RESEARCH ARTICLE

Prevalence of some Gram-Negative Bacteria and Adenovirus in Breast Cancer Patients in Kirkuk City

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Received: 16th March, 2020; Revised: 19th April, 2020; Accepted: 28th May, 2020; Available Online: 25th June, 2020

ABSTRACT

Background: Breast cancer is one of the most prevalent types of malignant tumors in the world. Bacteria have been linked with cancer through two mechanisms, the first, stimulating chronic inflammation, and the second, production of carcinogenic bacterial metabolites. The most common types of adenovirus in cancer patients are serotypes 7 and 11 of type B, and serotypes 1, 2, and 5 are of type C.

Methods: In this study, 98 blood and 50 urine, samples were collected from women with breast cancer at the Center for Oncology and Hematology in Kirkuk city in addition to 30 blood and 20 urine samples collected from healthy persons as control. Pathogenic gram-negative bacteria were isolated, identified in addition to checking their susceptibility to antibiotics. Antibodies (IgM, IgG) of adenovirus type-7 were involved in this study by the ELISA technique.

Results: In this study, 22 gram-negative bacterial species were isolated from patients with breast cancer, including (31.81% *Klebsiella pneumoniae*, 45.45% *E. coli*, 18.18% *Proteus mirabilis* and 4.54% *Acinetobacter baumannii*). Gram-negative bacteria showed high resistance to antibiotics (approximately absolute resistance) amoxicillin/clavulanic acid, ampicillin, ceftazidime, amoxicillin, cephalothin, vancomycin. Adenovirus type-7 antibodies were detected by using the ELISA technique (3.84% IgM and 91.02% IgG). Viral and bacterial co-infection were also included in this study caused by the involvement of *Escherichia coli* + ADV-7, *P. mirabilis* + ADV-7, and *K. pneumoniae* + ADV-7.

Keywords: Breast cancer, Gram-negative bacteria, Multi-drug resistance, Adenovirus Type-7 (IgM and IgG), ELISA, Co-infection.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.2.5

How to cite this article: Ali AA, Alabden SSZ, Zaman NAQ. Prevalence of some gram-negative bacteria and adenovirus in breast cancer patients in Kirkuk city. International Journal of Pharmaceutical Quality Assurance. 2020;11(2):224-227.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Breast cancer is one of the most common types of malignant tumors in cancer records; cancer caused by breast tissue and most common from the inner lining of milk ducts or lobules that supply ducts to milk. Breast cancer is one of the most common causes of death in women and is the most frequently diagnosed cancer in women.[1] Most breast tumors are benign. Breast cancer is a term given to malignant breast tumors and is more common in women and less frequent in men. Genetic factors cause 25% of breast cancer.[2,3] Breast cancer is one of the leading causes of mortality worldwide, affecting one in eight women worldwide. Regardless of the efforts spent through intensive research, the exact causes of breast cancer are still unknown, but many genetic, genetic, and environmental factors have been identified as a result. The origin of the vast majority of 70% of breast cancer cases is still unknown.[4] Cancer patients undergo intensive chemotherapy with immunosuppressive drugs and radiation therapy in an attempt to destroy the tumor. These measures also attack the immune system, which reduces the patient's immunity, which exposes the patient to opportunistic diseases.[5]

More than 15% of malignant tumors worldwide can be attributed to microorganism infection or about 1.2 million cases per year.[6]

Recent research suggests that microorganisms in women with breast cancer are different from healthy ones.[4] Women with breast cancer have a higher incidence of *Staphylococcus*, *Bacillus*, and *Enterobacteriaceae* than women who have not had breast cancer[7,8]

Multiple bacterial resistance increases mortality, as many bacteria have developed multiple resistance mechanisms. For example, the predominant mechanism of resistance to β-lactam antibiotics by the gram-negative bacteria is the production of the enzyme β-lactamase, while gram-positive bacteria are resistant to β-lactam antibiotics by altering the target location of penicillin-binding proteins (PBPs).[9]

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Adenovirus was first isolated before more than 60 years in humans from adenoid tissue.[10] The virus has a diameter of 70 to 90 nm and is not encapsulated and displays the symmetry of icosahedral, which has 20 triangular faces, 12 heads and 30 edges.[11] It was found that viruses cause 16.1% of human cancer worldwide, viruses associated with cancer include Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), Human Papilloma Virus (HPV), (HBV) Hepatitis B virus, Hepatitis C virus (HCV), John Cunningham virus (JCV), BK virus (BKV) and Molluscum contagiosum virus (MCV). Viruses have been central to modern cancer research and provide a deep insight into the etiology of cancer.[12] These adenovirus infections are usually mild and limited, but infections are more severe in people with immunocompromised. The most common serotypes of adenovirus in cancer patients are 7 and 11 type B and serotypes 1, 2, and 5 type C, with a mortality rate of 53.3%.[13]

MATERIAL AND METHOD

In this study 98 blood and 50 urine samples were collected from women with breast cancer at the Center for Oncology and Hematology in Kirkuk city in addition to (30 blood and 20 urine samples collected from healthy persons as a control in Kirkuk city for the period from 20/01/2019 to 20/03/2019 and distributed over different ages. Blood samples were collected by drawing blood from infected women by sterilizing with a 4% iodine intake area, then with 70% ethyl alcohol, and then drawing 7 mL of venous blood. The syringe needle was removed and replaced by a new and sterile needle; glass bottle caps containing brain heart infusion broth media were sterilized with iodine, with 96% ethyl alcohol, then 5 mL of drawn blood was injected into those bottles and then incubated at 37°C for 3 to 7 days.[14] The remaining volume of 2 mL drawn blood was injected into the serum tube and then placed in a centrifuge to obtain the serum, The resulting serum was distributed to two Eppendorf tubes and then kept at –20°C until it was used. Urine samples were collected in sterile plastic boxes where the first part of the urine was neglected and the medial urine was collected from patients and cultured on blood agar, MacConkey agar, and chocolate agar then incubated under 37°C for 24 hours.[15] The isolated colonies were identified and diagnosed according to (API 20 E and biochemical tests).

Table 1: Distribution of bacterial infection and adenovirus type-7 (IgM, IgG) antibodies positivity among the study group

Infection		*Bacterial infection*		*Viral infection*				*Bacterial and viral co-infection*	
				IgM		*IgG*			
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Breast cancer patients	Positive	22	14.86	3	3.84	71	91.02	6	3.84
	Negative	126	85.13	75	96.15	7	8.97	150	96.15
Total		148	100	78	100	78	100	156	100
Control	Positive	3	6	1	10	8	80	0	0
	Negative	47	94	9	90	2	20	20	100
Total		50	100	10	100	10	100	20	100

Table 2: Antibiotic sensitivity test for isolated Bacteria

Antibiotic	*Blood*			*Urine*			
	K. pneumoniae (%)	*E. coli (%)*	*P. mirabilis(%)*	*K. pneumoniae (%)*	*E. coli (%)*	*P. mirabilis (%)*	*A. baumanii (%)*
VA	0	0	0	0	0	0	0
CN	100	100	100	100	86	100	0
AM	0	0	0	0	0	0	0
CIP	100	100	100	100	43	33	0
AK	100	67	100	100	100	67	0
TMP	100	33	100	80	43	67	0
AMC	0	0	0	0	0	0	0
CAZ	0	0	0	0	0	0	0
CTX	100	33	100	40	29	33	100
CRO	100	33	100	60	43	33	100
KF	0	0	100	60	0	0	0
AX	50	67	100	0	0	0	0
CLR	50	0	0	80	57	33	0
AZM	0	0	100	80	29	33	100

AMC = Amoxicillin/clavulanic acid; CIP = Ciprofloxacin; AM = Ampicillin; TMP = Trimethoprim; CLR = Clarithromycin; AZM = Azithromycin; AK = Amikacin; VA = Vancomycin; CAZ = Ceftazidime; CN = Gentamicin; AX = Amoxicillin, KF = Cephalothin; CRO = Ceftriaxone; CTX = Cefotaxime

Table 3: Multi-drug resistance (MDR) of *E. coli*

No. of isolates	*Name of Antibiotic*	*Number of antibiotics*
10	AMC + AM + VA	3
10	CAZ + KF + VA	
6	AX + AZM + AMC	
6	AM + CRO + CTX	
5	TMP + CLR + AZM	
4	CIP + CRO + CTX	
1	AK + CLR + CTX	
1	CN + AZM + TMP	
10	AMC + AM + VA + CAZ	4
10	KF + CAZ + VA + AMC	
5	CLR + AZM + CRO + CTX	
5	TMP + CRO + CTX + AZM	
4	TMP + CIP + CRO + CTX	
3	CIP + CLR + AZM + AX	
1	CN + TMP + CLR + CTX	
1	AMC + AM + AK + CAZ	
10	AMC + AM + VA + CAZ + KF	5
5	TMP + CLR + AZM + CRO + CTX	
4	TMP + CIP + AX + CRO + CTX	
1	AK + AZM + CLR + AM + AMC	
1	CN + AX + KF + CAZ + AM	
8	AMC + AM + VA + CAZ + KF + AX	6
4	TMP + CLR + CRO + CTX + AZM + AX	
3	CIP + CLR + TMP + AZM + CTX + AMC	
1	CN + AMC + AM + CIP + CRO + CTX	
1	AK + AZM + CLR + AM + AMC + KF	
8	AMC + AM + VA + CAZ + KF + AX + AZM	7
3	CIP + AZM + CLR + CRO + CTX + AX + TMP	
1	AK + AZM + CLR + AM + AMC + KF + VA	
1	CN + AMC + AM + CRO + CTX + AX + KF	
4	AMC + AM + VA + CAZ + KF + AX + AZM + CLR	8
3	CRO + CTX + CIP + AZM + CLR + TMP + AX + AMC	
1	AM + TMP + CLR + AZM + AK + VA + CAZ + KF	
1	CRO + CTX + AX + KF + CN + CAZ + VA + AZM	
4	AMC + AM + VA + CAZ + KF + AX + AZM + CLR + TMP	9
3	CRO + CTX + CIP + TMP + CLR + AZM + AX + AMC + VA	
1	CN + CAZ + VA + AZM + CLR + TMP + CIP + AMC + AM	
3	AMC + AM + VA + CAZ + KF + AX + AZM + CLR + TMP + CIP	10
3	CRO + CTX + CIP + TMP + CLR + AZM + AX + AMC + VA + CAZ	
1	CN + AX + KF + CRO + CTX + VA + AZM + CLR + TMP + AM	

Table 4: Multi-drug resistance of *K. pneumoniae*

No. of isolates	*Name of Antibiotic*	*No. of antibiotics*
7	AMC + AM + VA	3
7	AMC + AM + CAZ	
6	CAZ + VA + AX	
3	KF + AM + AMC	
2	KF + CRO + CTX	
2	AM + CLR + AZM	
1	TMP + CRO + CTX	
7	AMC + AM + VA + CAZ	4
6	AMC + AM + VA + AX	
2	CRO + CTX + KF + AX	
2	AZM + CLR + AM + AMC	
1	TMP + AX + CRO + CTX	
6	AMC + AM + VA + CAZ + AX	5
2	CRO + CTX + KF + AX + CAZ	
2	AZM + CLR + AMC + AM + CAZ	
1	TMP + AM + CRO + CTX + CAZ	
3	AMC + AM + VA + CAZ + AX + KF	6
2	CRO + CTX + KF + AX + CAZ + VA	
2	CLR + AZM + AM + AMC + CAZ + VA	
1	TMP + AX + KF + CRO + CTX + VA	
2	AMC + AM + VA + CAZ + AX + KF + CTX	7
2	CRO + CTX + KF + AX + VA + CAZ + AM	
1	CLR + AZM + VA + CAZ + AMC + AM + AX	
1	TMP + AX + KF + CAZ + CRO + CTX + VA	
2	AMC + AM + VA + CAZ + AX + KF + CTX + CRO	8
1	AMC + AM + TMP + VA + CAZ + AX + KF + CRO	
1	AMC + AM + VA + CAZ + AX + KF + CTX + CRO + TMP	9

Kerby and Bauer's method was used to study the sensitivity of isolated bacteria to different antibiotics.[16]

RESULTS

In this study, 25 gram-negative pathogenic bacterial species were isolated and diagnosed according to (API 20 E and biochemical tests), 24% from blood samples, and 64% from urine samples in breast cancer patients, in addition to 12% from urine samples for healthy people.

Antibodies to adenovirus type-7 were included in this study: IgM detected by ELISA in 3.84% in breast cancer patients, with 10% in healthy people. IgG was also detected by ELISA in 91.02% in breast cancer patients and 80% in healthy people. Viral and bacterial co-infection caused by *E. coli* + ADV-7, *K. pneumoniae* + ADV-7, *P. mirabilis* + ADV-7 was also included in this study Table 1.

Gram-negative bacteria showed high resistance to antibiotics amoxicillin/ clavulanic acid, ampicillin, ceftazidime, amoxicillin, cephalothin, vancomycin (Table 2).

Tables 3 and 4 show the pattern of multi antibiotic-resistant of *E. coli* and *K. pneumonia*, the results showed that 10 isolates of *E. coli* showed resistance to 5 antibiotics as shown in Table 3, while in the table 4 regarding *K. pneumoniae* 6 isolates showed resistance to 5 antibiotics

DISCUSSION

In this study, the isolated gram-negative pathogenic bacteria include 31.81% *K. pneumoniae*, 45.45% *E. coli*, 18.18% *P. mirabilis*, and 4.54% *A. baumannii.*

In 2014, the researcher Urbaniak and his group conducted a study of breast cancer patients in Canada and Ireland, who isolated the *Enterobacteriaceae* family by 30.8%.[17] The study conducted to study the gram-negative bacteria in bloodstream infection of cancer patients including breast cancer patients were obtained in this study *K. pneumoniae* by 13.1%, which is comparable to the result of our study, and also obtained *E. coli* 29.5% and *P. mirabilis* by 1.6%.[18] Researchers Zorgani and his group (2012) in their study of cancer patients in Libya obtained many types of gram-negative bacteria in the urine samples, including, *E. coli* bacteria by 61.7% and bacteria *K. pneumoniae* by 18.8% and *P. mirabilis* by 6%.[19]

This study showed that *E. coli* was absolutely resistant to vancomycin, ampicillin, amoxicillin/ clavulanic acid, ceftazidime, cephalothin, clarithromycin, and azithromycin. In spite of this resistance, *E. coli* showed sensitivity to different antibiotics, as shown in Table 2, and these results are similar to the other study.[18] This study showed that *K. pneumoniae* absolutely was resistant to vancomycin, ampicillin, amoxicillin/ clavulanic acid, ceftazidime, cephalothin, azithromycin, Another study showed that *K. pneumoniae* is resistant to Ampicillin, while it is sensitive to amikacin and gentamycin.[19]

Antibodies to adenovirus type-7 included in this study: IgM detected by ELISA in 3.84% in breast cancer patients with 10% in healthy people. IgG was also detected by ELISA in 91.02% in breast cancer patients and 80% in healthy people. Adenovirus can reach tumors through the intravascular pathway.[20] A study of four people with cancer to detect adenovirus showed that they all had adenovirus. One of these cases was for a woman with breast cancer, and when the virus was detected, it is found to have ADV-7.[21]

Viral and bacterial co-infections were also included in this study, which showed the co-infection between *E. coli* + ADV-7 (33.33%), *K. pneumoniae* + ADV-7 (50%), *P. mirabilis* + ADV-7 (16.66%).

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