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Amoxicillin Impact on Electrophoresis Protein Profile of Tumor and Inflammatory Tissues

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ABSTRACT

Background: Inflammation is a critical component of tumor progression and cancer development. Objectives: To study amoxicillin effect as anti-inflammatory therapy on protein profile in human tissue with inflammatory and tumor diseases. Method: Three type of tissue were obtained from Ghazi al-Hariri Hospital: tumors of bladder, gall bladder, and thyroiditis tissues. Homogenization then extraction was carried for each tissue. Polyacrylamide gel electrophoresis analysis was also performed to detect protein profile in presence and absence different drugs. Results: The results indicated that the impact of drugs on tissues protein varies depending on the type of disease and tissue. Amoxicillin has been more influence on the malignant than benign tumors and least on the inflammatory tissue. Furthermore, Amoxicillin shows more effect than Ceftriaxone in parallel to Methotrexate on bladders tumor. Meanwhile in thyroiditis, Amoxicillin effect was parallel to Methotrexate. Conclusion: *In vitro* Amoxicillin drug has been effective impact on protein of tumor tissue, and it's necessary to do *in vivo* studies to evaluate the possibility of its use as a drug for the treatment of chronic inflammation and prevent its development into tumors.

INTRODUCTION

A tumor is a swelling or enlargement - a new growth of tissue forming an abnormal mass. It is either benign or malignant. Benign tumors do not invade neighboring tissues and do not spread throughout the body. In cancer, cells divide and grow uncontrollably and invading nearby parts of the body. Cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. [(1)]

The presence of infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders causes a large number of behavioral, physiologic, biochemical, and nutritional changes involving many organ systems distant from the site, or sites, of inflammation. [(2)]The most important component of these changes comprises the heterogeneous group of plasma proteins. [(3)]

Albumin synthesis is suppressed inflammation. [(4)] Reduction in its concentration established marker of poor prognosis in various diseases, including malignancy. By electrophoresis, globulins can be separated into different fractions, α_1, α_2, β and γ-globulins. Alpha and β globulins are synthesized in the liver. But γ-globulins are synthesized by plasma cells and B-cells of lymphoid tissues. [(5)]

In contrast to patients with hematologic malignancies, patients with solid tumors frequently undergo various surgical site infections (SSIs) which are the most common infections seen in such patients. [(6)] An analysis concluded that patient-related factors (e.g., advanced age, diabetes mellitus, and low serum albumin indicative of poor nutritional status, smoking, pre-existing colonization/infection with *S. aureus*) were predominant. [(7)]Surgical incisions develop secondary infections. Consequently, infection prevention is important and antimicrobial prophylaxis is recommended for most of these surgical procedures. The agents commonly used for prophylaxis (e.g., first- or second-generation cephalosporins; amoxicillin/clavulanate) were selected several decades ago, based on microbiologic data available. [(8, 9, 10)]

In previous study Kenneth *et al* reported that their data from cancer patients indicate that there have been significant changes in the epidemiology of the infections .The overall frequency of monomicrobial infections has declined, and the incidence of polymicrobial infections has increased considerably. [(11)]

Cancer's patients under chemotherapy are at high risk for febrile neutropenia (encloses oral temperature of > 38.3 °C). If fever occurs patient should receive immediate basic diagnostic procedures and broad-spectrum antibiotics have to be initiated. Quinolone with amoxicillin/clavulanic acid, while intravenous treatment recommendation: anti-pseudomonas cephalosporin. (12)

Immunocytochemistry ICC for the anticancer anthracyclines demonstrated that the drug accumulates in a characteristic pattern in the heart, liver, kidney, gastrointestinal tract, and hair follicles, which represent the sites targeted by the drug toxicity. ICC for amoxicillin demonstrated that the sites of the drug accumulation in small intestine, liver and kidney are closely correlated with the specific sites in which certain transporter systems for penicillin occur. Thus, an ICC method is a potential new tool for pharmacokinetic studies of wide variety types of drugs containing a primary amino group(s) in their molecules. (13)

Ceftriaxone is an antibiotic used to treat a wide variety of serious infections caused by organisms that are resistant to most other antibiotics. It is also a choice drug for treatment of bacterial meningitis, lastly in surgical (per operative). (14) Ceftriaxone inhibits bacterial cell wall synthesis by means of binding to the penicillin-binding proteins (PBPs). Inhibition of PBPs would in turn inhibit the transpeptidation step in peptidoglycan synthesis which is required for bacterial cell walls. Like other cephalosporins, ceftriaxone is bacteriocidal and exhibits time-dependent killing.(15) Methotrexate known as amethopterin, is an antimetabolite and antifolate drug.[16, 17] It acts as a competitive inhibitor of many enzymes that use the coenzyme folates due to the similarity in their structure.[16] Methotrexate was originally developed and continues to be used for chemotherapy, either alone or in combination with other agents. It is effective for the treatment of a number of cancers including breast, head and neck, leukemia, lymphoma, lung, osteosarcoma, bladder, and trophoblastic neoplasms.[18].Folic acid is needed for the *de novo* synthesis of the nucleoside thymidine, required for DNA synthesis.[19] Also, folate is essential for purine and pyrimidine base biosynthesis, so synthesis will be inhibited. Methotrexate, therefore, inhibits the synthesis of DNA, RNA, thymidylates, and proteins. (20)

MATERIALS AND METHODS

Three types of samples were obtained from patients undergoing surgery in Ghazi Al-Hariri Hospital: tumors of bladder, gall bladder, and thyroiditis tissues surgery .The final diagnosis was established by A histology (biopsy) exam .All samples were taken from the patients before any kind of therapy (this include chemotherapy, radiotherapy and hormonal therapy).

Tumor tissues were surgically removed from different tumor patients). The specimens were cut off and immediately rinsed with ice-cold normal saline (0.9% NaCl, pH 7) solution, & stored at (-20C) until homogenization.

The frozen tissue was thawed & worked as follow:

1) The blood & adipose tissue were removed with enough cold normal saline (0.9% NaCl).

2) Tissue was weighted.

3) Sliced finely with scalped in Petri dish standing on ice bath. The slices were further minced with scissors then homogenized by using a manual homogenizer in buffer solution (50 mM tris (hydroxyl methyl) methylamine – pH 7.6prepared by dissolving 1.514gm in 250 ml distilled water). The buffer was added in a ratio (1:3) (weight: volume) gradually to tissue. The homogenate was filtered through (4) layers of x-ray gauze size (90 cm*5 mm), then centrifuged at (3000 r.p.m) at (4^0C) for 10 min). An aliquot was removed (supernatant of the sample for determining protein concentration).

4) Sample immediately was frozen at (-20C) until assay, the supernatant was used for the study. [21] In each type of homogenate total protein was measured according to the Biuret method [22] albumin according to the Bromo Cresol Green (BCG) method [23, 24] and concentration of globulin was calculated indirectly from total protein and albumin as:

Globulin (g/dl) = Total serum protein – Albumin

Electrophoresis separates the proteins by using an electric current that separates homogenates' proteins into several fractions by size and electrical charge in an agarose gel[25].The technique was applied for each homogenate twice incubated with and without 1% drug (Amoxil, Methotrexate).

RESULTS AND DISCUSSION

This study was done on different types of tumors including malignant and benign of bladders and gallbladder respectively, furthermore an inflammation of thyroid .The tissues were collected from patients were attending Ghazi al-Hariri Hospital and throughout the surgery to reject the injured tissue (defected tissue). The biopsy for each the tissue was homogenate and determined the protein profile (total protein, albumin, and globulin) in each sample of the homogenate. Total protein had ranged between (9.76 -18.91) g/dl depending on the weight for each tissue. Table 1.

Table 1:-Illustrated the protein profile levels in the homogenates for different human tissues.

Samples No.	Samples name	Albumin g/dl	Total protein g/dl	Globulin g/dl
1	Benign tumor /gallbladder	8.75	9.76	1.01
2	Thyroiditis	6.97	10.16	3.19
3	Benign tumor/bladder	7.82	18.91	11.05
4	Malignant tumor/bladder	5.52	14.23	8.71

In order to identify the differences in proteins that are present in each homogenates samples after incubation with the antibiotic (Amoxil, Ceftriaxone) and anticancer (Methotrexate). Polyacrylamide gel electrophoresis was carried out the gel was stained with coomassie brilliant blue-R-250 .Figures-1, &3, show a digital analysis for the picture of the gels for comparing the difference in the patterns, J-image program was used in the present study to look for such differences. The tissue protein electrophoresis pattern using tris-glycine buffer, pH 8.9 as electrode buffer. Electrophoresis was carried out for 3 hours at 4^{o}C using a constant current of 40mA and a voltage of 20 V/Cm, the gel was stained by coomassie brilliant blue R-250.Figure -1 shows the electropherogram of bladder tissue protein .the Stain: lane {1} malignant tumor in bladder tissue treated with Methotrexate; lane {2} malignant tumor in bladder tissue treated with Amoxil; lane {3} malignant tumor in bladder tissue treated with Ceftriaxone; lane {4} malignant tumor in bladder tissue.

It is obvious from the from the comparison of the proteins profile of bladder cancer tissue treated with the antibiotic drugs; Methotrexate lane {1} was similar less more than Amoxil lane {2} and both lanes{1,2} were more than that for Ceftriaxone lane {3} in compare for that of the bladder

cancer tissue which not treated lane {4} , that the proteins of all group were separated into several bands with some differences in the globulin-regions for the tissue treated with the drugs while on differences in electrophoresis pattern of tissue not treated was observed for proteins.

The great advantage of electrophoresis compared with the quantization of specific proteins in the overview it provides. The electrophoresis patterns can give information about the relative increases and decreases within the protein population as well as information about homogeneity of a fraction.

This technique is recommended to be used to separate proteins into individual spots or bands and it can detect any variation in either the normal or disease specimen. [26]

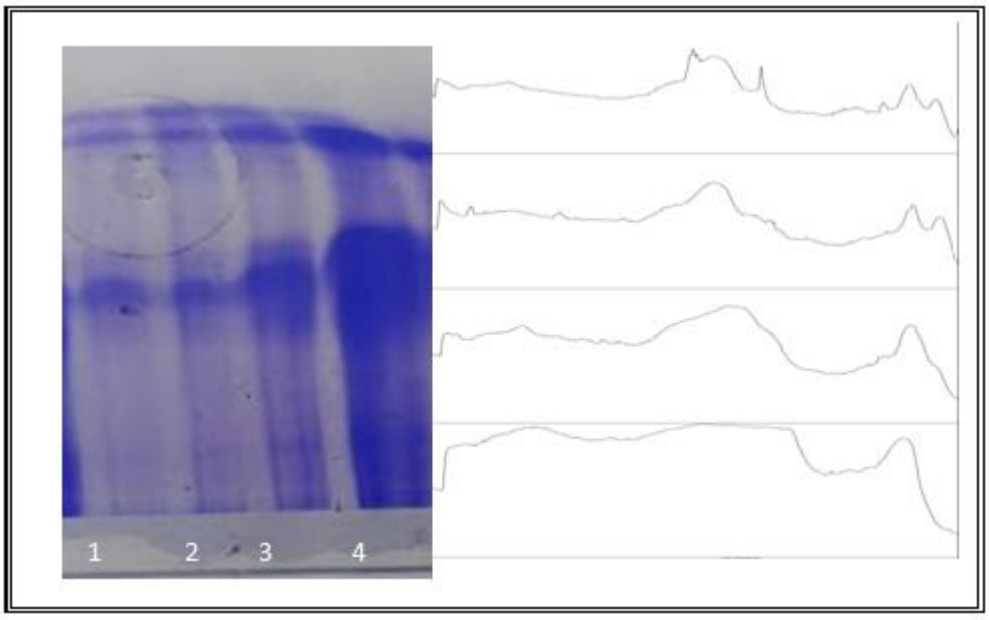

Figure 1: Electrophoregram of bladder tissue protein: (1) malignant tumor in bladder tissue treated with Methotrexate; (2) malignant tumor in bladder tissue treated with Amoxil; (3) malignant tumor in bladder tissue treated with Ceftriaxone; and (4)malignant tumor in bladder tissue.

In Figure -2 Illustrated the electropherogram of proteins in the gall bladder tissue not treated lane{ 1}and treated with Amoxil lane{2}.It can be observed that a big shift in the electrophoresis mobility and this shift is more obvious in lane {2} which referred to impact of Amoxil antibiotic on the protein of benign gallbladder tissue .Amoxil shows more effect than Ceftriaxone in parallel to Methotrexate on bladders .

Similar results obtained when incubated the Amoxil with thyroiditis tissue homogenate, lane {1} the shift in protein electrophoresis mobility was in parallel with that when incubation Methotrexate with thyroiditis tissue homogenate lane {2} as shown in Figure-3.

Amoxil is rapidly absorbed when one taken orally and it treats many inflammatory diseases with the less side effect on other tissues. [27]

The results of this study indicated that the impact of drugs on tissues protein varies depending on the type of disease and tissue. Amoxil has been more influence on the malignant than benign tumors and it gave a less effect on the inflammatory tissue. Furthermore, Amoxil as drug shows more effect than Ceftriaxone in parallel to Methotrexate on same malignant tumor in bladder tissue. Meanwhile, in thyroiditis tissue Amoxil effect was parallel to Methotrexate.

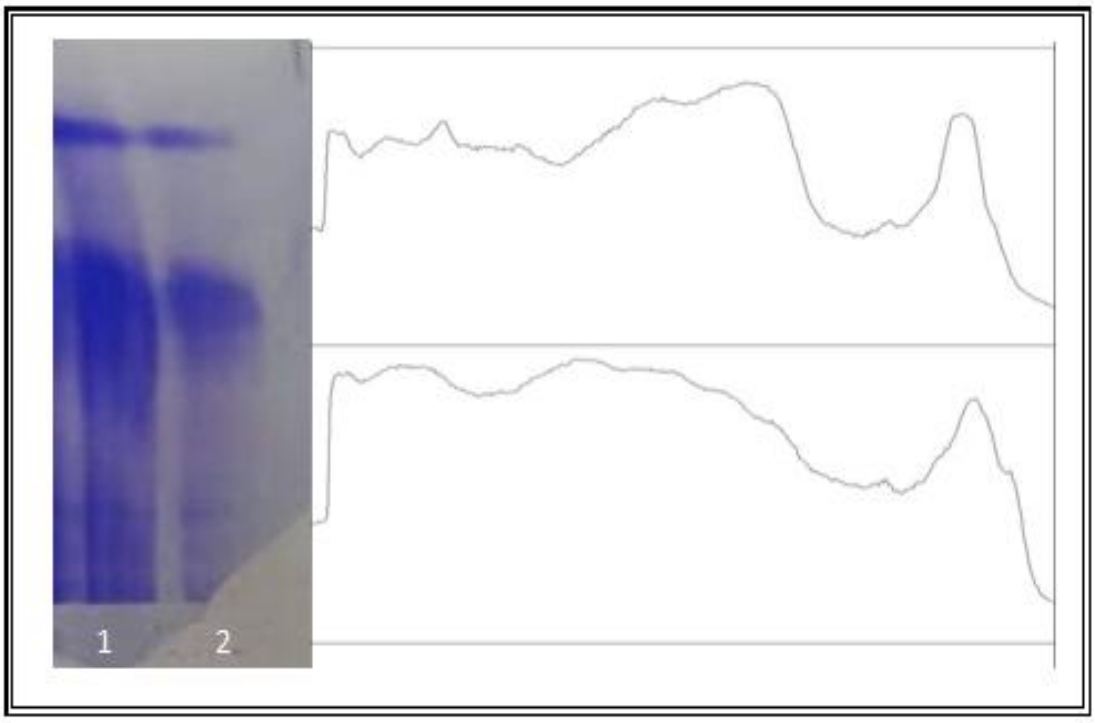

Figure 2: Electrophoregram of benign tumor in gallbladder tissue proteins (1) benign tumor in gallbladder tissue without treated; benign tumor (2) gallbladder tissue treated with Amoxil.

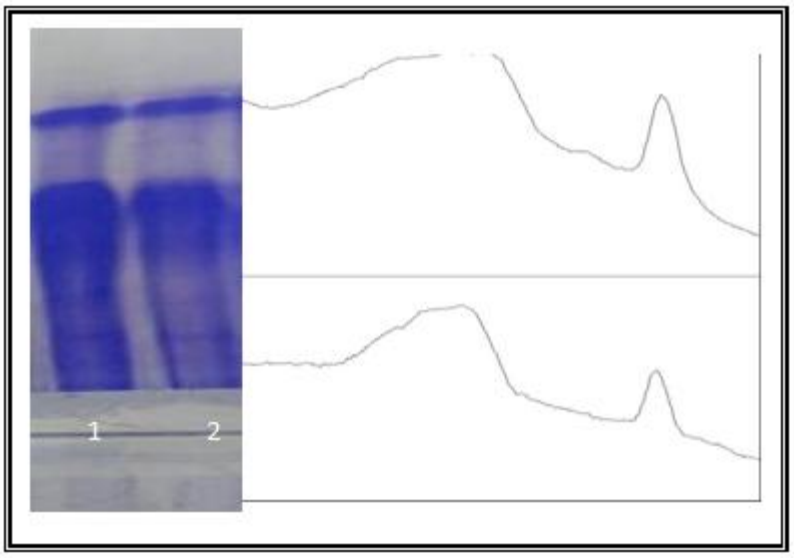

Figure 3: Electrophoregram of thyroiditis tissue proteins; thyroiditis tissue treated with Amoxil lane {1}; thyroiditis tissue treated with Methotrexate lane {2}.

CONCLUSION

In vitro Amoxicillin drug has been effective impact on protein of tumor tissue, and it's necessary to do *in vivo* studies to evaluate the possibility of its use as a drug for the treatment of chronic inflammation and prevent its development into tumors.

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Conflict of interest

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List of Abbreviations:

SSIs = surgical site infections;

ICC = Immunocytochemistry**;**

PBPs = penicillin-binding proteins;

BCG = Bromocresol green;