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RESEARCH ARTICLE

Assessment the Aurora a Kinas Overexpression among Colorectal Adenocarcinoma in Iraqi Patients

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Abstract

In the worldwide the colorectal carcinoma is represented the most common gastrointestinal malignancy The cancer represent one of major causes of death after cardiovascular diseases in Iraq. Aurora Kinase a (AURKA) is a member of the serine/threonine kinas family as well refers as (STK15) or (BTAK) regulates the function of centrosome, spindles, kinetochores in mitotic and chromosomal instability. AURKA overexpression is observed in various cancers including colon cancer and depended on our knowledge there is no in our country about the expression of AURKA colorectal carcinoma patients. This study included fifty five blocks embedded in paraffin was 31 (56.36%) males and 24 (43.63%) females which were obtained from colorectal adenocarcinoma patients and all samples composed of 25 (45.45%), 20 (36.36%) had moderate differentiated and (18.19%) had poorly differentiated colorectal carcinoma. The ages of patients had ranged from 20 to 80 years, with a mean of (50.6±7.44 SE).In this study, Iraqi patients showed that the protein overexpression of AURKA by immunohistochemical technique was significantly difference among these grades (p=0.0001,r=552) with high expression in well-moderate than poor differentiated while the AURKA protein overexpression was non- significant difference among different stage tumor sizes (P value =0.1746, r=0.254) as well as, the results showed there were no significant differences between AURKA protein overexpression with the gender, age and lymph-vascular and perinural invasions (P value =0.613,r=0.078), (P value 0.812, r=0.356), (p=0.711,r=0.192) respectively. Conclusion: The AURKA biomarker may be used as prognostic factor of tumor initiation in Iraqi patients with colorectal adenocarcinoma.

Key words: *Iraqi patients, Colorectal Adenocarcinoma, AURKA.*

Introduction

There is scarcity of data on cancer in Iraq and represent one of major causes of death after cardiovascular diseases in our country due to the abnormal growth of cells in the inner lining of the colon and rectum with ability to invade or spread to other parts of the body. Colorectal cancer is the most frequent cause of death among visceral malignancies and the seventh most common cancer in Iraq that accounts 4.7% of all malignant tumors and show rise in both genders. The incidence begins to rise at age 40 and peaks at age 40 to 75 yr with no strong correlation with age. In Iraq, the adenocarcinoma constituted about 84% of all cases. Cancer is not completely a genetic disease and characterized by uncontrolled cellular growth and rapid proliferation that have ability to invade the nearby parts of the body by forming metastases. Malignant tumors consist of heterogeneous populations of cancer cells [1]. The conversion of normal cell to a cancer cell is stepwise process that involves multiple mutations of oncogenes and inactivation of tumor suppressor genes. The colorectal carcinoma derived from the epithelial part of colon, colon is a site of multiple types of cancer including sarcoma, melanoma and lymphoma [2]. Colorectal Cancer (CRC) is ranking as the second and third most commonly diagnosed cancer among females and males respectively. CRC still contributes significantly to health-related mortality, in spite the advancement in methods of

diagnostic and treatment [3].The study of CRC still inspire many researchers worldwide due to its high incidence, advancement and in radiological imaging with the ability to take biopsy samples for histopathological a genetic studies [4]. Aurora Kinase-A or AURKA over expression is observed in tumor-enriched populations, including breast cancer (4-6), ovarian cancer [5], acute myelogenous leukemia (AML) [6] and mesenchymal stem cells (MSCs) from myelodysplastic syndromes (MDS) patients [7]. The overexpression of AURKA in cancer cells indicates a central role of Aurora-A in promoting cancer stem-like properties (e.g. therapy resistance, tumorigenesis, and EMT).

Materials and Methods

Study Group

Fifty five cases of colorectal carcinoma patients were selected randomly and their ages were ranging from 20 to 80 years. All cases were confirmed by the review of prepared H and E stained slides by certified pathologists. These cases were collected from Teaching Hospitals and private side laboratories in the Middle Euphrates region and in Baghdad. Fresh tissue sections of 4-micron thickness from all these paraffin blocks of colorectal carcinoma patients were taken for immunohistochemistry. Polyclonal Rabbit Anti-AURKA was used as primary antibody for detection of LGR5 protein. These primary antibodies were supplied from Elabscience Biotechnology Inc., United States.

The Immunohistochemical Study

The immunostaining method used in the current study was 2-step plus Poly-HRP technique which was applied for LGR5. Tissues 4 µm sections of multi-block with 10 % Neutral Buffed Formalin fixed and paraffin embedded human tissue. Mountedon Gharged slides. The sections were dried for 60 minutes at 60°C and the Primary is diluted in PBS Diluent. Tumor tissue slides were deparaffinized and rehydrated. The process of immunohistochemical including dewax and hydrate the paraffin section. Retrieve antigen of the tissue section according to special requirements of applied primary antibody and then incubate with 3% H_2O_2 for 10 min to eliminate endogenous peroxidase activity.

After that wash with PBS or TBS, 2 min× 3. The primary antibody added with proper dilution ratio, incubate at 20~37℃ for 1~2h or at 4℃ overnight (then rewarm at 37℃ for 30 min).Wash with PBS or TBS, 2 min× 3. The reagent 1 then added and incubated at room temperature or (37℃) for 20 min. Wash with PBS or TBS, 2 min×3. The reagent 2 Added and incubated at room temperature or 37℃ for 20~30 min. W ash with PBS or TBS, 2min×3.Color development with DAB. And wash with deionized water to make the section counterstained, dewatered, transparent, then seal the section.

The Results

In the colorectal carcinoma cases , AURKA immunoexpression was reported in 24 out of 31 cases of male group represented (77%) of the patients, seven cases were negative represented (23%) of the patients, while AURKA immunoexpression was reported in 20 out of 24 cases of female group represented (83%) of the patients and nine cases were negative represented (17 %) of the patients, with no significant difference among gender group (P value=0.613 r=0.078) (Figure 1), as well as the results showed no significant differences between AURKA protein overexpression with age group (P value 0.812, r=0.356) (Figure 2) and lymph-vascular and perinural invasions (p=0.711, r=0.192) (Figure 3).

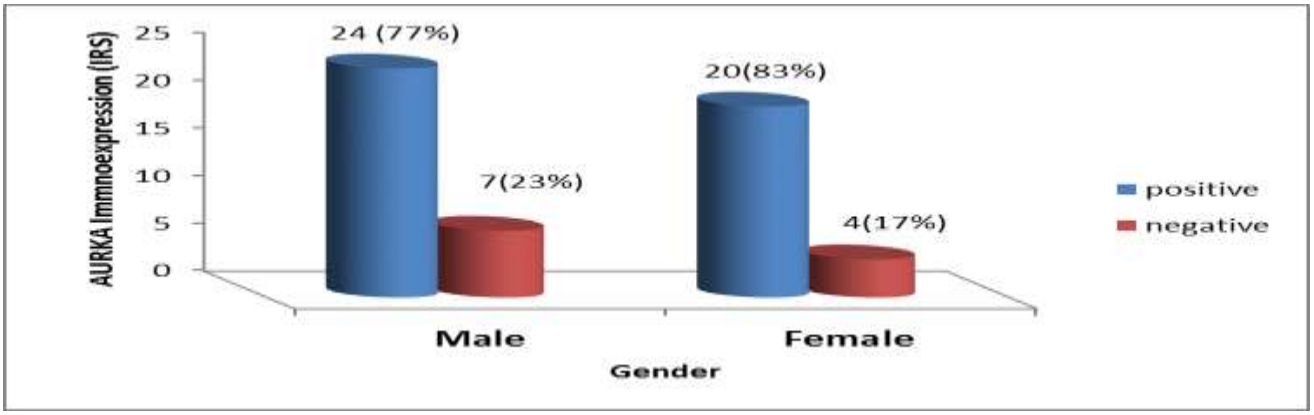

Figure 1: The comprise immunoexpression of AURKA in related to gender of colorectal carcinoma patients and there is no significant difference between them (t-student test)

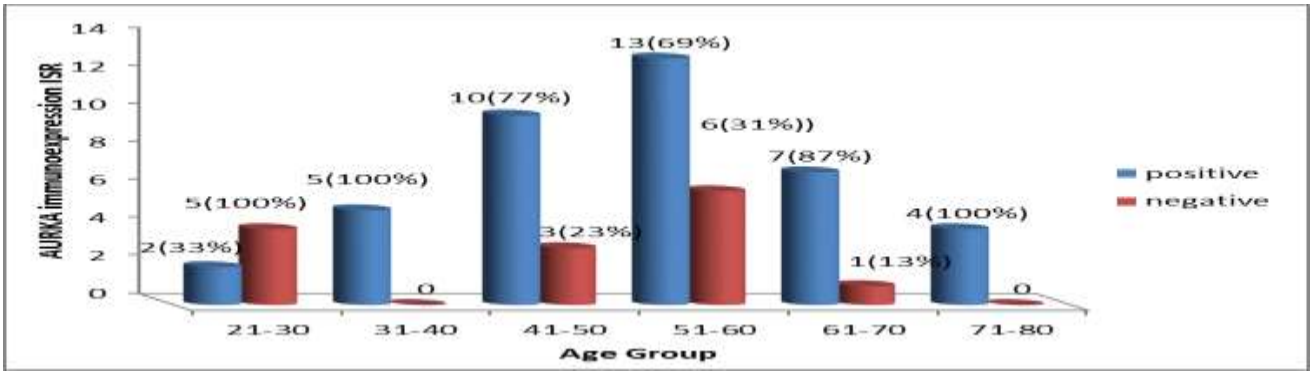

Figure 2: represented the correlation between AURKA protein overexpression and age group of colorectal carcinoma patients with non-significant among them when p value >0.05

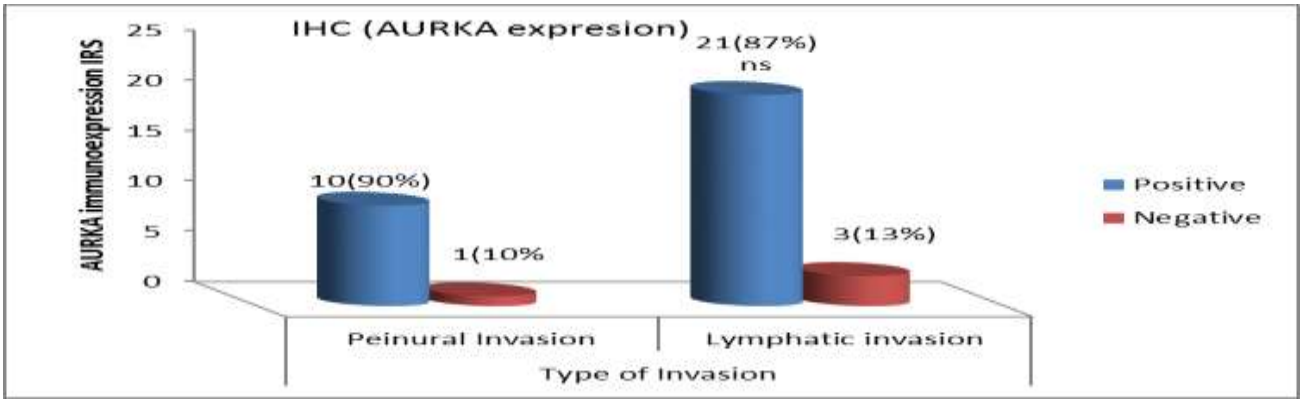

Figure 3: The association between the AURKA immunoexpression and the types of invasion of colorectal carcinoma patients and there was no significant difference among these invasion types

On the other hand, this study reported that AURKA protein expression is significantly differences with grades of tumor and there was more expression in well- moderated differentiation than poor differentiation (p=0.0001,r=552) (Figure 4), But there was no significant differences between this protein overexpression and stage extend of the tumor (P value =0.1746, r=0.254) (Figure 5).

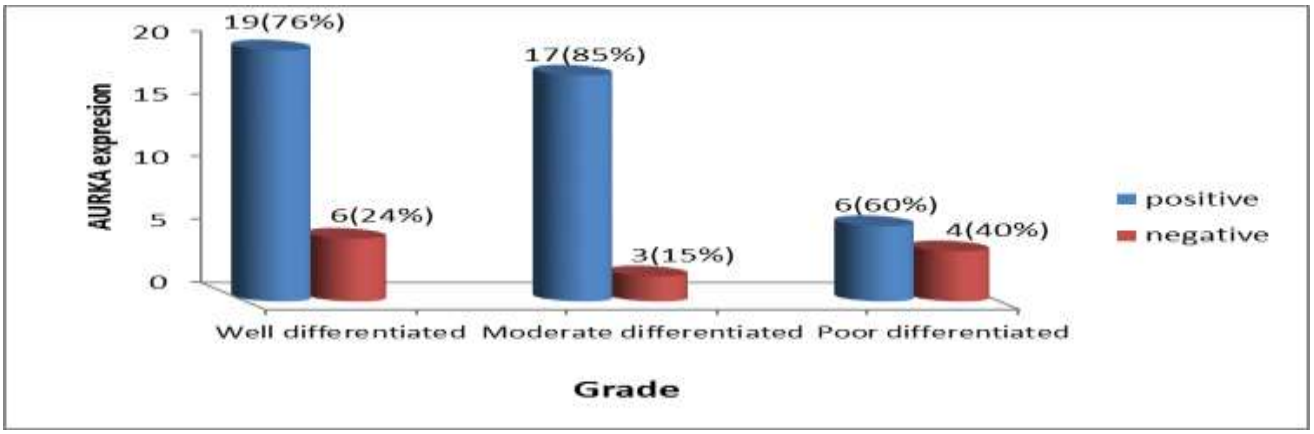

Figure 4: represent the correlation between AURKA immunoexpression and grade of tumor and show that significant differences among these grades

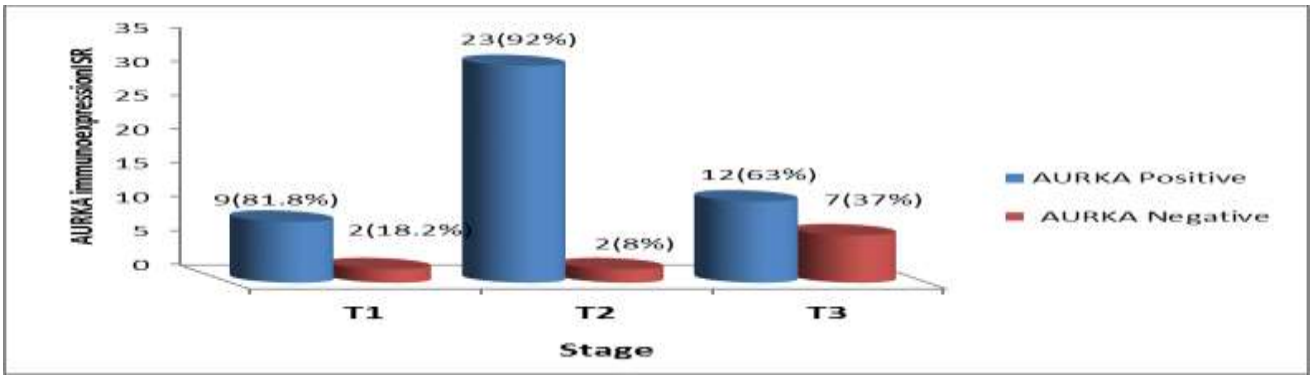

Figure 5: The association between AURKA immunoexpression and stage of colorectal cancer ,represent no-significant differences among the stages when p value >0.05

The immunostaining character and localization of ARUKA protein in colorectal carcinoma in our study is shown in the following figures:

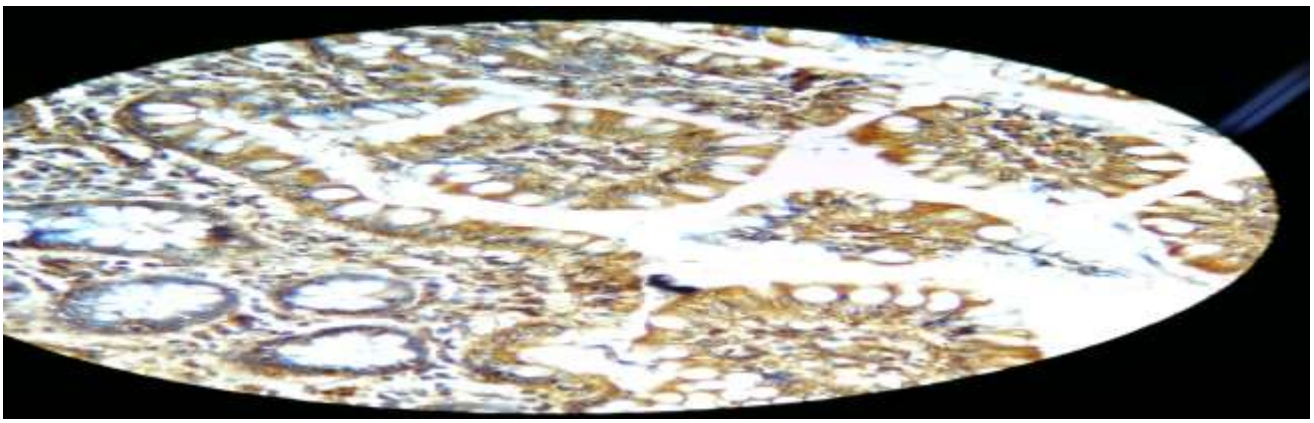

Figure 6: AURKA immunohistochemical cytoplasmic staining pattern score+3 in well differentiated of colorectal carcinoma tissues involved by colorectal carcinoma (X100)

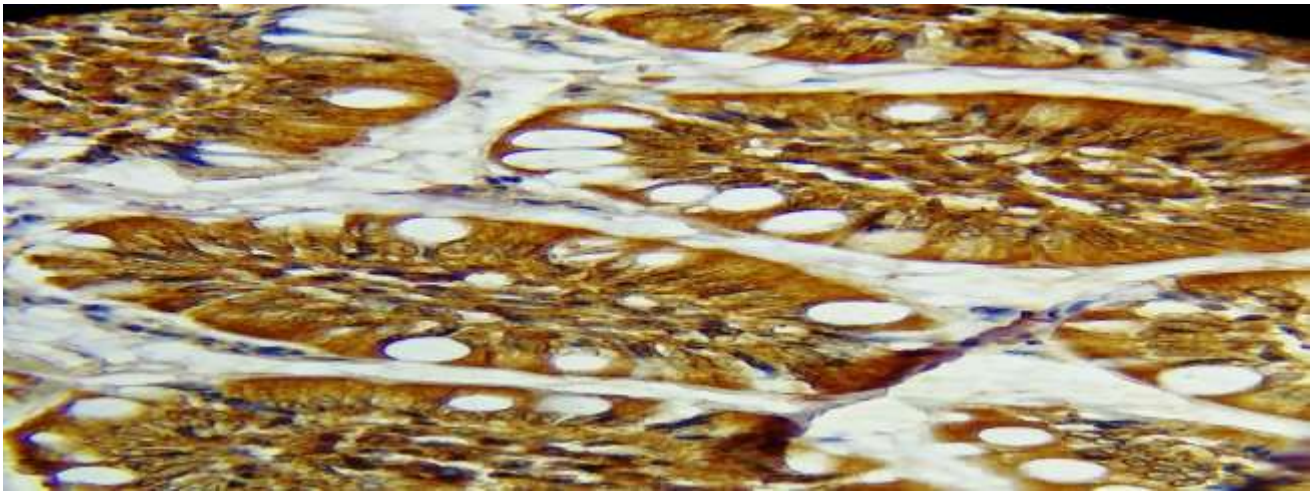

Figure 7: AURKA immunohistochemical cytoplasmic staining pattern score+3 in well differentiated of colorectal carcinoma tissues involved by colorectal carcinoma and show more details of AURKA protein cytoplasmic expression (X400)

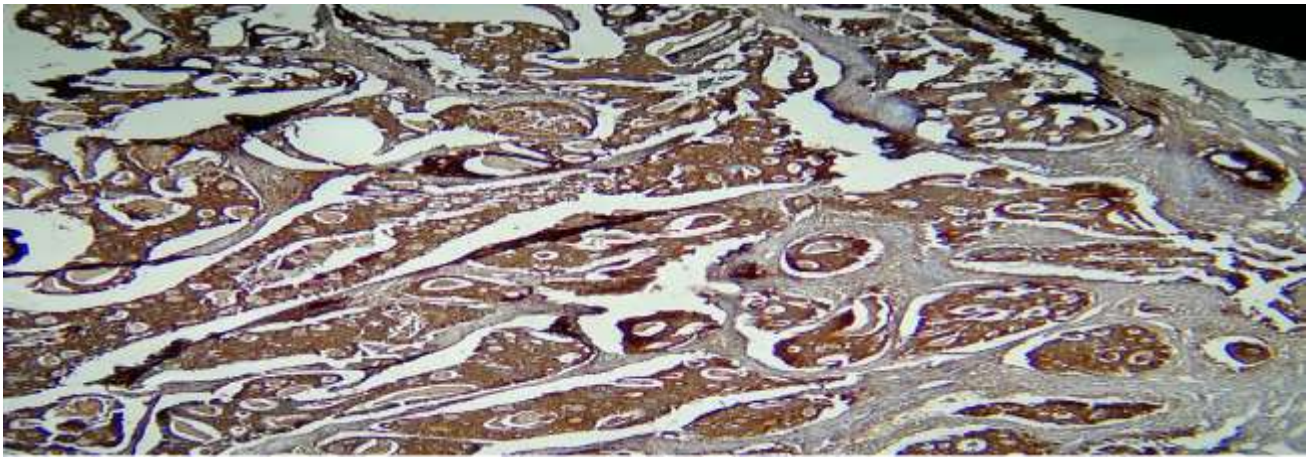

Figure 8: AURKA immunohistochemical cytoplasmic staining pattern score+3 in moderate differentiated colorectal carcinoma and showed the power of intensity (X100)

Discussion

The expression and activity of AURKA varies as the cell cycle progresses. The levels are low in the G1/S phase, up regulated during the G2/M phase and rapidly reduced subsequent to mitosis so that any aberrant expression of AURKA lead to induces oncogenic transformation and inhibition of AURKA activity leads to the failure of multiple events in mitosis, for example incorrect separation of centriole pairs, misalignment of chromosomes on the metaphase plate and incomplete cytokinesis [8]. Many studies done to investigated the relationship between AURKA protein expression or gene amplification and prognosis in solid tumor patients, including colorectal cancer (CRC) patients [9-12]. The

findings of the our present study showed there was no significant differences between AURKA overexpression with age, gender and type of invasions (lymphatic and perinural) of colorectal adenocarcinoma patients and this solidified with study done [13]. Our data demonstrate that AURKA overexpression is high at the early grade of the disease and gradually diminish in the poor differentiated grade. This result is similar to findings by [14] when reported that the overexpression of AURKA was high in patients with well or moderated differentiated colorectal carcinoma than in poorly differentiated grade, also there are significantly differences between overexpression of the AURKA protein and the stage.

In contrast, [12] revealed that AURKA over expression no have significant differences correlation with grade and stage of the colorectal adenocarcinoma but correlated with the gender of patients and this fully not identical with our present study. AURKA is an oncogene that contributes to colorectal adenoma to carcinoma progression and is associated with the malignant transformation of colorectal adenomas but not with serrated neoplasia progression [13]. In our opinion, cells began to divided in abnormal form in the early grades of cancer that because overexpression of AURKA and because the aberrant of AURKA gene and that lead to chromosomal instability or failure of chromosomal segregation and this lead to cell division under uncontrolled division and rapidly. The expression of AURKA protein in normal cells is predominant to the nucleus and subject to spatio-temporal regulation during mitotic progression, while the expression of the mentioned protein is in the cytoplasm of cancer cells [15] and that what we are noticed in our data. AURKA causes the activates Wnt/Akt signaling pathway simultaneously via reducing H3K4/H3K27 methylation on the promoter of (Twist), a famous negative factor of MET, and then promotes EMT [13].

Also, activation of oncogenic signaling, such as Raf-1 [8], Myc [9], OCT4 [10], all of these events lead to promote the EMT progression via the stabilization and accumulation of AURKA. In addition, AURKA overexpression can significantly enhance the expression of matrix metalloproteinases (MMP)-2, MMP-7 and MMP-10, leading to degradation of extracellular matrix proteins, which stimulates tumor cell mobility and metastasis [15, 20].

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