedly from hydropic abortion to be weakly or negatively expressed for choriocarcinoma cases (p=0.000). Serum β-hCG also increased in the same pattern as VEGF (P =0.000). BCL-2 & p63 and β-hCG are more sensitive (100% & 90% & 100%) and specific (56.4%, 100%, 99%, respectively) in diagnosis of choriocarcinoma. BCL-2 & p63- were highly sensitive markers (81.8% & 100%) in differentiating hydropic abortion from molar pregnancy. **Conclusion:** VEGF, BCL-2, p63 expression and elevated serum β-hCG level might have role in gestational trophoblastic disease pathogenesis and progression. Combined expression of BCl-2, p63 and high β-hCG, might be useful to differentiate choriocarcinoma from other gestational trophoblastic diseases and hence target therapy. BCl-2, p63 could be used as markers for early diagnosis of molar pregnancy.

Keywords: GTD, Choriocarcinomas, Partial mole, VEGF, β-hCG.

Introduction

Gestational trophoblastic diseases (GTD) encompass a spectrum of conditions, including hydatidiform moles (HMs) and gestational trophoblastic neoplasia (GTN). HMs are further categorized as partial (PHM) or complete (CHM). GTN comprises invasive mole, choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor. Despite their involvement in these pathological states, their specific roles of hydatidiform moles and gestational trophoblastic neoplasia remain unexplored [1, 2].

Hydatidiform mole is a benign condition, while choriocarcinoma is a highly malignant tumor that often spreads to other parts of the body. Choriocarcinoma can develop from the trophoblasts during any gestational event but most frequently arises after a complete mole [3].

Thankfully, the majority of HMs will naturally regress following curettage. However, 8–30% of patients will develop persistent GTD, particularly after CHM, necessitating specialized treatment, including chemotherapy. Current guidelines recommend measuring serum human chorionic gonadotropin (β-hCG) once a week after the evacuation of pregnancy products in order to forecast this aggressive behavior [4].

Choriocarcinoma (CC) is a highly aggressive type of tumor with a near 100% fatality rate [5-7]. Despite various molecular discoveries related to choriocarcinoma, there are no unique molecular signatures useful for diagnostic or prognostic purposes [8]. Hence, it is crucial to investigate new diagnostic and prognostic markers for innovative therapeutic approaches.

Despite the well-established histological criteria, it is nevertheless difficult in clinical practice to distinguish between non-molar HA, PHM, and CHM, particularly in the first trimester [9].

Important therapeutic and prognosis consequences arise from the clinical distinction of these lesions [10]. Numerous supplementary procedures- such as immunohistochemistry- have been suggested as solutions to the diagnostic problems associated with HM.

Vascular endothelial growth factor (VEGF) is a family of growth factors related to the platelet-derived growth factor superfamily. It includes several glycoproteins, such as placental growth factor. VEGF-A, commonly referred to as VEGF, promotes the formation of new blood vessels (angiogenesis) [11].

The trophoblastic tissue's normal development and carcinogenesis are significantly influenced by placental apoptosis. B-cell lymphoma 2 (BCL-2), is a member of the BCL-2 family of apoptotic regulatory proteins that function by either promoting or inhibiting apoptosis. It has been demonstrated that BCL-2 plays a part in both healthy placental formation and various pregnancy problems [9].

Tumor protein 63 (p63) is a transcription factor related to the p53 gene family. Unlike p53, p63 is not a typical tumor suppressor [12]. Studies suggest that p63 is vital for maintaining stem cells and developing various epithelial tissues in normal placentas. While p63 has been implicated in several cancers, its specific role in gestational trophoblastic disorders requires further investigation [13].

The present work aims to study the IHC expression pattern of VEGF, BCL2, p63 and serum β-hCG level in hydropic pregnancy, partial mole, complete mole and choriocarcinoma- to firstly; elucidate their role in detection of the risk of progression from benign (hydropic abortion and molar pregnancy) to choriocarcinoma ,secondly; for assessment of diagnostic utility of theses markers in discriminating malignant lesion and hence target therapy.

Patients and Methods

This retrospective study analyzed 65 selected cases of gestational trophoblastic

diseases, comprising: 11 cases of hydropic pregnancy, 23 cases of partial mole, 21 cases of complete mole, and 10 cases of choriocarcinoma. Fifteen cases were acquired through surgical resection (hysterectomy) and fifty via endometrial curettage. Ten normal placenta cases served as controls. The cases studied included archival formalin-fixed, paraffin-embedded blocks processed from January 2015 to December 2023 from the Pathology Department of Benha Faculty of Medicine and the Early Cancer Detection Unit (ECDU). The study received ethical approval from the Faculty of Medicine, Benha University (RC 35-1-2024).

Only cases with available blocks and clinic pathological data were included in this analysis. However, cases with previous history of chemotherapy or that without clinico-pathological information- were excluded from the current study.

Histopathological Evaluation

All cases were reviewed to confirm the original diagnosis using hematoxylin and eosin-stained slides. Clinicopathological parameters, including age, serum β-hCG level, gestational age, and ultrasound findings- were collected from patient records.

Immunohistochemical Evaluation

Sections of tissue, four microns in thickness, were prepared from formalin-fixed, paraffin-embedded blocks and then placed on coated slides. A labeled streptavidin-biotin system (Dako Cytomation A/S, Glostrup, Denmark) was employed as per the manufacturer's guidelines. Antigen retrieval involved heating the sections in a 10 mmol/L citrate monohydrate buffer (pH 6.0) for 15 minutes in a microwave, as specified in Table (1). The immunoreaction was visualized using 3,3'-Diaminobenzidine (DAB) as the chromogen. Negative controls were generated by excluding the primary antibodies and substituting them with saline or phosphate buffer (PB).

Table (1): Data for using antibodies.

Antibody	Type	Cat.No	Dilution	Retrieval solution	Incubation	Positive control
VEGF	rabbit polyclonal	ab137110, Abcam, Cambridge, UK	1:250	citrate monohydrate buffer (pH 6.0)	overnight at 4_C	Breast cancer
BCl2	rabbit polyclonal	ab137110, Abcam, Cambridge, UK	1:250	citrate monohydrate buffer (pH 6.0)	overnight at 4_C	lymph node
P63	Clone 4A4	Dako Cytomation, Glostrup	1:100	citrate monohydrate buffer (pH 6.0)	overnight at 4_C	prostatic tissue

VEGF: Vascular endothelial growth factor, BCL-2: B cell lymphoma -2, p63: Tumor protein 63.

Immunohistochemical assessment

Two pathologists independently evaluated the immunohistochemical staining. The stained cell types were identified as villous cytotrophoblasts and syncytiotrophoblasts. The immune-expression patterns of VEGF, BCL-2, and p63- were semi-quantitatively scored based on the percentage of positive cells and staining intensity, following the methods described by Missaoui et al , Anjum et al , Steurer et al & Hussein et al [10- 12 ,14].

Statistical analysis

Data were analyzed using SPSS version 16 software. Receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal sensitivity and specificity of study markers in diagnosing molecular subtypes. Categorical data were summarized as frequencies and

percentages and analyzed using the chi-square test or Fisher's exact test. Quantitative data were presented as mean ± standard deviation, median, and range. Normality was assessed using the Shapiro-Wilk test (α = 0.05). Parametric data were analyzed with Student's t-test.

Results

The present study included 65 cases of gestational trophoblastic disease: 21 (16.9%) cases of hydropic abortion, 23 (35.4%) cases of partial mole, 21 (32.3%) cases of complete mole and 10 (15.4%) cases of choriocarcinoma. The age of the studied cases ranges from 18-43 years with an average of 28.34± 8.9 years. There was high statistical significance between the age of the patient and gestational age among studied group (p=0.000).

Differences in expression of VEGF, BCL-2, p63 & β-hCG in the studied groups. VEGF was positively expressed in the cytoplasm of villous trophoblastic cells in 72.7% of hydropic abortion (Fig. 1A) and 65.2% of partial mole (Fig. 1B) while increased gradually to include 85.7% of complete mole (Fig. 1C) and all cases of choriocarcinoma (Fig. 1D) with high statistically significant direct correlation (P =0.000) as shown in Table (2).

On the contrary, BCL-2 was only expressed in cytoplasm of syncytiotrophoblasts as it was higher in 63.6% of hydropic abortion (Fig. 2A) and 82.6% of partial mole (Fig. 2B). Meanwhile It decreased markedly showing weak expression in 81% and 80% of the studied complete mole (Fig. 2C) and choriocarcinoma cases respectively (Fig. 2D) with highly significant difference (p=0.000) as detailed in Table (2). p63 was strongly expressed in nuclei of cytotrophoblast in 81.8% of hydropic abortion (Fig. 3A) and 82.6% of PHM (Fig. 3B). It shows moderate expression in 76.2% of complete mole (Fig. 3C) and negatively expressed in 90% of choriocarcinoma cases (Fig. 3D) with highly statistically significant difference (p=0.000) as detailed in Table (2).

Serum β-hCG level was increased significantly from hydropic abortion, molar pregnancy to reach its higher level in choriocarcinoma (p=0.000) as detailed in Table (2).

Table (2). Differences in expression of VEGF, BCL-2, p63 & serum β-hCG in the studied groups.

		Hydropic abortion N. %	Partial mole N. %	Complete mole N. %	Choriocarcinoma N. %	P value
VEGF	Positive	8 72.7%	15 65.2%	18 85.7%	10 100.0%	**0.000 (HS)**
	Negative	3 27.3%	8 34.8%	3 14.3	0 0.0%	
BCL2	Strong	7 63.6%	0 0.0%	0 0.0%	0 0.0%	**0.000 (HS)**
	Moderate	1 9.1%	19 82.6%	4 19.0%	0 0.0%	
	Weak	1 9.1%	2 8.7%	17 81.0%	8 80.0%	
	Negative	2 18.2%	2 8.7%	0 0.0%	2 20.0%	
p 63	Strong	9 81.8%	4 17.4%	5 23.8%	0 0.0%	**0.000 (HS)**
	Moderate	2 18.2%	19 82.6%	16 76.2%	0 0.0%	
	Weak	0 0.0%	0 0.0%	0 0.0%	1 10.0%	
	Negative	0 0.0%	0 0.0%	0 0.0%	9 90.0%	
β-hCG		370 (125-750)	120000 (84000-190000)	255000 (166500-330000)	780000 (665000-878000)	.000

VEGF: Vascular endothelial growth factor, BCL-2: B cell lymphoma -2, p63: Tumor protein 63, β-hCG: serum β-human chorionic gonadotropin level, HS: Highly Significant.

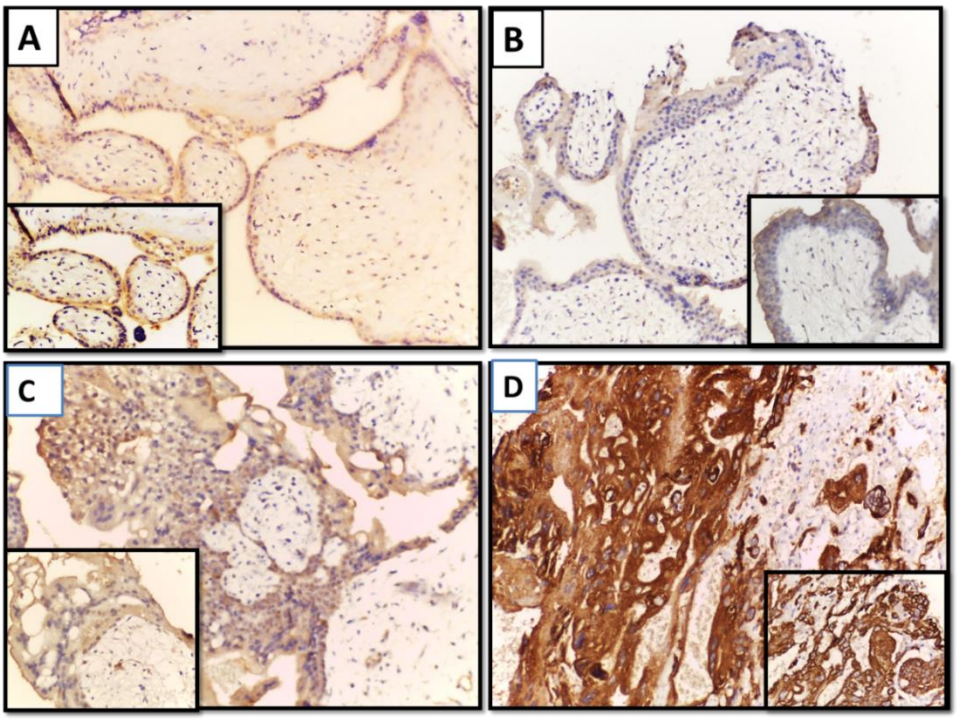

Fig. 1: VEGF expression among the studied groups: A) diffuse moderate cytoplasmic expression of VEGF in trophoblastic covering in hydropic abortion (inset, x400, IHC). B, C): focal weak cytoplasmic staining of VEGF in proliferated trophoblastic cells in PHM & CHM (Inset, x400). D) strong diffuse expression in malignant cells of choriocarcinoma (inset, x400, IHC) (IHC X200).

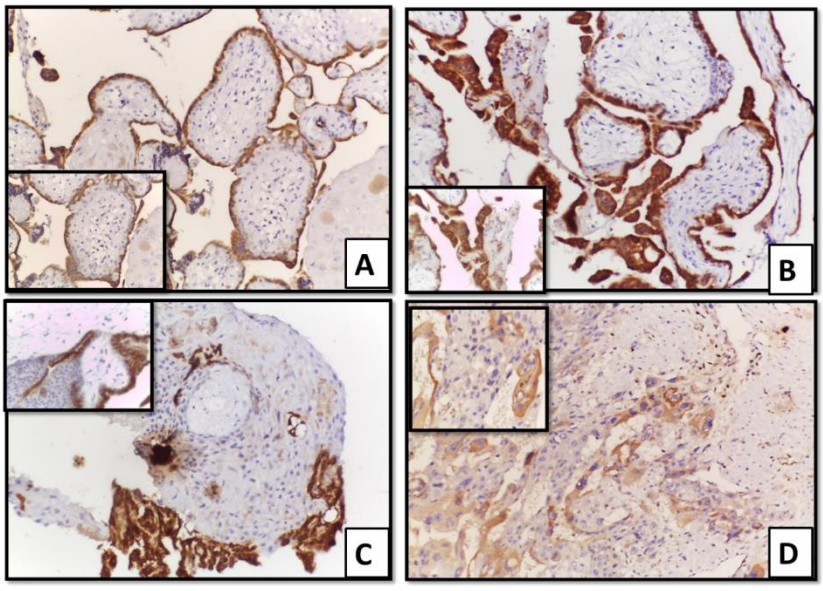

Fig. 2: BCL2 IHC staining among the studied groups. A) Diffuse strong cytoplasmic expression in syncytiotrophblast in hydropic abortion (IHC, X200, inset, x400). **B, C)**: Focal cytoplasmic expression of BCL2 in syncytiotrophoblast in PHM, CHM (inset x400, IHC). **D:** Focal moderate cytoplasmic expression in malignant cells in choriocarcinoma (IHC, X200, inset, x400).

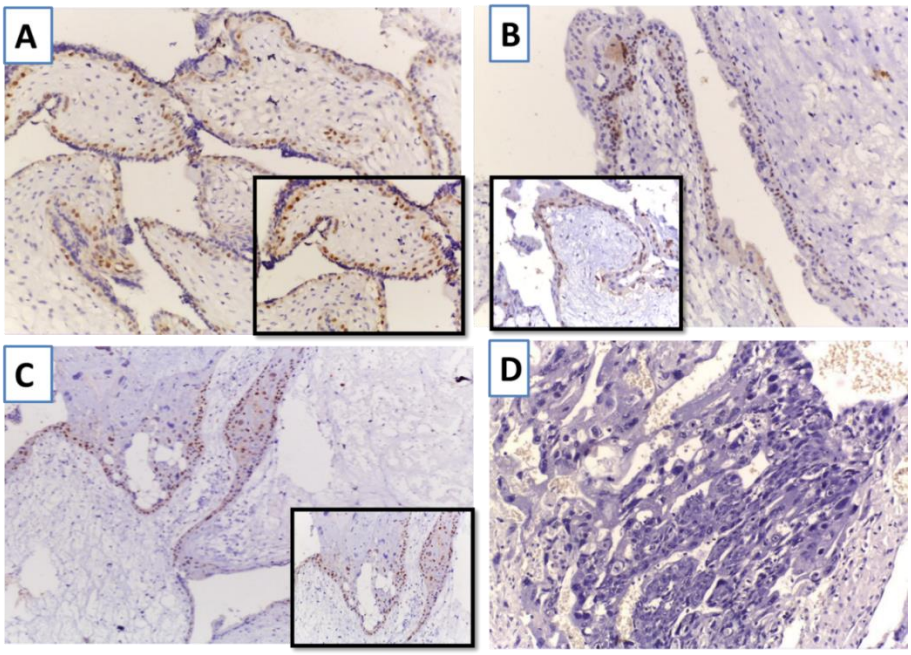

Fig. 3: p63 IHC staining among the studied groups. A) diffuse nuclear expression of p63 in cytotrophoblast in hydropic abortion (IHC, X200, inset x400. B,C): positive nuclear staining of p63 in cytophoblast in both PHM ,CHM (IHC, X200, inset x400, IHC). D) negative expression of p63 in choriocarcinoma (IHC, X200).

Table (3): Diagnostic value of VEGF, BCL2, p63 and serum β-hCG level markers for Choriocarcinoma

	Sensitivity	Specificity	95% CI	AUC
VEGF	80%	56.4%	.605-.868	.736
BCl2	100%	56.4%	.666-.897	.782
p63	90%	100%	.84-1.00	.95
Serum β-hCG level	100%	99%	94.4-100	.983
Positive VEGF &weak BCL2	80%	81.8%	.653-.965	.809
Positive VEGF &weak BCL2 &negative p63	70%	100%	.675-1.00	.850
Positive VEGF &weak BCL2 & positive serum β-hCG	80%	99%	.741-1.00	.891
Positive VEGF &weak BCL2 & negative p63 &positive serum β-hCG	70%	100%	.675-1.00	.850

VEGF: Vascular endothelial growth factor; BCL-2: B-cell lymphoma-2; p63: Tumor protein 63; β-hCG: serum β-human chorionic gonadotropin level; AUC: Area Under the Curve; CI: Confidence Interval.

Diagnostic Performance of VEGF, BCL-2, p63, and Serum β-hCG in Choriocarcinoma as shown in **Table (3)** and **Fig. 4**, BCL-2, p63, and serum β-hCG demonstrated superior sensitivity and specificity compared to VEGF in diagnosing choriocarcinoma. BCL-2, p63, and β-hCG achieved 100% and 90% sensitivity, respectively, 56.4%, 100%, and 99% specificity, respectively. In contrast, VEGF had 80% sensitivity and 56.4% specificity. The combined expression of VEGF, BCL-2, p63, and β-hCG exhibited 100% specificity and 70% sensitivity for

detecting choriocarcinoma, as summarized in Table (3).

Diagnostic utility of VEGF, BCL-2, p63 in discriminating hydropic abortion and molar pregnancy

VEGF has lower sensitivity in differentiating hydropic abortion from molar pregnancy was (72.7% in partial mole and 54.5% in complete mole, respectively). Moreover, very low sensitivity and specificity of differentiating partial mole from complete mole (52.2% ,32%) as shown in Table (4)

BCL2 has more sensitivity in differentiating hydropic abortion from complete mole (81.8%) than partial mole (72.7%), While, it was slightly more sensitive than VEGF in discrimination of partial from complete mole (69.6%) as shown in Table (4).

P63 was more specific and sensitive (100% sensitivity and specificity) in differentiating hydropic abortion from complete mole & in hydropic abortion Vs partial mole, the sensitivity was 90.9% with 74% specificity. While comparing P63 in discriminating partial and complete mole, it was the lowest sensitive one among the studied markers (26.1% and 64% specificity) as shown in Table (4).

Spearman correlation between the studied markers

There is a statistical correlation between VEG and both BCL2 & β-hCG as shown in **Fig .5** and (P =0.4 and p=0.20 respectively). There is a highly statistical correlation between BCL2 and both p63 and serum β-hCG (p = 0.00). There is a highly statistical correlation between p63 and serum β-hCG (p = 0.00).

Table (4): Diagnostic performance of VEGF, BCL-2, p63 in discriminating hydropic abortion from molar pregnancy.

VEGF	Sensitivity	Specificity	95% CI	AUC
Hydropic Vs partial	72.7%	31%	.302 - .714	.508
Hydropic Vs complete	54.5%	33.3%	.201 - .626	.413
Partial Vs complete	52.2%	32%	.233 -.566	.399
Bcl2	**Sensitivity**	**Specificity**	**95% CI**	**AUC**
Hydropic Vs partial	72.7%	30.4%	.473 -.961	.717
Hydropic Vs complete	81.8%	10%	.546 -1.00	.777
Partial Vs complete	69.6%	14%	.194 - .517	.356
P63	**Sensitivity**	**Specificity**	**95% CI**	**AUC**
Hydropic Vs partial	90.9%	74%	.675 -.974	.824
Hydropic Vs complete	100%	100%	.624 -.951	.788
Partial Vs complete	26.1%	64%	.279 -.618	.449

VEGF: Vascular endothelial growth factor; BCL-2: B-cell lymphoma -2; p63:Tumor protein 63; AUC: Area Under the Curve; CI: Confidence Interval

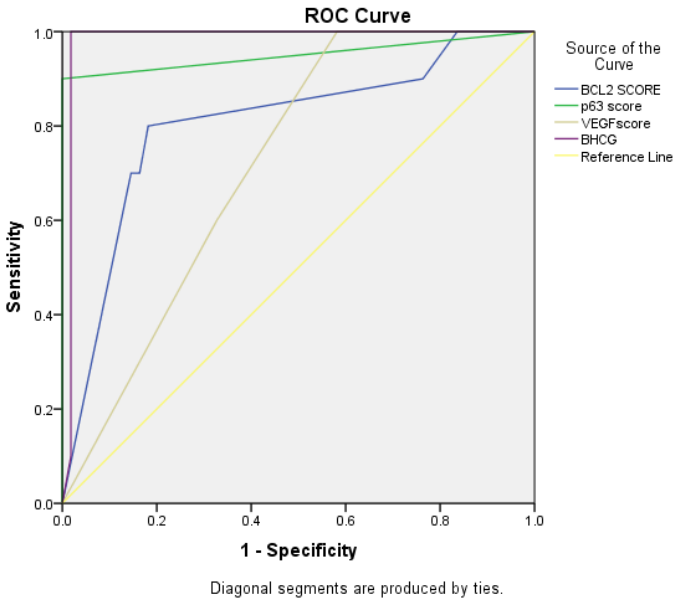

Fig.4:ROC curve of VEGF, BCL-2 and P63 and serum β-hCG diagnosis of choriocarcinoma.

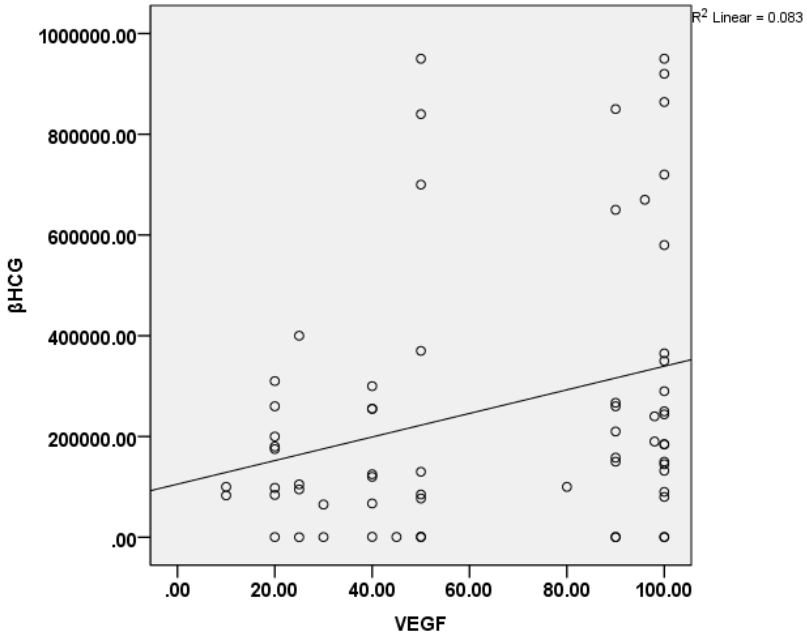

Fig. 5: Scatter diagram shows linear correlation between VEGF and serum β-hCG level.

Discussion

Gestational trophoblastic diseases is a diverse group of conditions characterized by abnormal growth of placental tissue. These disorders vary in their severity and can range from benign to malignant. The World Health Organization (WHO) classifies GTD into three categories: tumor-like lesions, molar pregnancies, and gestational trophoblastic neoplasms [15].

The present work aims to study expression pattern of VEGF, BCL2, P63 and serum β-hCG in hydropic pregnancy, partial mole, complete mole and choriocarcinoma to elucidate their role in detection the risk

of progression from benign trophoblastic lesions (hydropic abortion and molar pregnancy) to choriocarcinoma, and assessment of diagnostic utility of theses markers in discriminating malignant lesion and hence target therapy. In the current study, there was a highly significant difference in expression of VEGF among the studied cases (p=0.000).

This agrees with previous study [1 , 16] which found that VEGF expression in choriocarcinoma was higher than that in molar pregnancy suggesting that VEGF may be involved in the progression of trophoblastic diseases.

In the current study, highest levels of VEGF were found in the choriocarcinoma group when compared to hydropic abortion, partial, or complete mole with high sensitivity (80%) in discriminating this kind of Gestational trophoblastic neoplasms. This agrees with previous investigation ([8] demonstrated that VEGF was overexpressed in choriocarcinoma cell line inducing invasion and metastasis. Moreover, another study [16] demonstrated that choriocarcinoma tissue showed strong expression for VEGFR-3, confirming high expression of VEGF and its receptor in gestational trophoblastic disease.

Cytokines, including vascular endothelial growth factor (VEGF), play a crucial role in regulating the biological behaviors of trophoblasts, such as proliferation, differentiation, and invasion. VEGF- a potent mitogen and vasopermeability factor for endothelial cells- is expressed in both villous syncytiotrophoblasts and extravillous cytotrophoblasts during gestation, as well as in decidual cells [17].

Vascular endothelial growth factor (VEGF) is a crucial mediator of angiogenesis involved in various physiological and pathological processes, including embryogenesis, tumor growth, invasion, and metastasis. VEGF binds to corresponding receptors, leading to endothelial cell proliferation, tumor neovascularization, and promotion of tumor growth, invasion, and metastasis. While VEGF primarily targets endothelial cells, it can also influence non-endothelial cells, such as placental trophoblasts, which exhibit endothelial cell characteristics [18].

A previous study [19] discovered that VEGF and its receptors were highly expressed in trophoblastic tumors. As VEGF has a role in controlling angiogenesis. So, targeting VEGF and its receptors may be a promising therapeutic approach for patients with GTN.

In the present study, VEGF effectively differentiated between hydropic abortion and molar pregnancy, with a sensitivity of 72.7% in partial moles and 54.5% in complete moles. These findings align with previous work [1] reported the highest VEGF levels in the control group (abortion) compared to partial and complete hydatidiform moles, demonstrating a statistically significant difference ($p < 0.0001$). concluded that VEGF expression rates were elevated in spontaneous abortion materials compared to other GTD groups. In the first trimester of gestation, the simultaneous increase in VEGF and TGF-β1 expressions suggests their collaborative role in angiogenesis, a critical process for embryonic implantation and placentation.

Apoptosis plays a pivotal role in normal placental development and the pathogenesis of gestational trophoblastic neoplasia. BCL-2, an anti-apoptotic protein discovered decades ago, regulates cell death and survival without directly affecting cell proliferation [9]. In this study, BCL-2 expression was higher in hydropic abortion (63.6%) and partial moles (82.6%) compared to complete moles (81%) and choriocarcinoma cases (80%), with a highly significant difference ($p = 0.000$). Furthermore, BCL-2 demonstrated 100% sensitivity and 56.4% specificity in distinguishing choriocarcinoma from other gestational trophoblastic diseases.

A previous study [4] found that negative BCL-2 expression in choriocarcinoma cases is associated with increased

apoptotic cell death and necrosis. This aligns with our study's findings. Negative or weak BCL-2 expression in trophoblast cells may be due to the presence of microRNAs that inhibit BCL-2.

A previous study [20] identified 30 types of microRNAs involved in apoptosis, some with anti-apoptotic and others with pro-apoptotic functions. A previous study [21] discovered that miR-15a and miR-16-1 suppress BCL-2 expression, leading to apoptosis in leukemia cell lines.

A previous study [22] suggested that the lack of BCL-2 protein in choriocarcinoma syncytiotrophoblasts (STB) may contribute to the disorganization of villous structures and the prevalence of proliferation sites (weak p16 staining. In choriocarcinoma, increased apoptosis often coincides with increased proliferation.

Contrary to our study, previous study [23] observed significantly stronger BCL-2 expression in the syncytiotrophoblasts of CHM and choriocarcinoma compared to normal placentas and PHM. These discrepancies in expression patterns could be attributed to factors such as fixation, processing, antibodies used for immunohistochemistry (IHC), and antigen retrieval protocols.

Our data revealed also that BCL-2 has high sensitivity in differentiating hydropic abortion from both PHM and CHM (76.2% & 81.8%). This finding is in line with the previous works [4, 9 ,24 ,25]. In agreement with the current work, a study [26] found that a significantly increased BCL-2 expression pattern was observed in HA compared with PHM and CHM.

The decreased expression of BCL-2 in CHM compared to PHM and normal control tissues suggests an increase in apoptosis and excessive trophoblastic cell proliferation, characteristic of CHM. In addition to its anti-apoptotic role, BCL-2 can function as an anti-proliferative protein by preventing cell cycle progression to the S-phase. Therefore, decreased BCL-2 expression in CHM may indirectly lead to trophoblastic proliferation by making them more susceptible to mitotic stimuli [9]. This finding indicates that BCL-2 could potentially serve as a marker for differentiating between hydropic abortions and molar pregnancies in challenging cases.

However, these data contrast with another study [10] that found the inverse; increased BCL-2 expression in CHM. In this opinion, these discrepancies in the results can be attributed to the use of different antibody clones and retrieval methods.

In our series, p63 is expressed in cytotrophoblasts which decreased gradually from hydropic abortion that showed strong expression in (80.8%) to PHM highly expressed in 17% of cases and CHM in 23.5% of the studied cases to be negatively expressed in 90% of choriocarcinoma cases with highly significant correlation ($p = 0.000$).

Previous work [26] intriguingly observed that p63 expression was absent in choriocarcinoma but prominent in partial hydatidiform moles compared to hydropic abortions. While partial hydatidiform moles are theoretically pre-neoplastic lesions, hydropic abortions are not. They hypothesized that p63 stains well-differentiated cytotrophoblasts, which are more abundant in partial hydatidiform moles than hydropic abortions. Conversely, the neoplastic cells in choriocarcinoma are less differentiated, potentially explaining the lack of p63 staining in these cells [27].

Moreover, The Roc curve analyzes the diagnostic utility of p63 in discriminating choriocarcinoma from other gestational trophoblastic diseases with 100% specificity and 90% sensitivity. Curiously, p63 expression was absent in choriocarcinoma.

This agrees with the previous study [26] which observed a marked decrease in p63 positivity in cytotrophoblastic cells in choriocarcinoma in relation to hydropic abortion, partial hydatidiform mole and complete hydatidiform mole. They

suggested that the lack of p63 expression in choriocarcinomas indicates that the p63 gene may be downregulated in these neoplasms.

Our study identified p63 as the most specific and sensitive marker, boasting 100% sensitivity and specificity in distinguishing hydropic abortion from complete mole, and a sensitivity of 90.9% with 74% specificity when differentiating hydropic abortion from partial mole, though it was the least sensitive marker (26.1% sensitivity and 64% specificity) in discriminating partial from complete mole among those studied. A previous work [28] found increased nuclear immunoreactivity of p63 in molar over non-molar gestations, highlighting a significant disparity in p63 expression between molar and non-molar abortions but not between PHM and CHM, reinforcing its role in molar pathogenesis [10]. Therefore, p63 has been recommended even in routine clinical practice to enhance HM diagnosis, particularly in resource-limited settings [29].

In contrast to another study [27] which stated that p63 is negative in cytotrophoblastic cells in all abortions. It might be a different number of the studied cases or variable method of assessment of p63 in his study.

The present investigation revealed a notable variation in the blood levels of β-hCG between the groups under investigation, with an elevated level observed in cases of choriocarcinoma as opposed to molar pregnancy or hydropic abortion (p= 0.000). In agreement with studies [30] & [31] who concluded that a plateau or increase in serum β-hCG level would suggest persistent GTD. Moreover, weekly measurements of serum β-hCG level is an important and reliable biomarker to early detection of developing of molar pregnancy to persistent GTN.

β-hCG is one of the most sensitive and specific markers in our current study in discriminating choriocarcinoma from other gestational trophoblastic lesions (100% sensitivity, 99% specificity). In agreement with previous study [32] ,in their study which demonstrated that the serum β-hCG is most sensitive and specific for diagnosis of the trophoblast related conditions, i.e., pregnancy and GTDs. An increasing level of total β-hCG is diagnostic of invasive disease and choriocarcinoma.

In this study, we determined the combined sensitivity and specificity of VEGF, BCL2, p63 and β-hCG level in distinguishing choriocarcinoma from non-neoplastic molar pregnancy and hydropic abortion. Though the sensitivity was (70%) and specificity was (100%). So, these markers could be used in prediction of choriocarcinoma formation from non-neoplastic lesions.

This study found a significant link between VEGF and blood β-hCG levels (p = 0.02), aligning with previous study [33], who noted a close relationship between β-hCG and VEGF, with β-hCG influencing VEGF expression. A study [34] observed that in a human endometrial cell culture model, β-hCG binds to HCG/LH receptors to enhance VEGF protein and gene expression in human endometrial cells.

The current study found a statistically significant direct correlation between VEGF and Bcl2 (p=0.04), aligning with previous study [35], who demonstrated that in both murine (4T1) and human (MDA-MB-231) metastatic mammary carcinoma cell lines, VEGF upregulated Bcl-2 expression while anti-VEGF antibodies reduced it, reflecting the levels of tumor cell apoptosis. VEGF, therefore, not only plays a role in angiogenesis and vessel permeability but also acts as a survival factor for tumor cells by inducing Bcl-2 expression and inhibiting tumor cell apoptosis. Additionally, in agreement with previous work [36], who found strong molecular connections between Tap63 and the BCL2 anti-apoptotic protein with a significant correlation at both the mRNA and protein levels, this study also found a highly significant direct correlation between BCL-2 and p63 (p=0.000). Prior research has documented the

overexpression of these two proteins in various cancer types, including basal cell carcinoma and thyroid cancer.

Conclusions

Positive IHC expression of VEGF, BCL-2, p63 and increase serum level of β-hCG in molar gestation and choriocarcinoma suggesting their role in gestational trophoblastic disease pathogenesis and progression. Combined elevated levels of β-hCG, and positive IHC expression BCL-2 and p63 might be useful to differentiate a choriocarcinoma from other gestational trophoblastic diseases and hence target therapy. Positive IHC expression of BCl2, p63 could be used in differentiating hydropic abortion from partial mole or complete mole. So, they could be used as markers for early diagnosis of molar pregnancy.

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