Evaluation of Vascular Endothelial Growth Factor-A (VEGF-A) in Iraqi patients with urinary bladder cancer

قياس عامل النمو الوعائي البطاني في المرضى العراقيين المصابون بسرطان المثانة

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الخلاصة:
هدف الدراسة:إيجاد العلاقة بين مرض سرطان المثانة وعامل النمو الوعائي البطاني ودوره في تشخيص هذا المرض.
منهجية البحث:أجريت الدراسة الحالية في بريطانيا في قسم الباثولوجي في المستشفى الملكي للفترة من نيسان ٢٠١٢ ولغاية آب ٢٠١٣ على عينات الدم المأخوذة من المرضى الذكور العراقيين المصابون بمرض سرطان المثانة البالغ عددهم ٤٢ مريض وآخرين أصحاء عددهم ٣٨ استخدموا كمجموعة سيطرة.
الوسائل الإحصائية:تم استخدام نظام (**SPSS**) الكومبيوتري لتحليل النتائج إحصائيا.
النتائج :أظهرت نتائج الدراسة الحالية زيادة معنوية عالية(**p** < ٠.٠٥) في مستوى عامل النمو الوعائي البطاني لدى المرضى مقارنة بمجموعة السيطرة.
الاستنتاجات: من خلال الدراسة الحالية تم الاستنتاج بالإمكان اعتماد عامل النمو الوعائي البطاني كعامل تشخيص لمرض سرطان المثانة لاسيما في المراحل المبكرة من المرض.
التوصيات :١- قد يوصى من خلال الدراسة الحالية إجراء دراسة جينية متقدمة لمعرفة العلاقة بين عامل النمو الوعائي البطاني والتغييرات الجينية لسرطان المثانة. ٢- إجراء قياس العامل المذكور أعلاه للمرضى المصابون بسرطان المثانة في بداية ظهور المرض للتنبؤ بحدوثه.

Abstract:
Objective: The aim of this work is to study the alterations in the vascular endothelial growth factor (VEGF-A), and its role in pathogenicity of bladder cancer in serum of men who are initially diagnosed with this disease.
Methods: The current study was applied in pathology department of British United Kingdom kings college hospital during April ٢٠١٢ to August ٢٠١٣ by using Enzyme Linked Immuno Assay (ELISA) on serum taken from ٤٢ men infected with bladder cancer , and other ٣٨ benign considered as a control group to detect vascular endothelial growth factor type A (VEGF-A).
Data analysis: SPSS computerizing system has been used for statistically analysis of data**.**
Results: The results revealed a highly significant (P<٠.٠٥) level of VEGF-A in serum of patients in comparison with the control group.
Conclusion: It was concluded from the results of the present study that VEGF-A was good prognostic biomarker for urinary bladder cancer in men ,and these results significantly correlate with the early stages of disease.
Recommendation : It may be recommended that using VEGF-A to predict and diagnosis the bladder cancer.
Key words:VEGF-A, bladder cancer.

INTRODUCTION:

Vascular endothelial growth factor (VEGF) is a chemical signal produced by cells that stimulates the growth of new blood vessels (١).It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate (٢). VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, and new vessels (collateral circulation) to bypass blocked vessels. When VEGF is over expressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able t grow and metastasize (٣). VEGF is a sub. Family of growth

factors, specifically the platelet. Derived growth factor family of cystine. knot growth factors, they are important signaling proteins involved in both vasculogenesis (the "de novo" formation of the embryoniccirculatory system)and angiogenesis (the growth of blood vessels from pre.existing vasculature (٤).

"In vitro", VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration (Jones et al; ٢٠٠٠). VEGF-A is also a vasodilator and increases microvascular permeability and was originally referred to as vascular permeability fact (٥,٦). The VEGF receptors have an extracellular portion consisting of ٧ immunoglobulin. Like domains, a single transmembrane spanning region, and an intracellular portion containing a split tyrosine. kinase domain , VEGF-A binds to VEGFR-١ (Flt-١) and VEGFR-٢ (KDR/Flk-١), VEGFR-٢ appears to mediate almost all of the known cellular responses to VEGF(٧) . The function of VEGFR-١ is less well.defined, although it is thought to modulate VEGFR.٢ signaling, another function of VEGFR-١ may be to act as a dummy/decoy receptor, sequestering VEGF from VEGFR-٢ binding,VEGF.C and VEGF.D, but not VEGF-A, are ligands for a third receptor (VEGFR-٣)(٨ ,٩).

AIM OF STUDY:

To study the alterations in the VEGF-A in serum of men who are initially diagnosed with bladder cancer. Also in trying to measure the factor in future diagnosis for bladder carcinoma.

PATIENTS AND METHODS:

The current study included ٤٢ patients of Iraqi males with urinary bladder carcinoma and ٣٨ other healthy groups adjusted as control group. The blood were drawn to get the serum from the patients when coming to operation hall for cystoscopically checking and treating at AL-Sader medical city hospital in AL-governorate Najaf during period from April ٢٠١٢ until August ٢٠١٣. The patients group were classified into four stages and three grades, the stages are Ta,T١,T٢,T٣, and the grades are I,II,III (WHO, ٢٠١٠),also into four subgroups according to ages (٤٠.٤٩Y),(٥٠.٥٩Y), (٦٠.٦٩Y) ,(٧٠.٨٠Y) ,and two other subgroups smokers and non smokers.

The vascular endothelial growth factor.A(VEGF-A) ELISA Assay

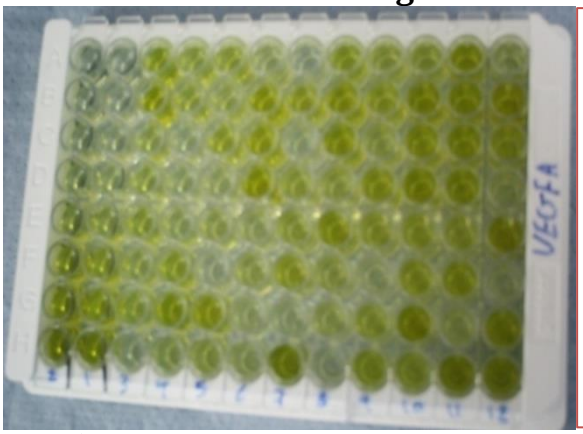
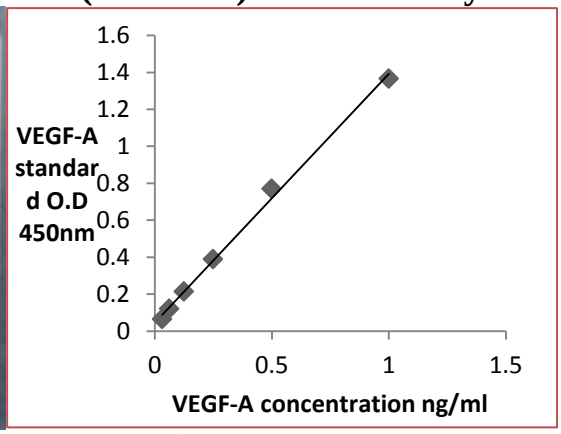


Figure (١):microwells of VEGF.A test with developing color.



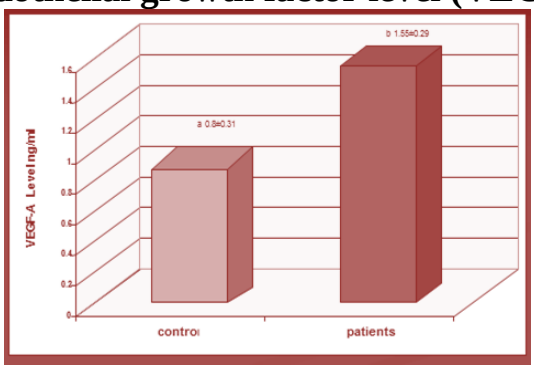
Figure(٢):VEGF.A standard curve.

This assay is based on the formation of coloured product in proportion to the amount of human VEGF-A present in the sample or standard. The reaction is terminated

by addition of acid and absorbance is measured at ٤٥٠nm. The specific Kit for this test is supplied by (eBioscience Company , Inc. Campus Vienna Biocenter ٢١٠٣٠ Vienna, Austria.human VEGF.A platinum ELISA.BMS٢٧٧/٢/BMS٢٧٧/٢TEN).

RESULTS:

١- The vascular endothelial growth factor level (VEGF-A).



Figure(٣):Comparison between VEGF-A level and control.

Serum analysis of VEGF-A levels revealed there was statistically significant increase (p<٠.٠٥) in urinary bladder cancer patients (١.٥٥±٠.٢٩) in comparison with the control group(١.١٧±٠.٣١) as in (Figure٣).

٢-Comparison between VEGF-A levels and stages of tumor.

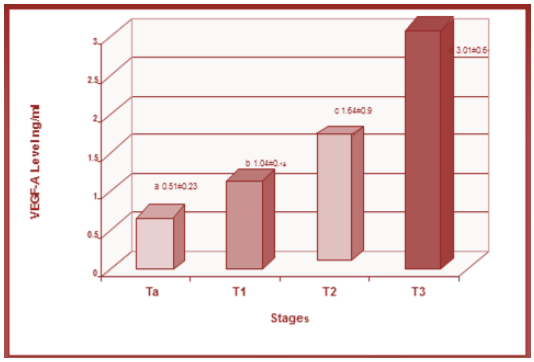
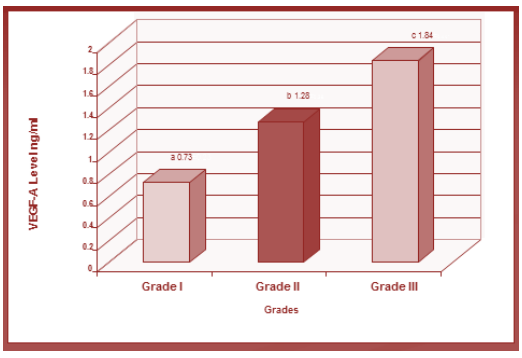


Figure (٤)Comparison between VEGF-A level and stages.

There were statistically significant differences(p<٠.٠٥) in the levels of VEGF-A between stages of bladder cancer patients ,significant increased(p<٠.٠٥)in T٣(٢.٠٩±٠.٦) compared with Ta(٠.٩١±٠.٢٣),T١(١.٠٤±٠.١٨) and T٢(١.٦٤±٠.٩) as shown in(Fig ٤).

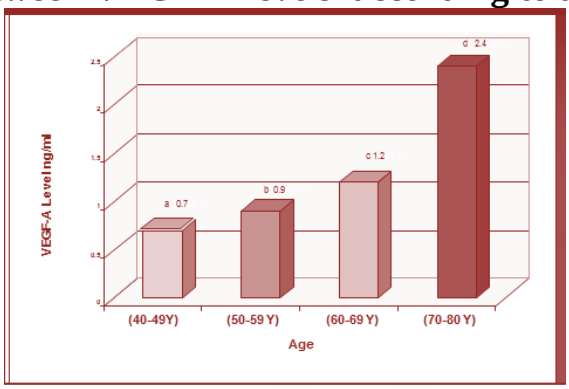
٣-Comparison between VEGF-A levels and grades of tumor.



Figure(٥):Comparison between VEGF-A level and grades.

The statistic analysis shows significant differences (p<٠.٠٥) in the levels of VEGF-A between grades of bladder cancer patients, significant increased(p<٠.٠٥)in VEGF-A level in GIII (١.٨٤±٠.٤٤) compared with GI(٠.٧٣±٠.٢٣), and GII (١.٢٨±٠.٣٨) (Fig ٥).

٤-Comparison between VEGF-A levels according to ages.



Figure(٦):Comparison between VEGF-A level and ages.

Figure(٦),shows acomparison between VEGF-A level in the different groups of bladder cancer patients according to their ages.This result revealed the significant increased (p<٠.٠٥) in the levels of VEGF-A at all ages, highly significant increase(p<٠.٠٥) of(٧٠-٨٠Y) (٢.٤±٠.٧٣) .

٥-Comparison between VEGF-A levels and to smoking:

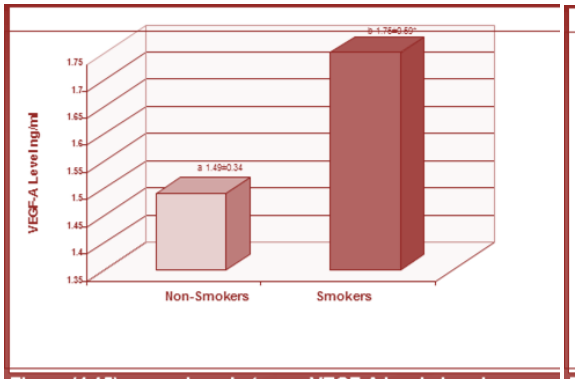


Figure (٧):Comparison between VEGF-A level and smoking.

The mean values and the standard errors of smokers and non smokers groups of bladder cancer patients are shown in figure(٧),this result demonstrate statistically significant increased(p<٠.٠٥) in serum VEGF-A level (p<٠.٠٥) in non-smokers (١.٧٥±٠.٥٩) compared with the smokers (١.٤٩±٠.٣٤) .

DISCUSSION:

The present study result in Figure (٣), revealed a significant increase(p< ٠.٠٥) in serum VEGF of urinary bladder cancer patients in comparison with the control.Many studies indicated that VEGF was the important angiogenic factor that play a vital role in the development and progression of tumor(١٠ ,١١).Previous study of Rahmani (١٢) contributed high expression of VEGF to malignant tumor and its promotion to tumor progression. VEGF is the key mediator of angiogenesis ,where it is up regulated by oncogenic expression and variety of growth factors also apotent stimulator of an

angiogenesis, inducer of endothelial cell migration and vascular permeability(١٣,١٤).Studies of Jelkmann(١٥) and Mylona (١٦) investigated VEGF as a prognostic serum marker in urinary bladder carcinoma and various types of cancer including astrointestinal, hepatobiliary,renal,and ovarian cancers.

Many studies indicated a VEGF as a prognostic marker in many types of human cancer such as gastric carcinoma, colorectal carcinoma, lung cancer, breast cancer, ovarian cancer, and pancreatic cancer (١٧,١٨). Two reports suggested that VEGF-C have basis functions of malignant cells and proliferation and apoptosis(١٩).Most studies that used serum or plasma samples confirmed increasing of free VEGF in malignant disease(١٥).The serum and plasma has been reported VEGF to be more correlated in cancer patients(٢٠).

The Figure (٤), (٥) showed a significant increase ($p<٠.٠٥$) in serum VEGF in high stage and grade of urinary bladder patients. Study of Vdeira (٢١) revealed the highly expression of VEGF. A was in the non muscle. Invasive bladder cancer cells (NMIBC). Other studies indicated that VEGF protein significantly over. expressed in all grades and stages of TCC (٢٢).Other study showed the VEGF.A transcript levels were greater in cancer tissues than in normal urothelium and more significantly in stages ($T^{٢}$-$T^{٤}$) than in stages (Ta,$T^{١}$) urothelial tumors(٢٣).Study of Swellam (٤) revealed a significant elevation of VEGF in late stage compared with early ones contributed that to direct angiogenic effect of the growth factor and proteolytic enzymes such as urokinase. type plasminogen activator in endothelial cells. Further studies showed that VEGF also appears to be a significant prognostic formation in association with tumor grade and average of apoptotic cells(٢٤).

The older ages of current study represented in Figure (٦) explained highly significant differences in serum VEGF level in patients of urinary bladder cancer. Previous study of Horstmann (٢٥) showed that age of ٥٠-٧٠ years has a peak incidence of bladder cancer and contributed the reason for cumulative effects of long time exposure to carcinogens, and failure of DNA repair mechanism with aging.

The smokers patients of urinary bladder cancer represented in Figure (٧)showed a highly elevation of serum VEGF in smokers than non-smokers patients. Study of Rizvi(٢٦) suggested a correlation between apoptosis with smoking and a risk of bladder cancer. Study of Baris (٢٧) proved that cigarette smoking stimulate apoptosis through a mechanism involving in the release of apoptotic related protein.

CONCLUSIONS:

High levels of vascular endothelial growth factor were detected in serum of men with bladder cancer indicating the important role of this marker as prognostic factor for this disease.

RECOMMENDATIONS:

١. Advanced study focusing on genetic DNA analysis of biomarker gene of bladder carcinoma patients to study the relationship between this biomarker proteins and the gene amplification.
٢. It may be recommended that, using this biomarker to predict and diagnosis the bladder cancer.

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