Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Beta- Catenin in Ovarian Tumors Among Sudanese Women

الكشف النسيجي الكيميائي المناعي عن البيتا- كاتينين في أورام المبيض لدى النساء السودانيات

A dissertation submitted for partial fulfillment for the requirements of master degree in medical laboratory science (Histopathology and Cytology)

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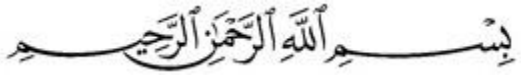
الآية



قال تعالى:

أَلَمْ نَشْرَحْ لَكَ صَدْرَكَ (1) وَوَضَعْنَا عَنْكَ وِزْرَكَ (2) الَّذِي أَنْقَضَ ظَهْرَكَ (3) وَرَفَعْنَا لَكَ ذِكْرَكَ (4) فَإِنَّ مَعَ الْعُسْرِ يُسْرًا (5) إِنَّ مَعَ الْعُسْرِ يُسْرًا (6)

صدق الله العظيم
سورة الشرح من الآية (1-6)

Dedication

To the soul of my father

Who taught me patience and success

Who miss him in the face of difficulties

To my mother

For his love, gentle care and support through life

Love you forever

To my sisters and brothers

Whose love flows in my veins, and my heart always remembers them

To my dear Mohammed

For his help and support

For all those times you stood by me

To my best friends

Thank you for the most beautiful moments I spent with you

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Abstract

This is hospital based analytical retrospective case control study was conducted at Soba university hospital, Omdurman military hospital, Omdurman maternity hospital (Khartoum state), and Sudan university of science and technology-college of medical laboratory science, during the period from September 2016 to February 2017. The study was aimed to detect beta-catenin expression in ovarian tumors, using immunohistochemistry.

Forty paraffin block samples were collected from patients previously diagnosed as ovarian tumors, 30(75%) were malignant and 10 (25%) were benign.

The paraffin blocks were cut by rotary microtome, then stained by immunohistochemical method (new indirect technique). The data obtained was analysed using SPSS program version 20.

The age of patients ranged between 16 and 88 with mean age of 44 years. Most patients were less than 50 years representing 31 (77.5%) and the remaining 9 (22.5%) were older than 50 years.

Beta-catenin expression was revealed positive result in 22 (55%) samples in malignant samples while 8 (20%) samples showed negative expression, while in benign ovarian tumors positive expression was found in 2 (5%) samples and 8 (20%) samples showed negative expression, this result showed significant association of beta-catenin expression and malignant ovarian tumors (P. value = 0.003).

Regarding the histopathological subtypes of malignant tumor, β-catenin expression was found positive in 18 (60%) samples of epithelial ovarian cancer, 1 (3.3%) sample of germ cell tumor, 2 (6.7%) samples of granulosa cell tumor and 1 (3.3%) sample of malignant mixed mullerian tumor, this result showed insignificant association between beta-catenin expression and histological subtype of malignant tumor (P. value = 0.796).

Regarding β-catenin expression and tumor grade, β-catenin were positive in 5 (16.7%) samples grade I, 2 (6.7%) samples grade II, 15 (50%) samples grade III, while negative expression was found in 3 (10%) samples grade I, 2 (6.7%) samples grade II and 3 (10%) samples grade III. This result showed insignificant association between beta-catenin expression and tumor grade (P. value = 0.284).

The study concluded that positive expression of β-catenin was associated with malignant tumors of ovarian and no association between β-catenin expression and histological subtype of malignant tumor as well as tumor grade.

المستخلص

أجريت هذه الدراسة المستشفوية التحليلية الإسترجاعية الحالة والحالة الضابطة في مستشفى سوبا الجامعي، المستشفى العسكري – أم درمان، مستشفى الولادة أم درمان (ولاية الخرطوم) وجامعة السودان للعلوم والتكنولوجيا- كلية علوم المختبرات الطبية، في الفترة من سبتمبر 2016 الى فبراير 2017 ، هدفت الدراسة للكشف عن ظهور البيتا – كاتينين في أورام المبيض بإستخدام كيمياء الأنسجة المناعية.

جمعت أربعون قالب شمعي من عينات مرضى كانو مشخصين مسبقا على أنهم مصابين بأورام المبيض، 30 (75%) منهم كانو مشخصين أورام مبيض خبيثة، و10 (25%) كانو مشخصين أورام مبيض حميدة. قطعت القوالب بإستخدام المشراح الدوار وصبغت بواسطة طريقة كيمياء الأنسجة المناعية (الطريقة الجديدة غير المباشرة) وأستخدم برنامج الحزم الإحصائية للعلوم الإجتماعية ،النسخة 20 لتحليل البيانات.

تراوحت أعمار المرضى بين 16-88 عام بمتوسط عمر 44 سنة. أظهرت الدراسة أن معظم المرضى كانت أعمارهم اقل من 50 سنة، وكان عددهم 31 مريض بنسبة (77.5%) و9 مريضا بنسبة (22.5%) كانت أعمارهم أكثر من 50 سنة.

أظهرت الدراسة أن البيتا- كاتينين كان موجب الظهور في 22 (55%) عينة من أورام المبيض الخبيثة وسالب الظهور في 8 (20%) المتبقية. بينما في أورام المبيض الحميدة كان موجب الظهور في 2 (5%) عينة وسالب الظهور في 8 (20%) المتبقية مع وجود علاقة ذات دلالة إحصائية بين إفراز البيتا- كاتينين وأورام المبيض (القيمة الأحتمالية =0.003)

فيما يتعلق بالبيتا- كاتينين والأنواع النسيجية للورم الخبيث، كان البيتا- كاتينين موجب الظهور في 18 (60%) عينة سرطان المبيض الظهاري، 1 (3.3%) عينة ورم الخلايا الجرثومية، 2 (6.7%) عينة ورم الخلايا الحبيبية وعينة واحدة (3.3%) ورم مولر المختلط . أظهرت الدراسة عدم وجود علاقة ذات دلالة إحصائية بين ظهور البيتا-كاتينين والنوع النسيجي للورم الخبيث (القيمة الأحتمالية = 0.796)

فيما يتعلق بالبيتا- كاتينين ودرجة تمايز الورم، البيتا- كاتينين كان موجب الظهور في 5 (16.7%) عينة من درجة التمايز الأولى، 2 (6.7%) عينة من درجة التمايز الثانية و15 (50%) عينة من درجة التمايز الثالثة. بينما كان سالب الظهور في 3 (10%) عينة من درجة التمايز الأولى، 2 (6.7%) عينة من درجة التمايز الثانية و3 (10%) عينة من درجة التمايز الثالثة. أظهرت الدراسة عدم وجود علاقة ذات دلالة إحصائية بين ظهور البيتا- كاتينين ودرجة الورم (القيمة الأحتمالية =0.284).

خلصت الدراسة إلى وجود علاقة بين إفراز البيتا- كاتينين وأورام المبيض وعدم وجود علاقة بين إفراز البيتا- كاتينين والنوع النسيجي للورم الخبيث أو درجة تمايز الورم.

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Chapter One
Introduction

1.1 Introduction

Ovarian cancer is any malignant tumor that develops in the ovarian tissues, ovarian cancer is commonly classified as epithelial ovarian carcinoma (EOC), ovarian germ cell tumor and sex cord-stromal tumor (Xiao-Ying and Pei-Ying, 2016).

Ovarian cancer is the 8th most common cancer among women, according to global estimates 225,000 new cases were detected each year (Saied, *et al*. 2016).

In Sudanese population ovarian cancer was the fourth most commonly diagnosed cancer among women after breast, leukemia and cervix (Elamin, *et al*. 2015).

The risk factors of ovarian cancer include age, hormone replacement therapy, hereditary breast and ovarian cancer, chronic inflammation, ethnicity, obesity, dietary factors and smoking (Monica, *et al.* 2009).

Methods of diagnosis of ovarian cancer is imaging tests (ultrasonography, computed tomography and magnetic resonance imaging), serum tumor biomarker (CA125, HE4), fine needle aspiration (FNA) and immunohistochemistry (IHC) (Melissa, 2015; Soumit, *et al*. 2016).

Treatments for ovarian cancer are surgery, chemotherapy, hormone therapy, targeted therapy (Michelle, *et al.* 2009; Danijela and Deborah, 2011).

β-catenin is a multifunctional protein plays important role in two pathways including cadherin-mediated adhesion at the plasma membrane by complexed with the cytoplasmic tail of E-cadherin, and as a main nuclear signal in the wnt/β-catenin pathway (Ai-hua, *et al*. 2015).

Ovarian tissue arrays were immunostained with β-catenin, results showed that β-catenin were significantly overexpressed in ovarian cancer samples as compared

with benign ovarian tumors and normal ovarian tissues. Positive expression rate of poorly differentiated was significantly higher than the well differentiated and moderately differentiated (Huanhuan, *et al*. 2015).

1.2 Objective:

1.2.1 General objective:

To detect β-catenin expression in ovarian tumors, by immunohistochemical method and its correlation with histopathological diagnosis and tumor grades.

Chapter Two

Literature Review

2.1 Scientific background:

Ovarian cancer is the most lethal gynecologic cancer. Less than one-half of patients survive for more than five years after diagnosis. Ovarian cancer affects women of all ages but is most commonly diagnosed after menopause. More than 75% of affected women are diagnosed at an advanced stage because early-stage disease is usually asymptomatic, the majority of cases are sporadic, and only 5% to 10% of ovarian cancers are familial **(**Christine, *et al*. 2000, Chyke, *et al*. 2016).

2.2 Structure of the ovary:

The ovaries are paired sex glands or gonads in female and are concerned with germ cell maturation, storage and its release. The ovary is covered by a single layer of cuboidal cells known as germinal epithelium. The substance of the gland consists of outer cortex which shows the structural changes during ovular cycle. The medulla consists of connective tissue, some unstripped muscles, blood vessels and nerves. Medulla also has hilus cells (Shivaji and Panchaksharayya, 2016).

The body of the ovary consist of spindle-shaped cell, fine collagen fibers and ground substance which together constitute the ovarian stroma which contain cells resemble fibroblasts and smooth muscle cells. In the peripheral zone of the stroma (cortex) are numerous follicles which contain female gametes in various stages of development. In addition, there may also be post-ovulatory follicles of various kinds, namely corpora lutea (responsible for estrogen and progesterone production), degenerate and former corpora lutea (corpora albicantes) and degenerate (atretic) follicles (Barbara and John, 2000).

2.3 Disorders of the ovary:

2.3.1 Benign disorders:

2.3.1.1 Serous cystadenoma:

Serous cystadenomas are common and account for approximately 25% of benign ovarian neoplasm, bilaterally in 12– 23% of cases, ovarian serous cystadenoma arises from the surface epithelium of the ovary depending on the amount of the fibrous tissue and are usually oval, about 3–10 cm in diameter, with a glistening surface and cystic fluid is clear to yellowish (Ahmad, *et al*. 2016).

2.3.1.2 Mucinous cystadenomas:

Mucinous cystadenoma are benign epithelial tumors that are typically multilocular, thin walled cysts with smooth external surface containing mucinous fluid. These are the largest tumors of ovary, of all ovarian tumors, mucinous tumors comprise 12% to 15% and 75% of all mucinous tumours are benign (Humera, *et al*. 2011).

2.3.1.3 Ovarian cystadenofibroma:

Ovarian cystadenofibroma is a relatively rare benign ovarian tumor that contains both epithelial and fibrous stromal components, that is seen in women aged 15–65 years. The routine imaging features of this tumor may mimic a malignant neoplasm, but the presence of the fibrous component often gives a specific/characteristic MRI appearance that may help differentiate it from malignant ovarian tumors (Ashish and Khaled, 2010).

2.3.1.4 Struma ovarii:

Struma ovarii is a very rare and usually benign ovarian tumor. It accounts for 0.3–1 % of all ovarian tumors and for 3 % of all mature teratomas. Struma ovarii is the most common form of monodermal teratoma and is characterized by the presence of macroscopically and histologically detectable thyroid tissue containing variable-sized follicles with colloid material, struma ovarii presents as a multi-cystic mass

with a peak incidence in the 5th decade of life and peak age at presentation of 50 years (Martine, *et al*. 2014).

2.3.1.5 Benign cystic teratomas:

Mature cystic teratoma is the most common ovarian neoplasm and affects mostly young patients. It is a benign germ cell tumor consisting of at least two of the three embryogenic germ cell layers, and usually contains ectodermal (skin, brain), mesodermal (fat, bone) and/or endodermal (thyroid tissue, gastrointestinal and bronchial epithelium) mature tissue (Pietro, *et al*. 2016).

2.3.1.6 Benign Brenner tumor:

Brenner tumors are composed of epithelial elements histologically resembling urothelium. Brenner tumors comprise less than 5% of all benign epithelial tumors. Most of them are found incidentally and are of less than 2cm in size. These tumors were seen in all age groups and 3.6% cases were seen in the pediatric age group. Microscopically, they show well defined small nests of transitional cells with intervening fibromatous stroma. Nuclei show typical grooved coffee bean appearance (Ghartimagar, *et al*. 2013).

2.3.1.7 Ovarian fibromas and fibrothecomas:

These are benign tumors of stromal origin. Fibromas originate from spindle cells producing collagen and can be associated with ascites. Fibrothecomas originate from both spindle and theca cells and may produce a small amount of estrogens. Fibromas and fibrothecomas can also show cystic areas, due to hemorrhage, edema or necrosis within the stromal tissue, frequently the lesions show little peripheral vascularity (Ahmad, *et al*. 2015).

2-3.2 Malignant disorders:

2.3.2.1 Serous cystadenocarcinoma:

Serous carcinomas are the most common form of ovarian carcinoma and make up 30-70% of all diagnoses, it is classified as low grade or high grade on the basis of

the extent of nuclear atypia and mitosis morphologically, low-grade serous carcinoma has minimal nuclear atypia, and mitoses are rare, high-grade serous carcinoma, on the other hand, is characterized by marked nuclear atypia and more mitoses. Low-grade and high-grade carcinomas are different at the genomic and molecular levels (Daniel, *et al*. 2009, Elham, 2013).

2.3.2.2 Mucinous cystadenocarcinoma:

The mucinous cystadenocarcinoma of the ovary accounts for 5-10% of all ovarian mucinous tumors even in adults. The mucinous tumors are filled with a mucus-like material, this mucus is produced by mucus-secreting goblet cells very similar to the cells lining normal intestine. These tumors may become very large. The cystadenocarcinomas contain a more solid growth pattern with the hallmarks of malignancy cellular atypia and stratification, loss of the normal architecture of the tissue, and necrosis. The appearance can look similar to colonic cancer. The mucinous ovarian cancer sometimes associated with pseudomyxoma peritonei (Jitendra, *et al*. 2015).

2.3.2.3 Clear cell adenocarcinoma:

Ovarian clear-cell adenocarcinoma (CCA) accounts for 10% of all epithelial ovarian cancers. It typically occurs at a younger age and diagnosed at an early-stage disease, clear cell carcinoma has also been associated with endometriosis and displays the following architectural and cytological features papillary, tubulocystic or solid architecture; hobnail tumor cells with clear cytoplasm (Catherine, *et al*. 2015, Lauren, *et al*. 2015).

2.3.2.4 Endometrioid adenocarcinomas:

Ovarian endometrioid carcinoma (ECs) comprises 10–20% of all epithelial ovarian cancer cases, highly resemble endometrioid carcinomas of the uterus in morphology, occur most frequently in women of premenopausal age, and most are found at an

early stage Most ECs are low-grade adenocarcinomas and seem to arise from endometriotic cysts, up to 42% of cases have evidence of endometriosis (Prat, 2012).

2.3.2.5 Malignant Brenner tumors:

Malignant Brenner tumors are very rare, they are large, friable tumors, partly cystic, which show histologically a continuity between benign mesonephric-type nests and malignant epithelial tumor tissue that is of transitional, squamous, or glandular type (Albert, *et al*. 2003).

2.3.2.6 Malignant germ cell tumors:

Ovarian malignant germ cell tumors (OMGCTs) are heterogeneous tumors that are derived from the primitive germ cells of the embryonic gonad. OMGCTs are rare, accounting for about 2.6% of all ovarian malignancies, and typically manifest in adolescence. Dysgerminoma, the most common malignant germ cell tumor, usually manifests as a solid mass. Immature teratomas manifest as a solid mass with scattered foci of fat and calcifications. Yolk sac tumors usually manifest as a mixed solid and cystic mass. Capsular rupture or the bright dot sign, a result of increased vascularity and the formation of small vascular aneurysms, may be present. Embryonal carcinomas and polyembryomas rarely manifest in a pure form and are more commonly part of a mixed germ cell tumor. Malignant ovarian germ cell tumors spread in the same manner as epithelial ovarian neoplasms but are more likely to involve regional lymph nodes. Suspicious areas may be sampled during surgery. Because OMGCTs are almost always unilateral and are chemosensitive, fertility-sparing surgery is the standard of care (Shaaban, *et al*. 2014).

2.3.2.7 Sex cord stromal tumor:

Sex cord–stromal tumors of the ovary are rare, making up approximately 8% of all ovarian neoplasms derived from the sex cords and specialized stroma of the developing gonad. Stromal tumors may arise from female-type (granulosa, theca) and male-type cells (Sertoli, Leydig) as well as other indifferent sex cord derivatives.

Sex cord–stromal tumors of the ovary affect all age groups and account for most of the hormonally active ovarian tumors that show estrogenic effects or virilization, approximately 70% of patients with these tumors are classified as having stage I lesions at presentation. The most common stromal tumor is the granulosa cell tumor (Seung, *et al*. 2005, Melissa and Daniel, 2010).

Granulosa cell tumours of the ovary are unique, hormonally active, oestrogen-secreting tumours accounting for 1 to 2% of all the ovarian malignancies. These are rare tumours, existing in two forms: the adult form and the even rarer juvenile form. These tumours present as predominantly solid lesions (Karan, *et al*. 2016).

2.3.2.8 Malignant mixed mullerian tumor:

Malignant mixed mullerian tumor of the Ovary (OMMMT), also referred to as carcinosarcoma is a very rare tumor accounting for less than 1% of all ovarian cancers, these tumors are composed of both malignant epithelial and mesenchymal elements, it occurs most commonly in postmenopausal women of low parity (Mustafa, *et al*. 2016).

2.4 Epidemiology of ovarian cancer:

Ovarian cancer is the eighth most common cancer among women, and it includes about 4% of all women's cancers this disease has high morbidity and mortality rates among cancers of the reproductive system according to global estimates 225,000 new cases were detected each year, and 140,000 people annually die from the disease (Saied, *et al*. 2016).

In a more recent data set (2009-2010) from the national cancer registry for Khartoum state alone, ovarian cancer was the fourth most common cancer in women, with an estimated incidence rate of 188 per 100,000 population, a gender-specific rate of 8.0 per 100,000 population, and an age-standardized rate (ASR) of 7.0 per 100,000 population furthermore, neither the morality rate for ovarian cancer nor the survival rate in Sudan has previously been described, the majority of patients presenting with

advanced stage disease were not thoroughly investigated or treated symptomatically (Dafalla, *et al*. 2016).

2.5 Risk factors of ovarian cancer:

2.5.1 Age:

The risk for ovarian cancer increases with age. The majority of patients are postmenopausal, with 80% of cases diagnosed being older than 50 years, and a peak incidence of 61.8 per 100,000 women is observed in the 60–64 year old age group (Jermaine, *et al*. 2015).

2.5.2 Family history:

The greatest risk factors of ovarian cancer are a family history and associated genetic syndromes, women with a strong family history of breast and/or ovarian cancer are ahigh-risk group who may carry a mutation of the *BRCA1* and *BRCA2* genes. These women have a risk of ovarian malignancy of up to 50 % (Cleola and Michael, 2003).

2.5.3 Hormone replacement therapy:

Application of hormone replacement therapy (HRT) was found to be a risk factor for ovarian cancer. An approximately 22% increased risk of ovarian cancer over 5 years was seen in postmenopausal women using unopposed estrogen as HRT. The risk was still significantly increased (by approximately 10%) by the application of a combination of estrogen and progestin, the incidence of ovarian cancer increased with longer duration of HRT therapy (Felicitas and Theresia, 2014).

2.5.4 Obesity:

Increased body weight was associated with an increased risk of ovarian cancer; particular, in the premenopausal period (Liu, *et al*. 2015).

2.5.5 Chronic inflammation:

Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors, such as the use of

talcum powder (talc) in the pelvic region and epithelial ovarian cancer, other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection) found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis (Merritt, *et al*. 2008).

2.5.6 Dietary factor:

Epidemiologic studies have suggested that some dietary factors may play a role in the etiology of ovarian cancer, suggested that ovarian cancer risk was positively associated with higher consumption of dietary cholesterol and eggs and inversely associated with higher intake of total vegetables and cruciferous vegetables and supplementation of vitamin E (Pan, *et al*. 2004).

2.5.7 Smoking:

Cigarette smoking regarding as a potential risk factor for ovarian cancer ,The strongest association appears to be with mucinous ovarian tumors, while the association with other histological types is less certain (Mette, *et al*. 2013).

2.5.8 Ethnicity:

The rates of epithelial ovarian cancer are higher in white women compared to black women. Ethnicity does play a role in incidence, as women of Ashkenazi Jewish descent are at greater risk for BRCA1/2 mutations, which gives them an over-representation in the numbers of hereditary ovarian cancer cases (Monica, *et al*. 2009).

2.6 Diagnosis of ovarian cancer:

2.6.1 Imaging tests:

2.6.1.1 Ultrasonography:

Transvaginal ultrasonography (TVS) has been shown to be the most effective means to screen for ovarian cancer. TVS is accurate in detecting abnormalities in ovarian volume and morphology, but is less reliable in differentiating benign from malignant

ovarian tumors, TVS is sensitive, but has a low positive predictive value (John and John, 2014)

2.6.1.2 Computed tomography (CT):

CT is the preferred technique in the pretreatment evaluation of ovarian cancer to define the extent of disease and assess the likelihood of optimal surgical cytoreduction. CT has been shown to predict suboptimal cytoreduction with sensitivity of 79% and specificity of 75%. However, accuracy varies considerably among institutions, CT is also particularly helpful in the identification of fat components in mature cystic teratomas (Veena and Susanna, 2010).

2.6.1.3 Magnetic resonance imaging (MRI):

MRI is an essential problem-solving tool to determine the site of origin of a pelvic mass, and is effective for the diagnosis and accurate characterization of a wide spectrum of ovarian masses as non-neoplastic or neoplastic (Pietro, *et al*. 2016).

2.6.2 Fine needle aspiration cytology (FNAC):

Image guided FNAC from ovarian lesions are being increasingly used for diagnosis of ovarian lesions. FNAC can reliably distinguish between benign and malignant ovarian lesions. FNAC helps in reaching an early diagnosis in advanced ovarian cancers (Soumit, *et al.* 2016).

2.6.3 Serum tumor biomarker:

2.6.3.1 Cancer antigen 125 (CA125):

Cancer antigen 125 (CA-125) is a high molecular weight glycoprotein that is expressed by a large proportion of epithelial ovarian cancers. Elevated CA 125 values most often are associated with epithelial ovarian cancer, so CA-125 has become well established as a tumor marker for epithelial ovarian cancer. However, the sensitivity and specificity of CA-125 is known to be poor. It is only raised in approximately 50% of stage I epithelial ovarian cancers and in 75% to 90% of

patients with advanced disease and false-positive results have been noted in many medical disorders, both malignant and benign (Jose, *et al*. 2011).

2.6.3.2 Human epididymis protein 4 (HE4):

HE4 is a glycoprotein secreted by müllerian epithelia of the female reproductive tract as well as male epididymis, its role as a potential biomarker for ovarian cancer and HE4 may serve as a useful prognostic biomarker for ovarian. Elevated levels of HE4 are associated with increases stage, grade, and is strongly expressed by the most common ovarian tumor subtypes (serous and endometrioid). HE4 is both more sensitive and specific than CA-125 in detecting early-stage ovarian cancer and is not associated with benign conditions to the same degree, enabling HE4 to distinguish malignant ovarian tumors from benign cystic lesions (Alison and Ronny, 2010, Archana, *et al*. 2013)

2.6.4 Immunohistochemistry:

Immunohistochemistry (IHC), which are useful in the diagnosis of ovarian tumors (mostly neoplasms but also a few tumor-like lesions), each of the three main categories of ovarian tumors has distinctive immunohistochemical features and stains can be used to suggest or confirm a diagnosis. IHC is often useful to differentiate between primary ovarian adenocarcinoma and metastatic adenocarcinomas specially those of colorectal origin (Divya and Mandakini, 2013).

2.7 Treatment of ovarian cancer:

2.7.1 Surgery:

Surgery is the primary treatment for ovarian cancer. It is used for staging and cytoreduction (debulking), surgical staging involves total abdominal hysterectomy, bilateral salpingo-oophorectomy (BSO), and the removal of pelvic and para-aortic lymph nodes and omentum; as well as other supplemental procedures; unilateral salpingo-oophorectomy, preserving the uterus and contralateral ovary, is an option for women with early stage invasive epithelial ovarian cancers, lesions with low

potential for malignancy (lesions with histologically abnormal cells that are judged to have a low likelihood of developing into cancer), germ cell tumors, or sex cord–stromal tumors (Michelle, *et al*. 2009, Chyke, *et al*. 2016).

2.7.2 Chemotherapy:

Chemotherapy is the main standard adjuvant treatment for ovarian carcinoma, chemotherapy-based platinum and paclitaxel is currently considered the standard treatment after surgical staging and resection of abdominal and pelvic disease. A high proportion of patients (60–80%) with advanced ovarian epithelial cancer respond to first-line chemotherapy, but most of these patients (about 70%) will later have disease progression and thus be candidates for second-line chemotherapy (Alberto, *et al*. 2010).

2.7.3 Targeted therapy:

The use of targeted biologic agents is the effective treatment of recurrent ovarian cancer, a variety of molecular targeted agents, the majority of which are monoclonal antibodies and small-molecule protein-kinase inhibitors, have been explored in the management of ovarian cancer. The antiangiogenic agent, bevacizumab, has been reported as the most effective targeted agent that target the vascular endothelial growth factor (VEGF) pathway (Hiroaki, 2010).

2.7.4 Hormone therapy:

Hormone therapy is classified under other drugs that are potentially effective as approved treatment for recurrent forms of epithelial ovarian cancer, which include tamoxifen (antiestrogen) (Yoshihito and Hideki, 2013).

2.8 Beta-catenin and its relation with ovarian cancer:

β-catenin is a multifunctional protein plays important role in two pathways, it interacts with E-cadherin at the cell surface, forming a cadherin–catenin unit which effect on the formation of intercellular adherens junctions, and it is a key mediator in the wnt signaling pathway which regulates cell proliferation and differentiation.

Dysregulation of these pathways allow β-catenin to accumulate and translocate to the nucleus, where it may activate oncogenes. Such nuclear accumulation can be detected by immunohistochemistry (Tony, *et al*. 2005).

Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer (Morin, 1999).

In previous study ovarian tissue arrays were immunostained with β-catenin, results showed that β-catenin were significantly overexpressed in ovarian cancer samples and low or no expression in benign ovarian tumors and normal ovarian samples positive expression rate of poorly differentiated was significantly higher than the well differentiated and moderately differentiated (Huanhuan, *et al*. 2015).

Carlos, *et al* reported that the immunohistochemistry (IHC) expression pattern of β-catenin has been correlated with β-catenin gene mutations, clinicopathological features, and disease outcome in 69 stage I and II ovarian carcinomas. β-catenin expression was localized in the nuclei, in addition to the cytoplasm and membrane (Carlos, *et al*. 1999).

Fatemeh, *et al* reported that the immunohistochemistry (IHC) staining of β-catenin demonstrated a defect in the wnt signaling pathway in 25% of ovarian endometrioid adenocarcinomas. The study concluded that, in all the different subtypes of ovarian carcinomas, observed a significant association with decreased β-catenin expression to tumor grade as well as in serous carcinomas with increased nuclear grade, mitosis, and tumor grade **(**Fatemeh, *et al*. 2015).

Chapter three

Materials and Methods

3.1 Materials:

Archived tissue blocks of ovarian tumors were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is hospital based analytical retrospective case control study aimed to detect β-catenin expression in benign and malignant ovarian tumors.

3.2.2 Study sample:

Forty paraffin block samples were collected from patients previously diagnosed as ovarian tumor**,** 30 (75%) of them were malignant and the remaining 10 (25%) were benign. Patient's identification information (age, histopathological diagnosis, malignant tumor grade) were obtained from patient's records.

3.2.3 Study area:

This study conducted at Omdurman military hospital, Soba university hospital and Omdurman maternity hospital (Khartoum state).

3.2.4 Sample processing:

Section was cut at 3μm thickness by rotary microtome, mounted in positively charged slides.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using new indirect-dextran polymer immune peroxidase technique. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol (100%, 90%, 70%, 50%) to water. The antigens were retrieved using Dako water path with tris EDTA buffer (pH 9) for 5 minutes and then cooled down to room temperature for 20 min. Endogenous peroxidase activity was blocked by 3% peroxidase blocker for 10 minutes. The slide then treated with anti β-catenin primary antibody for 20 min at room temperature in a humid chamber, then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then sections incubated in dextran polymer-HRB (horseradish peroxidase) secondary

antibody for 15 minutes then washed in three changes of phosphate buffer saline, after that incubated in 3, 3 diaminobenzidine tetrahydrochloride substrate solution for 5 minutes, then washed in running water. Then counter stained in Mayer's haematoxylin stain for one minute. After that dehydrated, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Result interpretation:

All quality control measures were adopted. Positive and negative control slides were used during immunohistochemical staining. Positive staining for beta-catenin appeared as brown particles at the nucleus, nuclear membrane or in the cytoplasm. Under microscopy, 5 representative high-power fields (×40 magnification) per tissue section were randomly selected and evaluated. Detection of more than 5 cells per one field considered as positive result.

3.2.7 Data analysis:

Data was analyzed using SPSS 20 computer program. Frequency, mean, standard deviation and chi-square test values were calculated.

3.2.8 Ethical consideration:

Samples were collected after taking ethical approval from each hospital to use the tissue blocks for research purposes.

Chapter four

4. Results

The study includes forty samples, 30 (75%) samples were malignant tumors and 10 (25%) samples were benign tumors.

The age of study population range between 16 and 88 years with mean age of 44 years. Most patients were less than 50 years representing 31 (77.5%) and the remaining 9 (22.5%) were more than 50 years as indicated in table (4.1).

The histopathological diagnosis of study population includes 24 (60%) epithelial ovarian cancer, 2 (5%) germ cell tumors, 3 (7.5%) granulosa cell tumor, 1 (2.5%) malignant mixed mullerian tumor and 10 (25%) benign ovarian tumor as showed in table (4.2).

The tumor grade of study samples revealed 8 (26.7%) grade I, 4 (13.3%) grade II and 18 (60%) grade III as showed in table (4.3).

Beta-catenin positive expression was found in (22/30) samples in malignant samples and (8/30) samples showed negative expression, while in benign ovarian tumors positive expression was found in (2/10) samples and (8/10) samples showed negative expression for beta-catenin. This result showed significant association (P. value = 0.003) as indicated in table (4.4).

Types of malignant tumor showed positive expression for beta-catenin in 18 (60%) samples of epithelial ovarian cancer samples, 1 (3.3%) sample of germ cell tumor, 2 (6.7%) samples of granulosa cell tumor and 1 (3.3%) sample of malignant mixed mullerian tumor. This result showed no association between beta-catenin expression and types of malignant tumor (P. value = 0.796) as showed in table (4.5).

Beta-catenin positive expression was found in 5 (16.7%) samples of grade I, 2 (6.7%) samples of grade II, 15 (50%) samples of grade III, while negative expression revealed 3 (10%) samples of grade I, 2 (6.7%) samples of grade II and 3 (10%) samples of grade III. This result showed no significant association between beta-catenin expression and tumor grade (P. value = 0.284) as showed in table (4.6).

Table (4.1): Distribution of age group among the study population

Age group	Frequency	Percentage
Less than 50 years	31	77.5%

More than 50 years	9	22.5%
Total	40	100%

Table (4.2): Frequency of histopathological diagnosis among the study population

Histopathological diagnosis	Type	Frequency	Percentage

Benign	Benign ovarian tumor	10	25%
Malignant	Epithelial ovarian cancer	24	60%
	Germ cell tumor	2	5%
	Granulosa cell tumor	3	7.5%
	Malignant mixed mullerian tumor	1	2.5%
Total		40	100%

Table (4.3): Distribution of malignant tumor grades

Grade	**Frequency**	**Percentage**
Grade I	8	26.7%

Grade II	4	13.3%
Grade III	18	60%
Total	30	100%

Table (4.4): Relation between histopathological diagnosis and β-catenin expression

	Beta- catenin expression	P. value

Histopathological diagnosis	Positive N (%)	Negative N (%)	
Benign	2 (5%)	8 (20%)	0.003
Malignant	22 (55%)	8 (20%)	
Total	24 (60%)	16 (40%)	

Table (4.5): Relation between β- catenin expression and types of malignant tumor

	Types of malignant tumor	Total	P. value

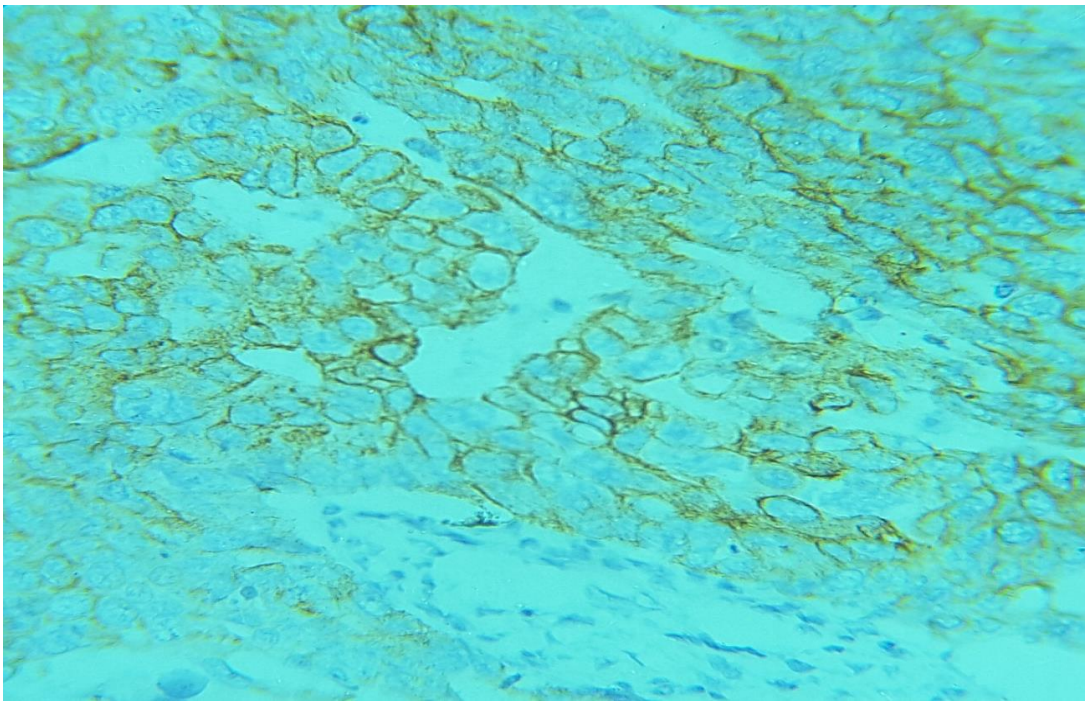
B- catenin expression	Epithelial ovarian cancer	Germ cell tumor	Granulosa cell tumor	MMMTs		
Positive	18 (60%)	1 (3.3%)	2 (6.7%)	1 (3.3%)	22 (73.3%)	0.796
Negative	6 (20%)	1 (3.3%)	1 (3.3%)	0 (0.0%)	8 (26.7%)	
Total	24 (80%)	2 (6.7%)	3 (10%)	1 (3.3%)	30 (100%)	

*MMMTs: malignant mixed mullerian tumors.

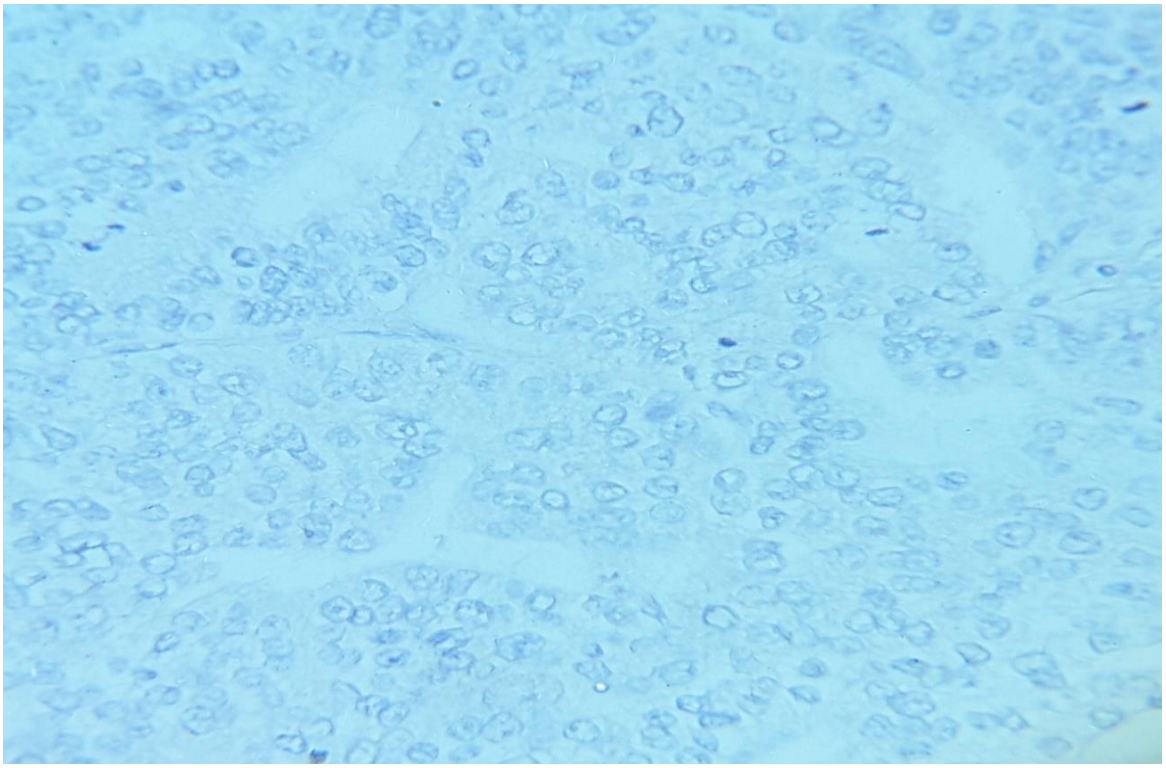
Table (4.6): Relation between β- catenin expression and malignant tumor grade

B- catenin expression	**Tumor grade**			**Total**	**P. value**
	Grade I	**Grade II**	**Grade III**		

Positive	5 (16.7%)	2 (6.7%)	15 (50%)	22 (73.3%)	0.284
Negative	3 (10%)	2 (6.7%)	3 (10%)	8 (26.7%)	
Total	8 (26.7%)	4 (13.3%)	18 (60%)	30 (100%)	



Microphotograph (4.1): Papillary serous cystadenocarcinoma showed membranous positive expression of β- catenin (40X).



Microphotograph (4.2): Papillary serous cystadenocarcinoma showed negative expression of β- catenin (40X).

Chapter Five

5. Discussion

The present study includes 40 samples of ovarian lesions stained by immunohistochemistry for beta-catenin. Concerning the age group of the study population, the study revealed that most of patients were less than 50 years indicating that women less than 50 years are more affected with ovarian cancer. This result is compatible with Mohammed *et al*. (2013), who reported that common involved age by ovarian carcinoma was the age group of 30-40 years. While disagree with Jermaine *et al*. (2015), who reported that risk of developing ovarian cancer increases with age. Also disagree with Dominic *et al*. (2013), who reported that 80% of all ovarian cancer cases are diagnosed after the age of 50 years.

The histopathological diagnosis of the study population revealed that more frequent type of ovarian cancer was epithelial ovarian cancer. This result is compatible with Robert, *et al*. (2011), who reported that epithelial ovarian cancer comprises most malignant ovarian neoplasms. Also agree with Colombo *et al*. (2010), who reported that about 90% of primary malignant ovarian tumors are epithelial carcinomas.

Beta-catenin expression was detected in (22/30) of malignant conditions compared to (2/10) in benign conditions with (P=0.003), which suggest that beta-catenin expression is more frequent expressed in malignant condition. This result is agree with Huanhuan *et al.* (2015), who reported that β-catenin was significantly overexpressed in ovarian cancers as compared with benign ovarian tumors. Also agree with Xiao *et al*. (2007), who reported that expression rates of beta-catenin in ovarian malignant and borderline epithelial tumors were higher than that in benign epithelial tumors.

The present study revealed that was no significant association between beta-catenin expression and type of malignant tumor. This result is agree with Huanhuan *et al*. (2015), who reported that was no significant association between the expression of β-catenin and pathological type. Also agree with Fatemeh *et al*. (2015), who reported that expression of β-catenin did not show a statistically significant relationship with tumor subtype.

This study found that no significant association between beta-catenin expression and cancer grade. This result disagree with Fatemeh *et al.* (2015), who reported that β-catenin expression was significantly associated with tumor grade. Also disagree with Huanhuan *et al*. (2015), who reported that clinicopathological correlation showed that overexpressed β-catenin were remarkably associated with histological grade. This may be due to the small size of our sample.

Chapter six

Conclusion and Recommendations

6.1 Conclusion:

From this study we conclude that:

- The age of ovarian cancer patients in our samples is commonly less than 50 years.
- Most histological type of ovarian cancer is epithelial ovarian cancer.
- Beta-catenin expression is associated with malignant tumors of ovary and no significant association between β-catenin expression and subtypes of ovarian malignant tumor as well as tumor grade.

6.2 Recommendations:

From this study we recommended that:

- Further study should be done for expression of β-catenin in ovarian tumors with large sample size.
- Beta-catenin should be used on panel of the epithelial markers to help in differentiation between benign and malignant ovarian tumors.

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Appendices

Appendix 1:

Materials and instruments for processing and staining of the specimens, include:

- Disposable gloves.
- Rotary microtome
- Microtome knives.
- Positively charged slides (thermo).

- Cover glasses.
- Dry oven.
- Water path (Dako water path)
- Coplin jars.
- Humidity chamber.
- Ethanol (100%, 90%, 70%, 50%)
- Xylene.
- Mayer's haematoxylin
  (Haematoxylin, DW, K or ammonium alum, sodium iodate, citric acid, chloral hydrate).
- Tris EDTA buffer (PH 9).
- Phosphate buffer saline (PH 7.4).
- Peroxidase blocker
  (0.3% hydrogen peroxide in methanol).
- Primary antibody (anti- human β- catenin).
- Secondary antibody (dextran polymer conjugated secondary antibody-HRP).
- DAB (3, 3 diaminobenzidine tetrahydrochloride) substrate solution.
- DPX

Appendix 2:

Dako

CE

Monoclonal Mouse
Anti-Human
Beta-Catenin
Clone β-Catenin-1

Code M3539

ENGLISH

Intended use
For In Vitro Diagnostic Use

Dako Monoclonal Mouse Anti-Human Beta-Catenin, Clone β-Catenin-1 (Anti-beta-catenin, β-catenin-1) is intended for laboratory use to identify qualitatively by light microscopy β-catenin positive cells in normal and neoplastic tissues using immunohistochemical (IHC) test methods. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made within the context of the patient's clinical history and other diagnostic tests by a qualified individual.

Summary and explanation
The catenins are structurally related cytoplasmic proteins which have been classified as alpha (α), beta (β), and gamma (γ) according to their electrophoretic mobility.[2,3] The β-catenin gene is located on chromosome 3p21 and encodes a 88 kD protein.[2,3] This cytoplasmic protein is multi-functional, playing an essential role in the cadherin-mediated anchoring and organization of the cytoskeleton.[2] Beta-catenin is also involved in regulation of gene expression as a mediator of the Wnt signaling pathway. Cellular β-catenin levels are tightly regulated by a multi-protein complex comprised of serine/threonine kinase GSK3β, the APC tumor suppressor gene product and axin, which facilitates phosphorylation and subsequent degradation of the β-catenin protein. Dysregulation of β-catenin degradation leads to cytoplasmic accumulation of the protein, followed by translocation to the nucleus. Nuclear β-catenin forms complexes with DNA binding proteins such as TCF and LEF, activating gene transcription.[4]

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Storage, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Reagent provided
Monoclonal Mouse antibody provided in liquid form as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clone: β-Catenin-1 Isotype: IgG_1, kappa
Mouse IgG concentration mg/L: See label on vial.

M3539 may be used at a dilution of 1:200 when performing IHC using the EnVision+, DAB (code K4006) detection system. These are guidelines only.

Optimal antibody concentrations may vary depending on specimen and preparation method, and should be determined by each individual laboratory.

Immunogen
Recombinant C-terminal β-catenin-GST fusion protein[1]

Specificity
Anti-beta-catenin, clone β-catenin-1 recognized human β-catenin protein in Western blots of human epithelial A431 cells and mouse β-catenin in blots of mouse fibroblast NIH/3T3 cells. No cross-reactivity with α and γ-catenin was observed.[1]

Materials required, but not supplied
Refer to Dako's *General Instructions for Immunohistochemical Staining* and/or the detection system instructions. Suggested diluent for IHC procedures:
Dako Antibody Diluent (code S0809).
The following negative control is recommended for IHC procedures:
Mouse IgG_1 (code X0931).

Precautions
1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN_3 may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[5]
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused reagents should be disposed of according to local, State, and Federal regulations.

Storage
Store at 2–8 ℃. Do not use after expiration date s tamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user.[5] There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation
Paraffin sections
Anti-beta-catenin, β-catenin-1 can be used on formalin-fixed, paraffin-embedded tissue sections.

The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure. Target retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95–99 ℃) or in a steamer (95–99 ℃). For greater adherence of tissue sections to glass slides, the use of silanized slides (Dako code S3003) is recommended. Target Retrieval Solution (code S1700) or 10x Concentrate (code S1699) is recommended using a 20-minute heating protocol.

Cryostat sections and cell smears
Anti-beta-catenin, β-catenin-1 can be used for labeling acetone-fixed cryostat sections or fixed cell smears. Target or antigen retrieval is not required.

Staining procedure
Follow the recommended procedure for the detection system selected.

Staining interpretation
The cellular staining pattern of anti-beta-catenin is mainly membranous, especially at the cell-cell boundaries. Positive nuclear staining and diffuse cytoplasmic staining have been reported in cancer cells.[7-16]

Performance characteristics
Normal tissues
Beta-catenin expression has been demonstrated in the membrane of normal epithelium. In normal urothelium, expression was found to be uniformly strong at the intercellular borders. More pronounced staining was observed at the apical junctional complexes of the superficial cell layer, whereas there was no expression at the luminal membrane and parts of the cells in contact with the basement membrane (*frozen and paraffin*).[17-19] In normal breast epithelium, normal ductal cells of breast within the lobular units stain in a peripheral and cytoplasmic pattern.[7] In normal mammary ducts and acini, β-catenin strongly localized to the basolateral surfaces of luminal epithelium; weak immunostaining was observed at lateral borders of myoepithelial cells (*paraffin*).[20] In normal colonic mucosa, β-catenin immunoreactivity was observed along the intercellular borders of all epithelial cells; no immunoreactivity was seen at the basal side facing the basement membrane or at the luminal cell border (*frozen and paraffin*).[8,9,15,21] In normal esophageal epithelium, staining was uniformly positive at the cell-cell boundaries (*frozen and paraffin*).[13,15,22] Expression of β-catenin, although present everywhere except in parakeratinized cells of normal esophageal epithelium, was more marked in the prickle cell layer than in the basal and parabasal layers.[22] In gastric epithelium, β-catenin immunoreactivity was in a membranous distribution throughout the epithelium of gastric crypts and glands, with increased intensity in deeper parts of antral, body, and cardiac glands.[10,15] Cytoplasmic and nuclear staining in normal gastric mucosal epithelium has also been observed *(paraffin*).[10] Normal thyroid follicular cells showed immunoreactivity mainly at cell-cell contacts, with the cytoplasm being weakly reactive (*paraffin*).[16]

Weak membrane-localized immunoreactivity has also been observed in endothelial cells, muscle cells, and neurons (*paraffin*).[10,18]

Abnormal cells
Beta-catenin expression has been demonstrated by immunohistochemistry in a variety of tumors. Neoplasms which have demonstrated positive immunoreactivity for β-catenin include bladder transitional cell carcinoma, colon adenocarcinoma and adenomas, breast adenocarcinoma, esophageal squamous cell carcinoma, primary squamous cell carcinomas of the head and neck, stomach adenocarcinoma, ovarian carcinoma and thyroid carcinoma.[7-24] In some studies abnormal β-catenin expression (weak, low percent positive cells or nuclear and/or cytoplasmic localization) has been associated with clinicopathological features such as high histological grade and metastasis.[13,17,18,22]

FRANÇAIS
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Utilisation prévue
Pour utilisation en diagnostic *in vitro*

L'anticorps Monoclonal Mouse Anti-Human Beta-Catenin de Dako, clone β-Catenin-1 (Anti-bêta-caténine, β-caténine-1) est conçu pour être utilisé en laboratoire en vue de l'identification qualitative par microscopie optique des cellules positives à la β-caténine dans les tissus sains et néoplasiques, en utilisant des méthodes de test immunohistochimiques (IHC). L'interprétation clinique de tout marquage positif ou de toute absence doit être complétée par des études morphologiques et histologiques à l'aide de témoins appropriés. Les évaluations doivent être réalisées uniquement par un professionnel agréé dans le contexte de l'historique clinique du patient et d'autres examens.

Résumé et explication
Les caténines sont, d'un point de vue structural, apparentées aux protéines cytoplasmiques et ont été classifiées comme alpha (α), bêta (β), et gamma (γ) en fonction de leur mobilité électrophorétique.[2,3] Le gène de la β-caténine est localisé u niveau de la région chromosomique 3p21 et code pour une protéine de 88 kD.[2,3] Cette protéine cytoplasmique a de multiples fonctions, jouant un rôle essentiel dans l'ancrage arbitré par les cadhérines et dans l'organisation du cytosquelette.[2] La bêta-caténine est également impliquée dans la régulation de l'expression génique comme un médiateur de la voie de signalisation Wnt. Les niveaux de β-caténine cellulaire sont étroitement régulés par un complexe multiprotéique comprenant la sérine/thréonine kinase GSK3β, le produit du gène suppresseur de tumeur APC et l'axine, qui facilitent la phosphorylation et la dégradation ultérieure de la protéine β-caténine. Un dérèglement de la dégradation de la β-caténine conduit à une accumulation cytoplasmique de la protéine, suivie par une translocation vers le noyau. La β-caténine nucléaire forme des complexes avec des protéines se liant à l'ADN telles que le TCF et le LEF, activant ainsi la transcription de gènes.[4]

(schwach, geringer Prozentsatz positiver Zellen oder nukleäre und/oder zytoplasmische Lokalisierung) mit klinisch-pathologischen Merkmalen, wie z.B. einem hohen histologischen Grad und Metastasen, assoziiert.[13,17,18,22]

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