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About Journal

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CONTENTS

Survey of Female Breast Cancer in Waist Province - Iraq for the Period From (2010-2019)

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Abstract

Breast Cancer is Iraq's most that prevalent form of malignancy. The Iraqi Cancer Archive demonstrates an apparent tendency for the disease to affect advanced-stage women younger. the Objective of study, In Waist Province/ Iraq, to I provide an that overviews of the occurrence of female I breast cancer. A retrospective research was performed over the 10 year period from 2010-2019 to classify all new cases of breast cancer from all over Wasit. The Patient Methods: Breast cancer statistical information was collected from the publicly accessible cancer registry/ Wasit province/ Iraq. Results: Between January 2010 and December 2019, 274 female I breast cancer cases were detected and registered in the Wasit governorate. A recent study showed the age group 45-59 years is high affected, the percentage was (38.68%). According to life stylethe highest incidence of female breast cancer was for female who live in the city 204 (74.45%).Conclusions: Obviously, these findings reflect the general population's poor health education and their misunderstanding. Clinical breast examinations, breast self-examinations, and early physician consultations are all beneficial.

Keywords: Breast Cancer, incidence ,Wasit province /Iraq, data bases

1. Introduction

The female Breast cancer Malignancy is the most frequent cancer among women from glob, and it is also one of the causes the mortality among women [1] Cancer of the breast is a multifactorial

disorder. [2] And multiple variables contribute to its occurrence. Although the disease occurs worldwide, its prevalence, mortality, and survival rates vary greatly across various areas of the world, due to many factors, such as population structure, lifestyle, genetic factors, and environmental factors.[3]In Iraq, according to the findings of the latest Iraqi Cancer Registry, breast cancer is the most common cancer site in Iraqi women, surpassing even bronchogenic carcinoma in general.[4] The World Health Organization recommends registered approaches to the treatment of breast cancer.[5]

1.1 Breast cancer type

There are several different that breast cancer types, and ductals carcinoma in situ (DCIS) and invasive carcinoma are common types. There are 15 to 20 parts of each breast called lobes, which have several tinier parts called lobules. thin tubes, called ducts, the connect the lobes and lobules. Ductal cancer is the most common form of breast cancer. In the cells of the ducts it is located. Lobular cancer is a cancer that begins in the lobes or lobules. Cancers are also known as invasive (in situ). The term in situ refers to cancer that not spread beyond the region where it originally evolved. There is a potential for breast cancer to metastasize to other breast tissues or other regions/ area of the body. Inflammatory breast cancer, characterized by overall breast inflammation, is a less common breast cancer in its various forms. Others are the less common, such as tumors of that phyllodes and angiosarcomas[6].

1.2 How breast cancer spreads

As Cancer cells enter the bloodstream or lymphatic system and spread throughout the body, I breast cancer may spread. The lymphoid system composition of vessel network and clear fluid (lymph) is similar to that of plasma , but the ingredients have some additional substances(macroparticles and, damaged cells). [7]When they are transferred fluid lymph to breast through Lymphoid veins or breast. Cancer that cells, can invade those I lymph vessels Breast.Most of the breast vessels of the lymph that drain into, nodes of axillary ,lymph nodes around collarbones, the nodes inside the chest. There is a greater risk cells may has passed through the fluid lymph and spread (metastasized) to body if cancer cells have spread to the lymph nodes. The more lymph nodes harboring breast cancer cells, the more likely cancer will be found in other organs[8].

1. 3 Risk factor of breast I cancer

Globally, the prevalence of breast cancer seems to be growing. [9]Breast cancer is a multi-factorial that disorder in which genetic risk, environment, diet and other risk factors for lifestyle interact. Improved recognition of modifiable risk factors and risk mitigation for breast cancer will allow the implementation of helpful preventive strategies [10].

2. Methods

Breast cancer statistical information was collected from the publicly accessible cancer registry/Wasit/ WasitA r e t r o s p e c t i v e r e s e a r c h w a s p e r f o r m e d b y t h e h e a l t h d e p a r t m e n t t o descriptiveepidemiological of all Wasit breast cancer cases during that a period of 10 years (2010-2019). In this study that following information was collected from the patients under study Table1describedthisinformation.

Table 1. Examination of the breast- information collected from patient data.

Gender	Female			
education level	Education	non-education		
Address	Villagers	citizens		
Age	15_29	30- 44	45-59	60 - ≥ 70

3. Results

Between January 2010 and December 2019, a total of 274 female cases that of breast cancer were diagnosed and reported in the Wasit governorate.In this, in the study, Female breast that cancer cases were divided into age groups, and the correlation between theage group and the incidence. The groups mentioned are 15-29, 30-44, 45-59, 60-75 years of age.

In figure (1) the age groups 15-29 years Less affected by breast cancer at (5.56%), those aged 45-59 years recorded the highest percentage (38.68%), and the other age groups 30-44, 60-75 representing(30.65%,24.08%), respectively the estimated number of cases of female breast cancer.

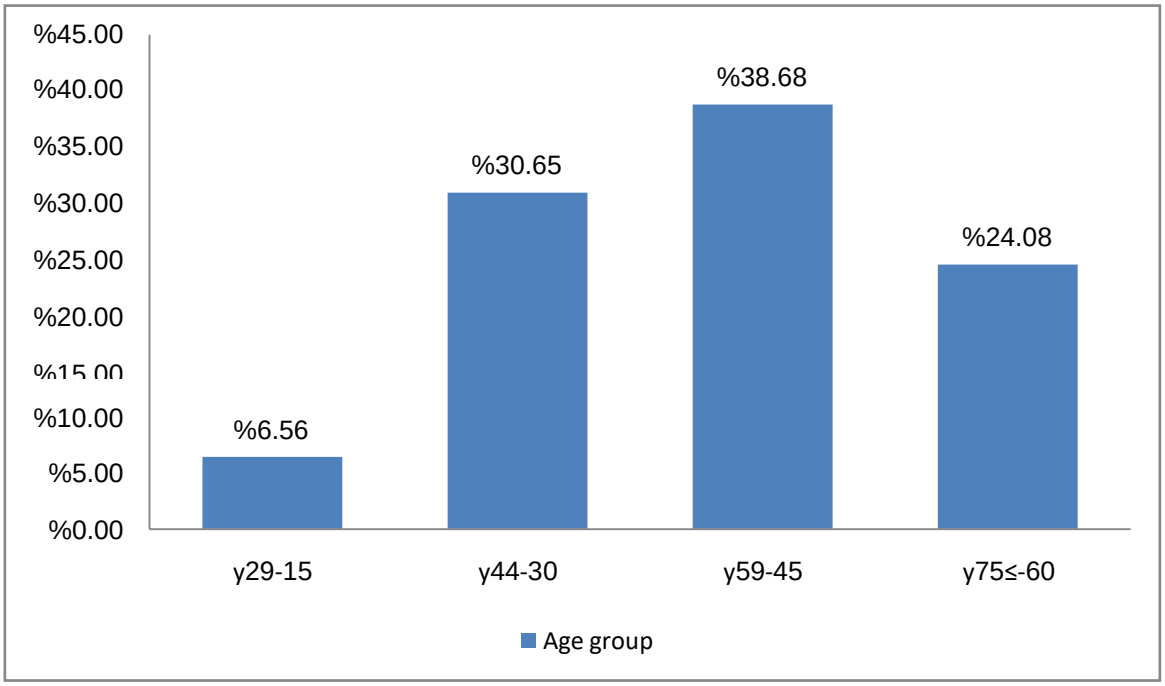
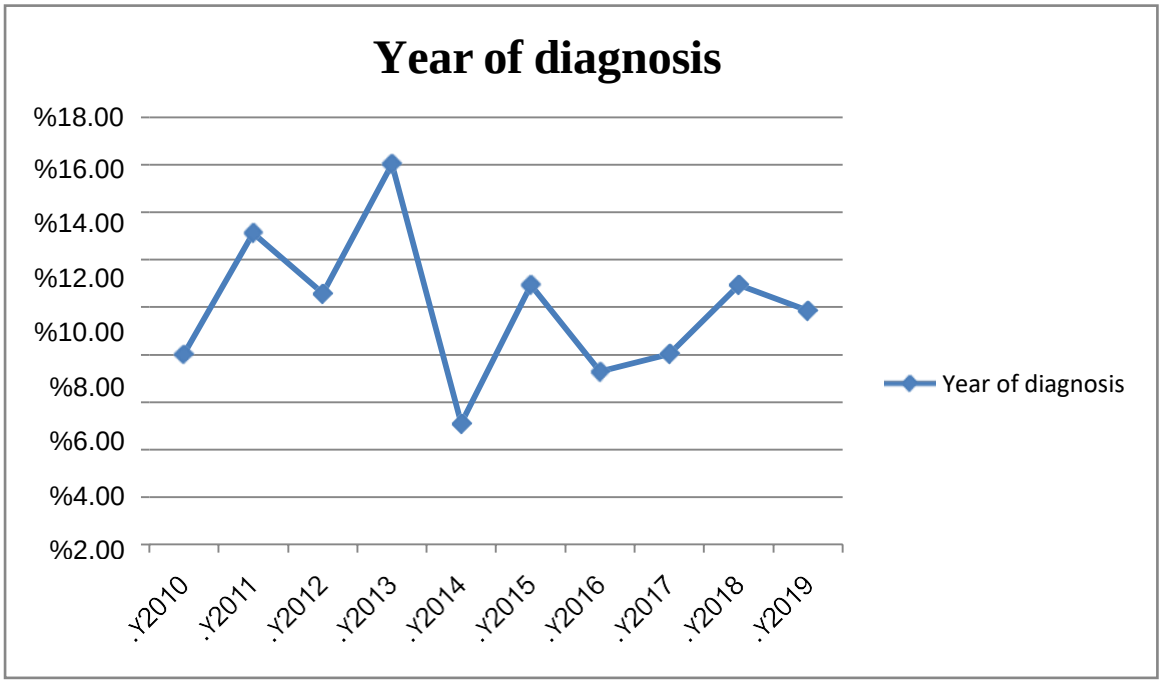


Figure 1. Correlation between breast cancer in females and women's age

Figure (2)The highest incidence of female breast cancer was recorded in year 2013, where 44 case (16%) were affected with breast cancer. While the lower incidence of breast cancer in year 2014 were14 case (5.1%), and the remaining years were closed to the incidence rates.



Figure(2): A bar chart showing the distribution of all cases of female breast cancer is attributed to the duration (2010-2019).

Most patients were not well educated at all. In the cases, the proportion of education in the non-

educated community was 201(73.72%) vs. 72(26.27%) and the outcome was statistically important ,Table (2) and Figure (3).

Table (2) relationship between Brest cancer and two variation (life style region and education level)

Year	education level		Total	address		Total
	non education	education		citizens	villagers	
2010	14(63.63%)	8(36.36%)	22(8.02%)	17(77.27%)	5(22.72%)	22(8.02%)
2011	23(63.88%)	13(36.11%)	36(13.13%)	25(69.44%)	11(30.55%)	36(13.13%)
2012	25(86.2%)	4(13.79%)	29(10.58%)	22(75.86%)	7(24.13%)	29(10.58%)
2013	31(70.45%)	13(29.54%)	44(16.05%)	38(86.36%)	6(13.63%)	44(16.05%)
2014	10(71.42%)	4(28.57%)	14(5.1%)	12(85.71%)	2(14.28%)	14(5.1%)
2015	23(76.66%)	7(23.33%)	30(10.94%)	23(76.66%)	7(23.33%)	30(10.945)
2016	15(75%)	5(25%)	20(7.29%)	19(95%)	1(5%)	20(7.29%)
2017	17(77.27%)	5(22.72%)	22(8.02%)	14(63.63%)	8(36.36%)	22(8.02%)
2018	21(70%)	9(30%)	30(10.94%)	19(63.33%)	11(36.66%)	30(10.94%)
2019	23(85.18%)	4(14.81%)	27(9.85%)	15(55.55%)	12(44.44%)	27(9.85%)
Total	201(73.35%)	72(26.27%)	274(100%)	204(74.45%)	69(25.18%)	274(100%)

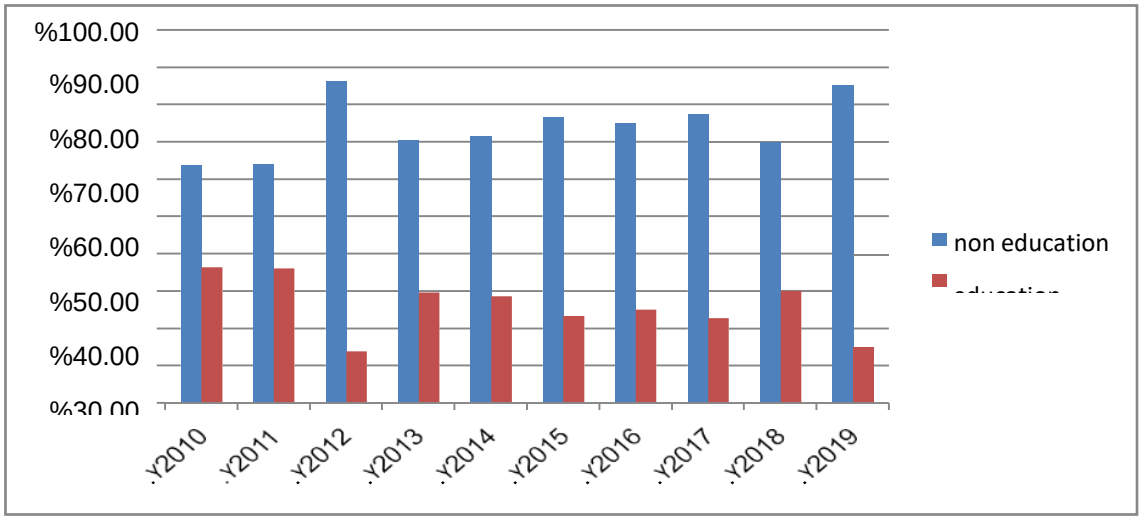


Figure (3) relationship between Brest cancer and education level

Figure (4) showed the highest incidence of female breast cancer was for female who live in the city 204 (74.45%)compared to the patient who live in the village was 69 (25.18%).

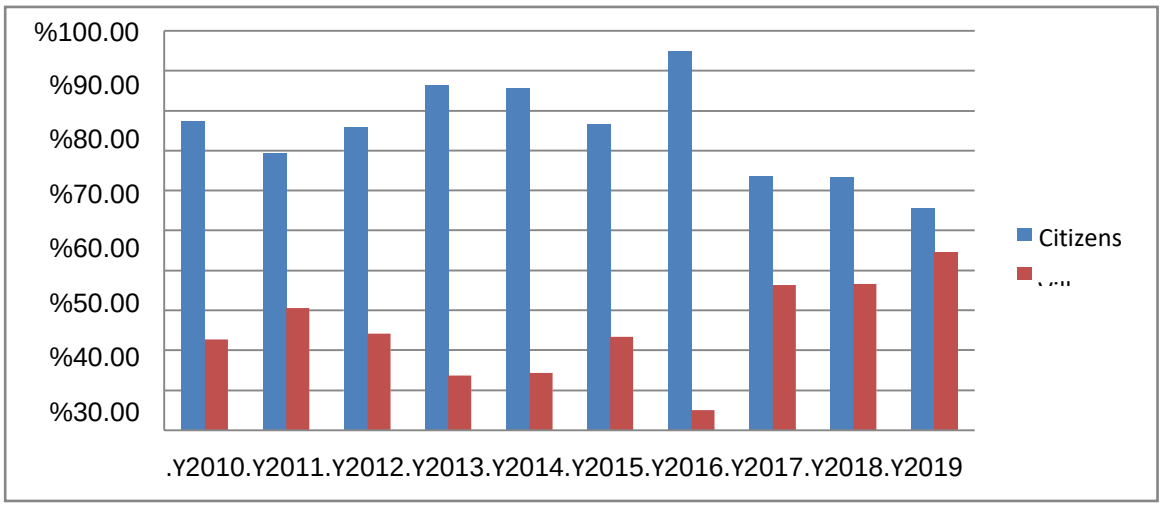


Figure (4) correlation between the female breast cancer and ad dress (citizens, villagers) of cases from period 10 years .

4. Discussion

A recent study showed the age group 45-59 years is high affected and these results were similar to [11,12].Non-educated women were highly affected than educated women and these results similar for[13]In those women linked to variables such as hormone replacement therapy use, lifestyle changes, reduced exercise, obesity, late the age at first birth and reduced that breast-feeding, this increased risk was due to the western life style.

We observed that schooling was related to a reduced risk of breast cancer, in comparison to these results. These findings may be due to certain cultural differences based on the fact that, compared to other women in the world, trained Iraqi women may be less influenced by the western life style or due to increased knowledge of their diet and physical activity and early detection of disease.[13].

5. CONCLUSIONS

Obviously, these findings reflect the general population's poor health education and their misunderstanding of the value clinical breast examination, breast self-examination, and early medical consultation..

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Detection of Rotavirus Infection in Iraq's Children and its Correlation with Rotarix Vaccine.

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Abstract

This study was aimed to determine the prevalence of Rotaviruses in Karbala and Basrah provinces in Iraq, the genetic recombination of Rotavirus strains in human with another hosts and release a novel strains may lead limited activity of Rotarix vaccine. Rotaviruses were detected by Immunochromatography Test (ICT) then the positive samples were tested by reverse transcription PCR (RT-PCR) using specific primers to vp4gene, this gene is responsible for stimulation the immune system to produce neutralizing antibodies about of 200 diarrheal stool samples, 100 samples from pediatrics hospital of Karbala province and another 100 samples from Maternity and children hospital in Basrah province . The vp4 gene was implemented in rotavirus vaccine of Iraqi immunization program. The RT- PCR results showed that 56.3% (27/48) of samples positive in children under five years of age in Karbala province while Basrah province revealed 58.5% (31/53) positive samples in children. The results of sequencing of human and Rotarix vaccine revealed that there was relation between them and animal strains according to genetic reassortment between human and another species while in comparing with international strains there were closely related with Lebanon and Turkey human strains. Necessarily for further studies are needed to study the genetic alterations with viral recombination of rotavirus to detect the Rotarix vaccine activity against novel Rotaviral strains.

Keywords: Rotaviruses, Rotarix, vaccine, RT- PCR

1. Introduction

Reoviridae family included Rotaviruses which are naked viruses with double stranded RNA (dsRNA). And about eight species from Rotavirus A to Rotavirus H were detected in this family [1] .The rotavirus genome consists of six non-structural (NSP1-NSP6) proteins and 11 dsRNA segments which code for the six structural proteins (VP1-VP4, VP6, VP7), VP4 with VP7 responsible for attached of virus with host receptors [2]. VP4 cleavage into two fragmentsVP8 and VP5 when a activated by proteolytic.at the tip of the VP5* attached in circular form with VP8 [3]. Binary system was classified RotavirusesA into 37 P and 27G kinds depend on vp4 (Protease sensitive) and VP7

(Glycoprotein) [4]. The infectious rotavirus particle and complete is about 100 nm in diameter, virion is genomefully infective particle surrounding by three-layered icosahedral protein capsid (TLP) similar to wheels (lat. rota) and this form gave the Rotavirus term [5]. Next icosahedral arrangement has been identified the single layered particle (SLP = core shell) consist of 120 particles of the viral protein2 (VP2) [6]. Newly, The three-dimensional (3D) structure of virion RV was detected by cryomicroscopy (cryoEM) with 4.3˚A determination of tomography [3]. The mechanism of RV entry was explained by communications of VP4 with other proteins of RV [7] Meanwhile VP4 spikes developed either in presence or lack of trypsin [8]. The faecal–oral route main transmition way of The virus, chiefly by direct contact of persons with other and less changes are wanted to induce disease in predisposed [9]. Outer capsid protein VP4 during its VP8 domain causes Rotavirus connection to host cells [10] and attachment partners on the host cell surface, containing by sialoglycans and histo-blood group antigens (HBGAs) [11]. Rotavirus–HBGA connections are influenced by protease sensitive P genotype, and studying these connections has induced new visions into variation of host species and transmission between divers species of different rotavirus strains [12]. In fact, alterations in rotavirus epidemiology among populations had been affected by genetic differences in HBGA expression [13].The function of HBGAs in the related effect on vaccine efficacy and susceptibility to rotavirus vaccine strains are important developing areas of research attended with public health [12]. Rotavirus attached and neutralized their antigen through the structural protein in outercapsid which are vp4 and vp7 genes Estes and [14]. TM (Wyeth-Lederle Vaccines) was pullout according to its side effect with intussusception in 1999. Then in 2006 another vaccine was improved with pentavalent reassortant human –bovine and finally RotatixTM [15]. Immunoglobulin A (IgA) give a good protection against infection with rotavirus , this suggestion was conducted by studies achieved on hygienic children infected with usual rotavirus [16].The total serum anti rotavirus IgA level, determined shortly after infection, generally reflects intestinal IgA levels and seems to be the best marker of protection [17]. The first immunity induced in human after birth for 6 months was obtained from mothers then the role of vaccination have been achieved for active immunization through the first years of children may expos to dangerous infection. In 2019 this vaccine induced in many country like Swedish national immunization program [18].The extra intestinal influence of the rotavirus vaccine, which is called the “rotavolution”, has recently become a growing field of debate and research. It has been proposed that the effect of rotavirus infections depend on host susceptibility, host genetics and the interaction with the intestinal microbiota. The effects has been proposed going far beside the classic gastrointestinal symptoms which are just the top of an iceberg. An evaluation from 2019 stated that the currently known rotavirus infection pathology is just the tip of an iceberg and that new questions about the influence of the rotavirus vaccine influence have

emerged. For example, it has been proposed that rotavirus infections can activate autoimmune diseases. Therefore, rotavirus vaccines might lead to reduced incidences of autoimmune related conditions such as diabetes mellitus and coeliac disease, due to immunological susceptibility and mechanisms. These hypotheses need to be explored further [19].

2. Materials and Methods

2.1 Materials

Rotavirus Detection
Immuno Chromatography (IC)Kit
RNA purification kit
FAVROGEN FavroPrep™Viral Nuclic Acid Extraction Kit
Reverse Transcription Kit
Bioneer Accupower® RocketScript™RT PreMix

Table1. Amplification Primers

Target gene	Primer Sequence 5´- 3	Product Size (bp)	Reference
Vp4	Forward, TATGCTCCAGTNAATTGG Reverse, ATTGCATTTCTTTCCATAATG	663	Durmaz *et al.*, 2014

2.2 Methods

A total of 200 diarrheal stool samples, 100 samples from pediatrics hospital of Karbala province and another 100 samples from Maternity and children hospital in Basrah province all children were under 5 years of age and suffering from diarrhea. were collected from hospitalized patients during the period from November 2018 to August 2019.

3. Detection of Rotavirus Antigen

3.1 LumiQuick, Adeno-Rota Virus Antigen Comb Test Card

Immuno Chromatography (IC)

The specimens were screened for Rotavirus antigen through Immuno Chromatography Test (ICT) according manuscripting company.

3.2 RNA Extraction Kit

FavroPrep™ for isolation viral nuclic acid from supernatant stool samples were used . It based on mini spin column (silica matrix) for the isolation of viral RNA according manuscripting company.

3.3 Reverse Transcription Kit

according manuscripting company.

3.3.1 cDNA Synthesis

Reagents and samples were brought to room temperature before starting with the reaction, 10 ul of RNA were added to the reaction tube and the final volume is completed up to 20 ul with RNase free water and mixed gently and spun-down by microcentrifuge at 3000 rpm. The reaction tube then placed in the thermal cycler programmed at 37 ,42, 95 respectively as manuscripting company. During cDNA generation random priming gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Synthesized cDNA is immediately used as template for PCR, or stored at -20°C for farther use.

3.3.2 PCR Amplification of vp4 gene

PCR reaction tube containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers from Bioneer company (Korea), adding 2.5 µl from forward primer TATGCTCCAGTNAATTGG (10 pmols/µl) and 2.5 µl from reverse primer, ATTGCATTTCTTTCCATAATG (10 pmols/µl) for vp4 gene. Seven microliter of cDNA template was added for each reaction tube, and the volume was made up to 20 µl with Deionized Nuclease –Free water and tubes were then spun down with a micro centrifuge for the adequate mixing of the components of the reaction. The thermo-cycling conditions were done with initial denaturation stage at 92°C for 5 min then denaturation stage also at 92°C but for 30 sec while annealing stage done at 45°C for 30 sec finally elongation stage done with72°C for 1 min.

3.3.3 DNA Sequencing

Sequencing of samples was done by Macrogen company (Korea). And sequencing result were viewed and compared with reference DNA sequence using Blast software.

3.3.4 Statistical Analysis

The Molecular Evolutionary Genetics Analysis version 7 (MEGA7) Software is used for both DNA and protein phylogenetic analysis using neighbor joining algorithm (20).

3.3.5 Phylogenetic Tree Analysis

Sequencing of samples was done by Macrogen company (Korea). And sequencing result were viewed and compared with reference DNA sequence using Blast software.

4. Results

4.1 Prevalence of Rotavirus in Human Stool Samples

The characteristic features of study population are summarized in table 2. All samples were tested from diarrheic children with acute gastroenteritis by imunochromotography test (ICT). In this study, stool samples of all children were less than 5year old with acute gastroenteritis were screened for Rotavirus antigen. The result showed 48 samples out of 100 were positive Rotavirus infection from pediatric teaching hospital in Karbala province. While in Basrah province 53 out of 100 samples were positive in diarrheic children, this difference was not significant (P>0.05) between two provinces .

Table 2. The result of Human Rotavirus Detected in Karbala Province and Basrah Province by Imunochromotography test (ICT).

Province	Total samples	Positive	Negative
		no.(%)	no.(%)
Kerbala	100	48 (48)	52 (52)
Basrah	100	53 (53)	47 (47)
Total	200	101 (50.5)	99 (49.5)

X2 = 0.5 df= 1 P=0.479

4.2 Prevalence of Rotavirus in Human Stool Samples

4.2.1 Diarrheic Human Samples

In order to Immuno Chromatography assay results, conventional PCR was carried out to detection the two genes of outer layer protein's VP4 663bp. After RNA was extracted then transcribed to cDNA these samples were amplified with primers for the 3' and 5' ends of VP4 gene. The study enrolled 27 samples from 48 Rotavirus positive samples (by an Immuno Chromatography assay) in Kerbala province shown in table 3, while in Basra province the result of RT-PCR results demonstrated 31 samples from 53 Rotavirus positive samples (by an Immuno Chromatography assay) as shown table 3 this difference was not significant (P>0.05) between two provinces. The positive results for VP4 gene were shown in figure 1. Also Rotarix vaccine were checked for gene of VP4 663 bp that demonstrated in this study as shown in figure 2.

Table 3. The results of human Rotavirus detected in Kerbala province and Basrah province by Polymerase Chain Reaction (PCR).

Province	Total samples	PCR (VP4) Positive no.(%)	PCR (VP4) Negative no.(%)
Kerrbala	48	27(56.3)	21(43.7)
Basrah	53	31(58.5)	22(41.5)
Total	101	58(57.4)	43(42.6)

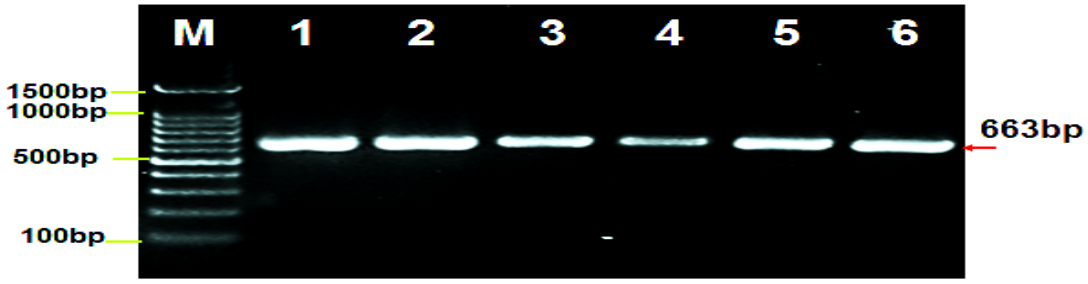

Figure (1): Agarose gel electrophoresis image showed RT-PCR product analysis for VP4 gene M (Marker ladder 100bp). Lane (1-10) some positive VP4 gene in Rotavirus at 663bp product size.

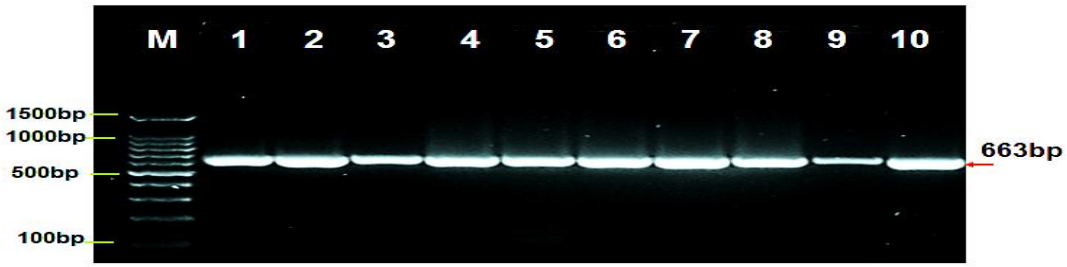

Figure (2): Agarose gel electrophoresis image showed RT-PCR product analysis for VP4 gene M (Marker ladder 100bp). Lane (1-6) positive VP4 gene in Rotavirus at 663bp product size.

4.2.2 Sequencing of DNA detected

The accession number of local Iraq's strain in present study demonstrated in (Table 4).

Table 4.Clinical sample from human amplified using Rotavirus vp4 gene specific primers

No	Type of gene	Current study strain (GeneBank accession No.)	Source
1	Vp4	MT074691	human
2	Vp4	MT074692	human
3	Vp4	MN995336	vaccine
4	Vp4	MN995337	vaccine
5	Vp4	MT074687	vaccine

4.2.3 Sequencing of DNA detected

The amplified vp4 gene with 663bp from human and vaccine. Using Blastn software for all local samples of vp4 gene derived from diarrheic human samples derived nucleotide sequences were aligned for matching with the reference database (GenBank) revealing the local samples from human and vaccine having slightly mismatch (99% identity) with reference human vp4 gene genbank sequence accession ID: MH59138 in human sample while with ID: Kx228855 in Rotarix vaccine as shown in figure 3 and figure 4 respectively.

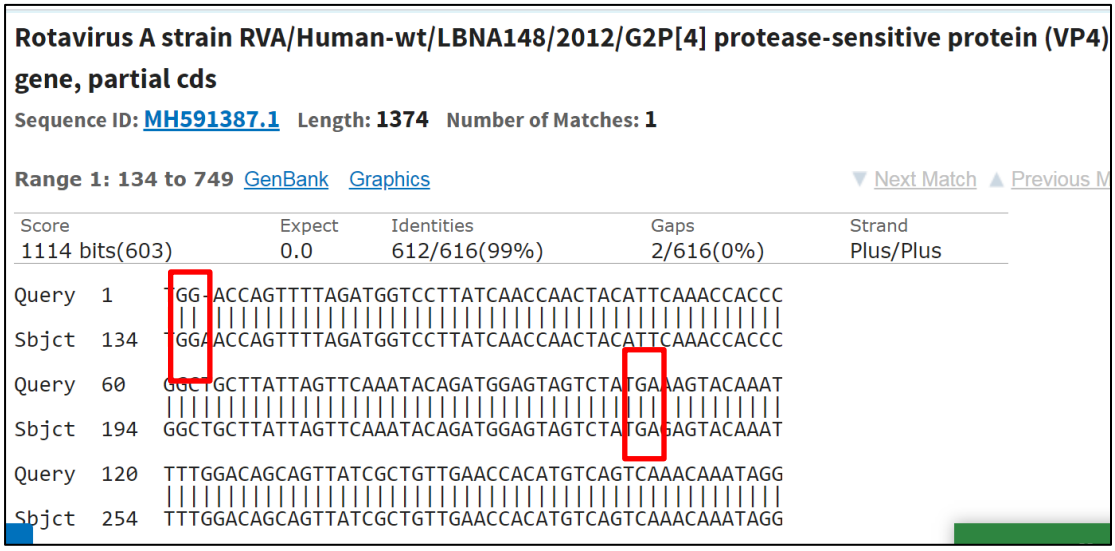

Figure (3): GenBank database alignment of VP4 gene DNA sequence from human sample showing 99% match with match with reference human Rotavirus, Mismatch sites indicated with red boxes, using Blastn.

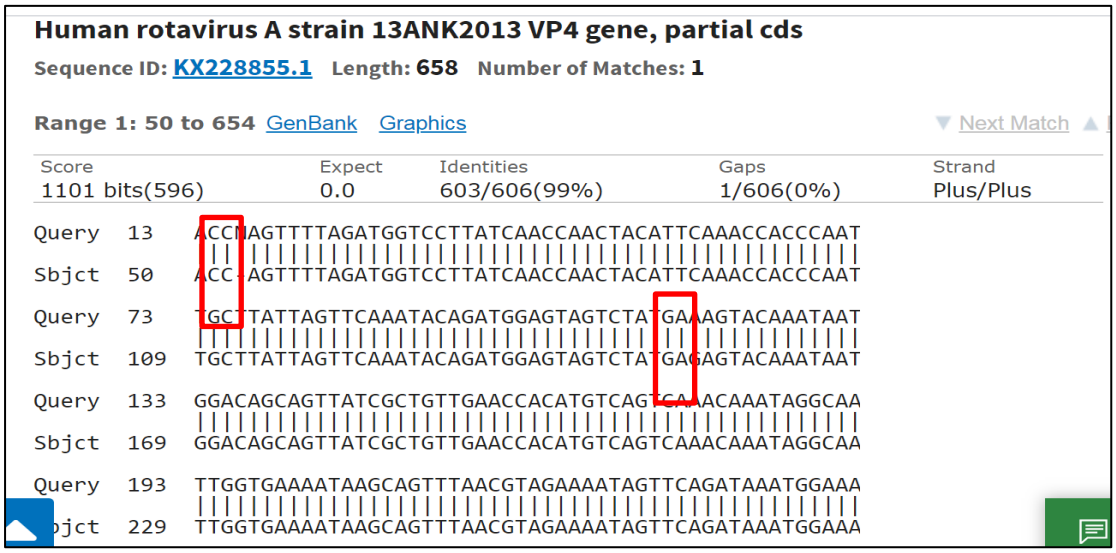

Figure (4): GenBank database alignment of VP4 gene DNA sequence from vaccine sample showing 99% match with match with reference human Rotavirus, Mismatch sites indicated with red boxes, using Blastn.

4.2.4 Phylogenetic Tree Analysis

Phylogenetic analysis where done using MEGA7 softwere , analysis done for vp4, the tree is drown to scale, with branch lengths measured in the number of sub situation per site. the analysis involved 30 nucleotide sequences for VP4 gene, figure 5.

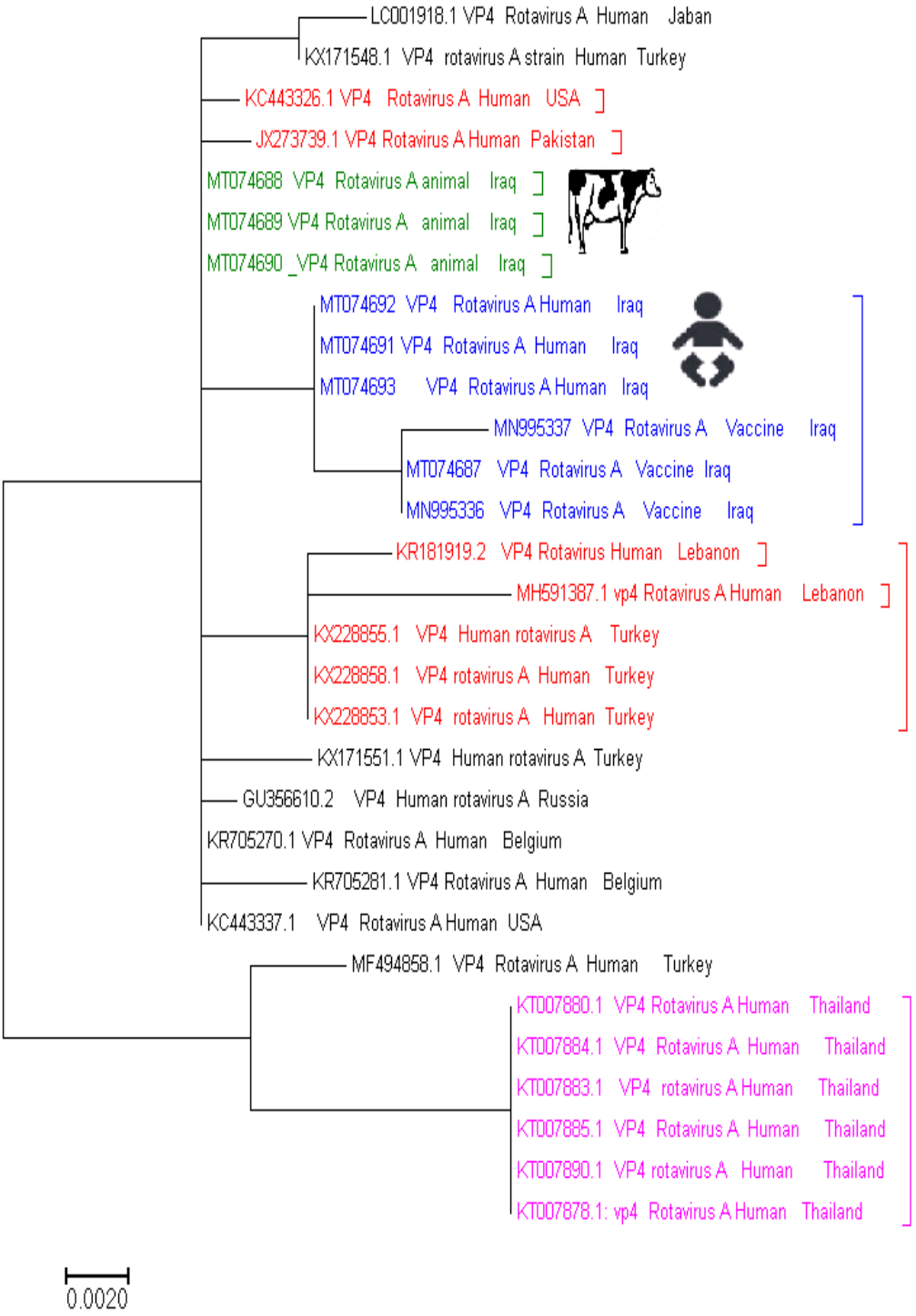

Figure (5): Rotavirus A *VP4* gene segment phylogeny for both local and international sequences molecular phylogenetic analysis was done by maximum likelihood method. The boot strap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the sequences

analyzed. blue color = current human and vaccine strains, green color = related animal strains, red color = closely related strains, pink color = less related strains, the rest samples are international sequences.

5. Discussion

Young children under 5 yeas more susceptible for gastroenteritis symptoms with Rotaviruses infection (2) in another hand diarrheal disease record about 90% in developing countries although the improvement in medical treatment (21). Rotavirus infection in children were confirmed with infection by rapid Immunochromatographic test (ICT) that showed 48% and 53% in Kerbala and Basrah provinces respectively (table 2) this difference was not significant , our results agree with (22) who collected the samples from five Iraqi provinces (Babil, Kerbala, Missan, Qadissiya, and Wasit) they found a similar prevalence (47.2%), by using (IC) test. The international scenario of rotavirus prevalence exhibited by (23) who used conventional methods (eg, IC test and ELISA test) for detection of Rotavirus also recognizing the varying manner after vaccination with common serotypes in children about 24%. Truly the picture is highly complex in human and animal may haven a diversity of genotypes with current period or in prospectively in a far time appear the dramatic of related host species that may release a unique strain rotaviruses patterns according to credited genomic diversity in numerous hosts species (24). There is an profusion of molecular epidemiological statistics on the opportunity out of both usual and unusual VP7 and VP4 proteins determined by Reverse transcription PCR amplification in world, so the present study by using RT- PCR technique for intensification of vp4 and vp7 genes (by rotavirus specific primer) from human ,animal with desired amplicon size is designed to detect the occurrence of Rotavirus and its molecular properties to detect the specific genotype which are distributed between the human and animal populations, Rotavirus was detected in (56.3%) and (58.5%) of human samples in Kerbala and Basrah provinces respectively (table 3), therefore, the results of present study were somewhat consistent with many Iraq's studies, (51.98%) in Mid Iraq was conducted by .(25)

While neighbored countries RT-PCR conducted in Turkey found (78.2%) of Rotavirus in tested samples while in children age range from thirteenth to twenty four months (38.7%) infective rate whereas higher than (28.3%) monitoring in children with twenty five to thirty six menthes (26). On other hand, Arab countries disagree with the incidence of Rotavirus accomplished in present study resembling by (27) who revealed (44%) in Kuwait while (28) reveled (61%) in Syria whereas (29) showed (50%) in Oman .These varying in incidence statistics may be due to season of specimen taken and approaches of specimen detection.

Rotaviral VP4 DNA Sequence Analysis

The present study by Blastn significant the similarity of genetic features between human and Rotarix vaccine that applied to advance studies are desired to understand the undercurrents of Rotavirus relationship between circulating strain and Rotarix strain .

Blastn showed related of vp4 gen in human, animal samples and vaccine sample related with MH559138.1 for both human and Rotarix vaccine and KX228855.1 for animal sample from GenBank (NCBI) as shown in figure (3) and figure (4) respectively. Since 2016 year Iraq has implemented the final Rotavirus vaccine Rotarix vaccine but continuous circulating novel strains according re-assortment habitat of Rotavirus encourage the observation of variations in Rotavirus genotype circulation and detection of vigorous vaccine compatible with this unique variants strains become necessary to develop vaccination protocol and induce of modern Rotavirus vaccines (30).

However, it is essentially check methods periodically to develop vaccines efficacy against Rotavirus infection in order obtain the perfect benefits from immunization with vaccine implemented (31).

Rotaviral VP4 DNA Sequence Analysis

Phylogenetic tree and correspondence analyses were achieved to conclude the association between most recurrently identified rotavirus, The close relation of local samples sequences to each other as it is clustered in one branch away from other international derived sequences. The figure (5) demonstrate the genetic diversity of local strains from different sources human, animal and vaccine strains for vp4 gene, human an vaccine strains are closely related with Lebanon at KR181919.2 and KX22885 from Turkey and with animal strains which are closely related with Pakistani strain have ID JX273739.1 and KC443326.1 (NCBI) from USA whereas less related with strains from Thailand have ID KT007880 . The differences of human strains identity with variant strains give indication about Rotavirus modification (32).The genotypic diffusion of Rotaviruses with different strains according to production of novel strain with affected conditions as changing of temperatures and geographical terrain of infected region all these variations corresponding with vaccine improvement and approach of diagnostic vaccine efficacy (33). However, in present study analysis of both vp4 gene in middle and south Iraq was detected in partial genome while in future analysis for complete genome will need to understand the molecular characterization of Rotavirus.(35 , 34)

Full complete genome analysis could be made in the upcoming to improved describe strains socializing in the pre- and post-vaccination dated. Though, to the greatest of our information, no revision from nations or areas with high vaccination attention have described in unimmunized or immunized children. No suggestion of vaccine derivative variations in vp4 were detected while slight

genetic difference in human and animal sequences throughout the study dated (36). Therefor current study, also realized about extremely probable that a choosy effect of vaccination occasioned in a different molecular outline of common G1P[8] Rotarix vaccine strains and the correlation of human strains with animal strains show the origin of human strain may be from animal sources according to recombination property of rotavirus while Rotarix vaccine prepared from human strain only this analysis lead to indicate the limited protection of Rotarix vaccine (37).

6. CONCLUSIONS

In present study the local strains are different from other local Iraqi strains while data of Rotaviruses prevalence with international strains showing closely related and continuous observing of reassortment of Rotavirus genotypes is needed in the upcoming to evaluate the Rotarix vaccine efficacy with variant Rotavirus strain. Also, full molecular analysis of genome fragments should be done frequently to track strain variety and improved outlines of vaccine preparation and insurance with vaccine update periodically in this types of recombinant viruses.

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Epstein - Barr Virus: Correlation of the Presence of Associated Genes and Antibodies with Rheumatoid Arthritis

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Abstract

The study's goal is to find a link between the existence of Epstein-Barr virus nuclear antigens (EBVNA) and rheumatoid arthritis in patients. The present study was conducted on a study population with a sample size of 148 participants, comprising of two groups: the first (74) rheumatoid arthritis patients (case) and the second (74) healthy persons with no symptoms of autoimmunity (control).

The existence of the virus was investigated in both case and control groups. Patients visiting Marjan Medical City in Babil Governorate between November 2017 and May 2018 between the ages of 15 and 75 years were recruited. The patients and control samples were serologically screened using ELISA techniques prior to identification of EBV genes (EBNA1 and EBNA2) was conducted using nested polymerase chain reaction (nPCR).

The results obtained indicated that 3 samples were positive for EBNA1, while 5 were positive for EBNA2 in the patients group. No positive EBNA1 and EBNA2 positive sample was obtained in the control group. This study found that patients with rheumatoid arthritis had detectable levels of EBNA-1, EBNA-2 genes while the control group had none. These findings suggest that the virus has a role in the increased prevalence of rheumatoid arthritis.

Keywords: RA , EBV , PCR, EBNA1 and EBNA2

1. Introduction

Epstein-Barr virus (EBV) is a human herpesvirus that is carried by more than 90% of the adult population globally. EBV has a preference for B cells, and in healthy carriers, latently infected B cells serve as the viral reservoir (1). EBV are very common infectious pathogens that infect around 95 percent of the world's population in a dormant state (2). The majority of EBV infections develop during infancy and might result in a moderate, generally asymptomatic illness. Primary infection in adolescence results in infectious mononucleosis (3). It's also linked to endemic Burkitt lymphoma (4), Hodgkin lymphoma (5), diffuse large B cell lymphoma and two epithelial cancers: gastric carcinoma

and nasopharyngeal carcinoma (6). EBV-associated cancers occur most likely in immunosuppressed persons .Where up to 20% of B-lymphocytes are infected with EBV . (7)

During latent and transitory infections, the first viral protein identified was Epstein–Barr viral NA1 (EBNA 1). (8). The sole protein required for EBV genome persistence is EBNA1, which contributes to replication and histological separation of the viral genome.(9)

This study adds to the evidence that EBNA1 is involved in gene expression control (10). For example, lymphoma is required to avoid cell death.(11)

Epstein–Barr virus NA1 stimulates replication activity via its promoter location (Sp1) and causes protein expression survival. As a result of the suppression of caspase pathways in EBV-positive cells, the regulation of survival in expression will prevent cell death (12). The genome's stability, including DNA damage, chromosomal abnormalities, and double-stranded DNA, can be promoted by EBNA1, (RAG-2) and (RAG-1) and ROS (raised by transcription activity activation) (nX2) / gp91phox, catalytic sensitivity to NADPH oxidase.(13)

EBNA1 has the ability to suppress the production of tyrosine receptors r-kappa, the target gene TGF-b, and aids in the development and survival of HD cells.(14)

EBNA1 can control the malignant tumor cell in nasopharyngeal cancer by boosting expression and nuclear localization (15). EBNA1 stimulates AP-1 transcription activity and enhances vascular endothelial growth factor (VEGF) production in nasopharyngeal cancer cells, indicating that EBNA1 may contribute to blood vessel development and necrosis in nasopharyngeal carcinoma.(16)

Because of the dispersion of PML nuclear bodies, EBNA1 may be crucial for the development of nasopharyngeal cancer and then prevent malignant transformation (17).Through the activation of cellular genes and many viral genes, Epstein–Barr virus NA2 initiated a cascade transcription of primary and secondary targeted genes.(18)

These changes eventually govern B cell activation by triggering cell cycle induction of proliferation in altered growth cells.(19)

EBNA2 has the capacity to express release (c-MYC), which stimulates cell proliferation by regulating cyclin Ds and E and regulates CDK2 inhibitors at the bottom, such as p27KIP1 and p21CIP1.(20)

Epstein–Barr virus NA2 also affects Bcl6 expression, a key regulator of germinal centers difference, and B cells lymphomagenesis has been linked to the involvement of EBNA2.(21)

According to a recent research, the survival of EBNA2 positive DLBCL patients is considerably lower than that of EBBC2-negative DLBCL patients, implying that EBNA2 plays an essential role in lymphoma formation.(22)

Rheumatoid arthritis (RA) is an autoimmune disease that primarily affects the joints. This term (rheumatoid arthritis) is based on the Greek for watery and sore joints. A affects about 24.5 million people of the worlds in 2015 (23).This is between 0.5 and 1% of adults in the urban globe with 5 and 50 per 100,000 new cases is developing the condition each year (24). This condition occurred most frequently during the middle age with the women affected 2.5 times most than the men. In 2013, it resulted in 38,000 deaths up from 30,000 in 1990 (23).

2. Material and methods

Seventy-four patients consisting of 58 females and 16 males which were diagnosed with RA (according to rheumatologist physicians and serological tests) were recruited. The control group consisted of 74 individuals without any symptoms of autoimmune disease. Blood samples were collected at Marjan Medical City in Babil /Iraq, between the periods of November, 2017 to May, 2018. All the samples were diagnosed by ELISA technique, using ELISA kits EBV-VCA (IgM , IgG) antibodies (Euroimmune, Germany).

The control group were 74 case without any symptoms of autoimmune disease. All the samples were diagnosed by ELISA technique, by using ELISA kits EBV-CA (IgM , IgG) antibodies (Euroimmune , Germany).

PCR technique: The DNA was extracted from blood RA patients using extraction kit (Sacase ,USA) .

Primers: EBNA1 Inner and Outer (Lin JC et al ., 2001) ,and EBNA 2(Chen CH et al .,2009).

Nested Polymerase Chain Reaction (nPCR)

Table 1. The PCR profile included

Primer	Sequence	Amplicon
EBNA 1 Inner	F AG.ATGA.CCCA.GG.AGA A.GG C.CC A.AG C	308 bp
	R CA.AAGGG.GA G.ACG.AC T.CA A.TG. GTG T	
EBNA 1 Outer	F GT.AGAA.GGCC.ATT.TTT.CC.AC	609 bp
	R CT.CCAT.CGTC.AAA.GCT.GC. A	
EBNA 2	F AGGCTGCCCACCC.TGAG.GAT	186 bp
	R GCCACCTGGCAGCC.CTA.AAG	

3. Results and discussion

3.1 EBV Specific Primers Detection by PCR According to Age Groups in Whole Blood Sample

The study of the special genes of the EBV (EBNA 1 & EBNA 2) primers was conducted in the blood samples of the RA patients sample by using Nested - PCR technique. The results showed that 5 were given positive results for EBNA1 genes and the most of the positive results were seen in age groups (61-75) and (46-60) years.

Depending on the primer, the second gene that was used in the PCR technique EBNA2 genes from blood samples for the same patients. The results showed that there were 6 samples that gave a positive result results, also all within the same denominator (Table 2).

In case of the control group, the detection of specific primer of EBV genes (EBNA1 & EBNA2) showed that negative results and there were no DNA primer detected in all diagnosed samples. (Table 3).

Table 2. EBV Primers Detection by PCR According to Age Groups in Whole Blood Sample

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	24	0	**24**
31 – 45	19	0	19	0	**19**
46 – 60	20	2	18	3	**15**
61 - 75	11	3	8	3	**5**
Total	74	5	69	6	**63**

Table 3. EBV Primers Detection by PCR According to Age Groups in Whole Blood Sample (Control group)

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	0	0	**0**
31 – 45	19	0	0	0	**0**
46 – 60	20	0	0	0	**0**
61 - 75	11	0	0	0	**0**
Total	74	0	0	0	**0**

3.2 EBV Primers Detection by PCR According to Age Groups in Serum Sample

The study also involved an investigation of some special genes of the EBV such as (EBNA 1 & EBNA 2) primers were conducted in the blood samples of the RA patients sample by using Nested - PCR technique .

The results showed that (5) samples were given positive results for EBNA1gens and the most of the positive results were seen in age groups (61-75) and (46-60) years.

Depending on the primer, the second gene that was used in the PCR technique was EBNA2 genes from blood samples for the same patients .

The results showed that there were (6) samples gave a positive result. Table(1)

In case of control group. The detection of specific primer of EBV genes (26) showed that negative results and there were no DNA primer detected in all diagnosed samples. Table (2)

The study investigated the primers of special genes in blood samples of rheumatoid patients in order to study the relationship between some viral genes and the incidence of rheumatism. Several studies have shown that these genes have a direct relationship with rheumatism.

(27)refer to that EBNA-1 has the capacity to cellular chromosome anchoring, for viral DNA replication action and facilitates the evasion of immune response. This process lead to viral maintenance mechanisms such as latency.

The PCR test showed that some positive samples results were observed for the presence of these parameters while the results of the control group were 0 indicating a correlation between these genes and incidence of disease .

The study also included the investigation of the EBV virus genes primers (26) in serum samples of rheumatoid patients using nested PCR.. The results showed that out of 74 serum samples, three samples were given a positive result of a EBNA1 in age groups (61-75) and (46-75).Table (4.3.6 A)

The investigation of the EBV viral primer EBNA2 in the serum sample of RA patients revealed 5 cases were positive results, these positive samples belong to the age groups(61-75) and (46-75).Table: 4.3.6 B.

Table 4. EBV Primers Detection by PCR According to Age Groups in Serum Sample.

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	24	0	**24**
31 – 45	19	0	19	0	**19**
46 – 60	20	1	19	2	**18**
61 - 75	11	2	9	3	**8**
Total	74	3	71	5	**69**

Table 5. EBV primers detection by PCR according to age groups in serum sample (Control group).

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	0	0	**0**
31 – 45	19	0	0	0	**0**
46 – 60	20	0	0	0	**0**
61 - 75	11	0	0	0	**0**
Total	74	0	0	0	**0**

The study also included the investigation of the EBV virus genes primers (EBNA1 & EBNA2) in serum samples of rheumatoid patients using nested PCR. The results showed that out of 74 serum samples, three samples were given a positive result of EBNA1 in age groups (61-75) and (46-75).Table (4)

At the time, the investigation of the EBV viral primer EBNA2 in the serum sample of RA patients revealed 5 cases were positive results. These positive samples belonged to the age groups (61-75) and (46-75).Table (5)

The same special parameters in the viral genes as in the results and the previous tables were also investigated in the serum samples of the disease in order to as certain the presence of these genes in the serum of the patients. The study showed that some serum samples contain the viral gene primers while the results were controlled .

The virus has the high access to serum and not only blood and also indicates the high activity of the virus in patients with rheumatism, and that these viral genes have a close relationship with rheumatism, the same documents did not show these genes in serum samples of healthy people. It is found lost and associated with patients with rheumatism. The same viral genes evaluated in the whole blood samples were also investigated in the serum samples of the diseased and control groups in order to ascertain the presence of these genes in the serum of the patients. The study showed that some serum samples contain the viral gene primers while others do not.

4. Conclusions

According to the results of the study the importance of viral genes are present in the patients' serum. Specific primers EBNA 1 and EBNA2 was detected in some samples of rheumatoid arthritis patients and these primers have a role in the pathogenesis of rheumatoid arthritis. Viral genes EBNA1

and EBNA2 are carcinogenic proteins and play a main role in causing different types of cancers and their presence in patients with rheumatoid arthritis may signal future cancers in these patients.

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CD20 and CD10 of Chronic B-Cell Neoplasms in Correlation with Morphological Diagnosis

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Abstract

Chronic B-cell neoplasms include a number of disease entities arising from mature B lymphocytes which involve primarily the blood, bone marrow (BM) and lymphoid organs such as the lymph nodes and spleen. Chronic lymphocytic leukemia (CLL) is characterized by absolute lymphocytosis in the peripheral blood and BM. Non-Hodgkin lymphomas (NHL) also may have extensive BM and peripheral blood involvement at initial presentation like follicular lymphoma (FL), mantle cell lymphoma (MCL) and splenic marginal zone lymphoma (SMZL).

Although there are difficulties in separating CLL from some NHL, the distinction is important because prognostic and therapeutic differences exist. Immunophenotyping (IPT) has become an essential tool to confirm the diagnosis and to separate CLL from other lymphoid malignancies. Evaluate the role of immunostained CD10 and CD20 in the subclassification of chronic B-cell neoplasms and confirm the significance of these markers as a complementary test to morphological features of chronic B-cell neoplasms using BM aspirate and biopsy. BM biopsies of fifty five adult patients with CLL and leukemic phase of NHL were collected from December 2010 to April 2011; fifty of them were retrospectively while five of them were prospectively collected. Current study revealed that CLL cases (27) showed weak positive reaction with CD20 (96%) while no reaction with CD10 (0%). While NHL (28) cases showed positive reaction with CD20 (100%) & CD10 (71.5%). This study revealed significant statistical difference between morphology and IPT diagnosis at the level of P.value <0.05.

This study revealed that the CD markers has important diagnostic role in the subclassification of chronic B-cell neoplasms also revealed that the IPT technique in conjunction with morphology have more precise role than morphology alone in the diagnosis of chronic B-cell neoplasms.

Keywords: CD10, CD20, CLL, NHL

1. Introduction

Within the broad category of B-cell lymphoproliferative neoplasms a number of disease entities arising from mature B lymphocytes which involve primarily the blood, BM and other lymphoid organs such as the lymph nodes and spleen. All these disorders are classified by the World Health Organization (WHO) 2008 on the basis of their histopathological features.[1]A constant finding in all these entities is the presence in peripheral blood of leukemic cells in various degrees. Some of these conditions could be considered as CLL and others represent the leukemic phase of NHL and their recognition is important for differential diagnosis and patient management.[2]IPT markers play a key diagnostic role by enabling demonstration of the B or T cell nature of the neoplastic cells; by establishing clonality in B-cell disorders by immunoglobulin light chain restriction analysis, thus, distinguishing between neoplastic and reactive B lymphocytosis.[3]

Lymphocyte malignancies compose a wide spectrum of different morphologic and clinical syndromes. Lymphocyte neoplasms can originate from cells that are at a stage prior to T and B lymphocyte differentiation from a primitive stem cell or from cells at stages of maturation after stem cell differentiation. Thus, acute lymphocytic leukemias (ALL) arise from a primitive lymphoid stem cell that may give rise to cells with either B or T cell phenotypes. On the other hand, chronic lymphocytic leukemia arises from a more differentiated B lymphocyte progenitor and myeloma from progenitors at even later stages of B lymphocyte maturation. Variability in expression of a lymphopoietic stem cell disorder may result in the spectrum of lymphocytic diseases, such as a B lymphocyte or T lymphocyte lymphoma, and different types of diseases, such as hairy cell leukemia (HCL), prolymphocytic leukemia (PLL), natural killer cell (NK-cell), large granular lymphocytic leukemia, or plasmacytoma. [6]

WHO Classification of mature B-cell neoplasm: [11]

- Chronic lymphocytic leukemia/Small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia)
- Splenic marginal zone lymphoma
- Plasma cell neoplasms:
    - Plasma cell myeloma
    - Plasmacytoma

- Monoclonal immunoglobulin deposition diseases
- Heavy chain diseases

- Extranodal marginal zone B cell lymphoma, also called MALT lymphoma
- Nodal marginal zone B cell lymphoma (NMZL)
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B cell lymphoma
- Mediastinal (thymic) large B cell lymphoma
- Intravascular large B cell lymphoma
- Primary effusion lymphoma

Burkitt lymphoma/leukemia

The chronic/mature B-cell leukaemias include CLL, which is by far the most common, the rare B- PLL and HCL. The B- cell NHLs include FL, SMZL and MCL that most frequently affect the blood and BM and may spill over to the peripheral blood[1] . Antibodies that used in this study are CD10 and CD20[4].

2. Markers

In general, there are no surface markers that are diagnostic of malignancy in lymphocytes. Both flow cytometry or paraffin block IHC can be used to identify specific cell surface or intracellular protein expression. Flow cytometry has the advantage of simultaneous semiquantitative analysis of multiple markers.(112) However, not only it requires fresh sample for cell suspension preparation, but it is also expensive and cannot be correlated with cytoarchitectural findings. With advances in antigen retrieval techniques most antibodies can be successfully applied to paraffin blocks. Furthermore, IHC can easily identify a small population of target cells. CD20 was chosen to determine the lineage of the cells (B and T-cells). After presence of B cell is demonstrated by positive staining for CD20 (or other B cell markers). CD10 was selected for its putative role in FL. The B-cel NHLs phenotypically correspond to normal cells in the mid stages of normal differentiation. More specifically, by their expression of B-cell activation antigens, these tumors are the neoplastic counterparts of normal activated B cells[11].

2.1 CD10

Common acute lymphoblastic leukemia antigen (CALLA) is a cell-surface endopeptidase. CD10 is belonging to a family of type II transmembrane metallo proteases that also include the leucocyte antigens CD13 and CD26 as well as aminopeptidase. The gene encoding CD10 is located on chromosome 3.(12)

CD10 is a zinc-dependent enzyme and is thought to down-regulater cellular responses to peptide hormones (120) .

On lymphoid cells, CD10 is expressed on immature T-and B-precursor cells but lost as the cells reach maturation. It is re-expressed on proliferating B cells and mature neutrophiles (12) .

In lymphoid malignancy, CD10 is expressed in acute lymphoblastic leukemia(ALL) arising from precursor B cells, but also observed in a portion of T-cell ALL. Additionally, the antigen may also be expressed in FLs, Burkitt lymphoma and subsets of DLBCL. CD10 may be expressed infrequently in MCL; CD10 is expressed also in renal cell carcinoma.(12) Beside hematopoietic cells, it is also expressed in epithelial cells of the liver bile canaliculi, renal tubules, tonsil, germinal center B cells, stem cells in the BM, a subset of immature B cells in BM and neutrophiles.(13) The antibody also labels germinal center in lamina propria of colon, brush borders of the enterocytes in the small intestine, gallbladder brush border epithelium, interstitial stromal cells of the lung, Schwan nervecells and stromal cells in striated muscles, fibroblasts, trophoblast and cytotrophoblast of the placenta, prostate, stromal cells in the endometrium and breast myoepithelial cells. The staining pattern is membranous. (14).

2.1 CD20

CD20 is a transmembrane, glycosylated protein expressed on B-cells precursor and mature, but is lost following differentiation into plasma cells. The long N-and C-terminal ends of the protein are located on the cytoplasmic side of the membrane and only a minor portion of the protein is expressed on the cell surface. It is suggested that CD20 plays a direct role in regulating the transmembrane conductive Ca flux of B-cells which indicates a possible function for CD20 as a regulator of proliferation and differentiation. (125)

In normal lymphoid tissues, the antibody labeled germinal center cells, mantle zone lymphocytes, and scattered interfollicular lymphocytes but not T cells, histiocytes and plasma cells. No labeling was observed in epidermis, sebaceous glands, hair follicle and eccrine glands in the skin, follicular epithelium in the thyroid, pneumocytes and bronchial epitheliam of the lung[16].

CD20 is expressed on the great majority of mature B-cell lymphomas. This is a very useful “pan-B” cell marker. The CD20 antigen is not expressed on very immature lymphoid cells (acute

undifferentiated leukemias) but begins to be expressed on early maturational stages and then CD20 is fully expressed on mature B-cells (CLL, PLL, HCL and B-cell NHL).The staining pattern is membranous and or cytoplasmic[17].

Evaluation of CD20 expression has therapeutic importance.A humanized monoclonal antibody (Rituximab) against CD20 is now available for treatment of B-cell lymphomas expressing this molecule. Thus, CD20 expression is used as a criterion for administering Rituximab. Following treatment of FL with anti -CD20 monoclonal antibodies, the trephine biopsy sections may show disappearance of the B-cell infiltrate but persistence of reactive T cells). In some patients, there is a change of IPT shortly after such immunotherapy with B cells failing to express CD20; this may be persistent for several months and does not correlate with a failure of response[18]

The aim of current study was to evaluate the role of immunostained CD10 and CD20 in the subclassification of chronic B-cell neoplasms and confirm the significance of these markers as a complementary test to morphological features of chronic B-cell neoplasms using BM aspirate and biopsy.

3. Materials

BM biopsies of fifty five adult patients with CLL and leukemic phase of NHL were collected from December 2010 to April 2011 with age range of 28-80 years; fifty of them were conducted retrospectively from archive files of the Department of Hematology of the Medical City Teaching Laboratories while five of them were conducted prospectively from private laboratories. For each case one section was stained with hematoxylin and eosin and two other sections were stained immunohistochemically (IHC) for CD10 and CD20.

Primary antibody kit as shown in table 2.13.

Table 1. Primary antibody kit used in this study

Primary antibody	Source	Type	Code number
CD10	Dako Cytomation	Monoclonal Mouse Anti-Human CD10	M7308
CD20	Dako Cytomation	Monoclonal Mouse Anti-Human CD20	M0755

Scoring systems for markers: The scoring system for all markers was scored positive if 25% or more of the cells within an aggregate showed cellular membrane and/or cytoplasmic staining pattern[5]

Staining patterns: The cellular staining pattern is membranous and/or cytoplasmic for CD10[7] and CD20[8].

4. Results

Current study revealed that CLL cases (27) showed positive IPT reaction with CD20 (96%) while no reaction with CD10 (0%).Moreover, NHL (28) cases showed positive reaction with CD20 (100%), CD10 (71.5%).

Furthermore, FL showed positive reaction with CD10 and CD20 in 100% of cases while MCL showed 100% positive reaction with CD20 and no reaction with CD10. So significant statistical difference between morphology and IPT diagnosis at the level of P.value <0.05.

Table 2. IHC findings in CLL patients

CD10		CD20	
positive	negative	positive	negative
0%	100%	96%	3.7%
0/27	27/27	26/27	1/27

Table 3. IHC findings in NHL patients

CD10		CD20	
positive	negative	positive	negative
71.%	28.5%	100%	0%
20/28	8/28	28/28	0/28

Table 4. subtypes of NHL diagnosed by IPT

FL	MCL	Other Low grade B-cell NHL
20/28 (71.5%)	4/28 (14%)	4/28 (14%)

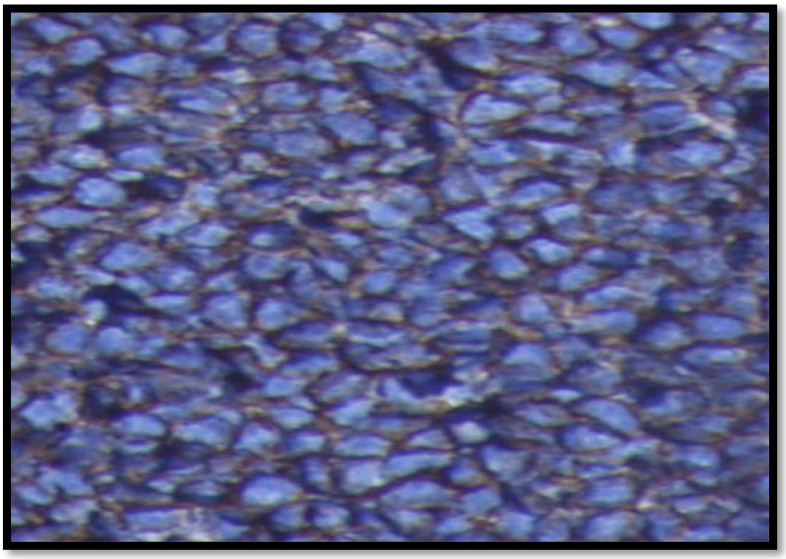

Figure 1. Photomicrograph shows brown membranous and cytoplasmic IHC stain with CD20 in bone marrow tissue of NHL patient (x40)

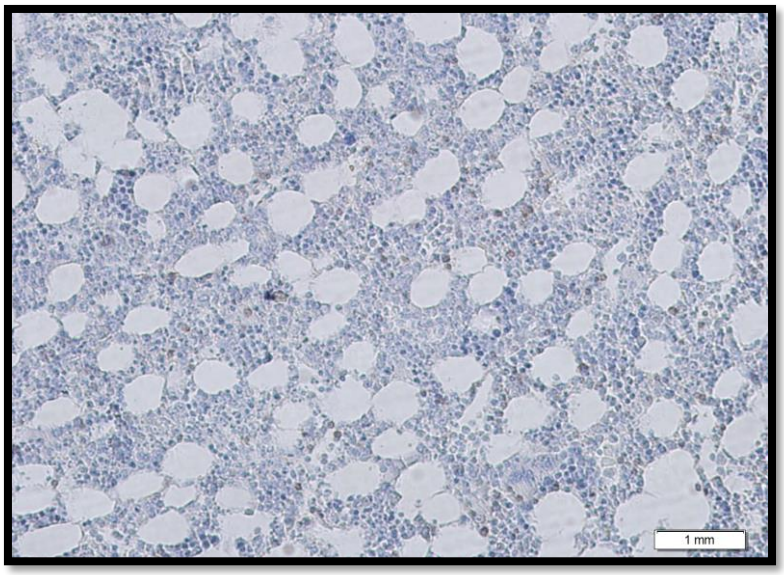

Figure 2. Photomicrograph shows no brown membranous or cytoplasmic IHC stain with CD20 in BM tissue of CLL patient (x10)

4. Discussion

The chronic/mature B-cell neoplasms include CLL, which is by far the most common, the rare B-PLL and HCL. The B-cell NHLs include FL, SMZL, and MCL that most frequently exhibit circulating lymphoma cell(1) .

IHC is useful in distinguishing between reactive and neoplastic lymphoid infiltration. The availability of a broad panel of antibodies suitable for paraffin-embedded tissues enables us to perform complete IPT on BM trephines and allows for classification of lymphoid infiltrates.[4]

CD20: In this study CD20 present in more than 25 % of cells within aggregate as cellular membrane and/or cytoplasmic stain in 26 cases of CLL so it is 96% positive in CLL group.

So twenty seven cases of CLL showed CD20 positive membranous and/or cytoplasmic stain reaction and negative reaction with CD10 as agreement with most studies (16, 17), so only one case of CLL was negative for CD20.

Twenty eight cases of NHL showed CD20 positive membranous & or cytoplasmic reaction and majority (71.5%) showed CD10 positive membranous reaction, this agree with other workers(20 ,19) .

CD10: twenty cases of NHL showed CD10 positive membranous staining reaction were diagnosed as FL [5].

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Effect of Crude Plant Extracts on Some Liver Enzymes in Mice Exposed to Ethanol

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Abstract

The aim of this a study was to evaluate the possible effects of the Methanolic and aqueous extracts decreased the liver enzymes (ALP, SGPT and SGOT) to the normal level after the its values by the treatment with ethanol liquid diet. The level of GOT, GPT and ALP in animals treated with ethanol after 25 days were increased in comparison with control group, results showed that animals treated with Crude Plant Extracts caused decreased in liver enzymes activity to reach to normal level in comparison with control group.

Keywords: Crude Plant Extracts, GOT, GPT, ALP and ethanol liquid diet.

1. Introduction

Plants are an important source of medicine and this importance comes from their medical prevention of many diseases and its increase of the body's immunity. Even now, the World Health Organization reports that up to 80% of people will use herbal treatments as their primary source of medication. Medicinal plants contain chemical compounds with great interest for its physiological effect with medical activity. This is because they contain more than one active substance that Synergy naturally available in the plant, " the effect of active substance with the other " have a very important effect on the events of healing [1]. Different chemical constituents derived from plant crude extracts have been assigned pharmacological and medicinal properties. Chemical constituents with antioxidant function, in particular, can be present in large amounts in plants and are thought to be responsible for their protective effectiveness against a variety of degenerative diseases, including cancer, neurological and cardiovascular diseases [2], as well as pulmonary, urinary, skin, gastrointestinal, and liver disease [3].

Herbs are important in the treatment of a variety of liver disorders, as well as other disease-related processes [4]. The liver is a vital organ that regulates homeostasis in the body via a variety of functions. Toxic chemical and drug-induced caused by liver damage has been identified as a

toxicological problem. Natural products found in plants, such as flavonoids, terpenoids, and steroids, have gotten a lot of attention in recent years because of their wide range of medicinal properties, including antioxidant and hepatoprotective ability.

2. Materials and Methods

2.1 Plant samples collection and drying

M. piperita and O. basilicum were purchased from a local market and M. communis from a local garden in Holy city of Karbala, Iraq. Leaves were dried at room temperature with darkness and powdered by electrical grinder.

2.2 Preparation of leaf crude extracts

2.2.1 Alcoholic extracts

The powdered material (20 g) was mixed with 200 ml of methanol for 24 hrs at room temperature. The suspensions were then filtered by filter paper (whatman No.1) and ground at room temperature. The extracts were stored at -4 °C until being used[5].

2.2.2 Aqueous extracts

The powdered materials (20 g) were mixed with 400 ml of D.W for 24 hrs at room temperature. The crude extract then was evaporated at 60°C using oven and the resultant crude extract was collected and stored at -4 °C until being used[6].

2.3 Animals

One hundred female adults and fifty male adults were bought from the National Centre for Drugs Control and Research, Ministry of Health, Iraq, varying in age (8-12) weeks, and weighting (22-28) g. They were kept in plastic cages in a managed animal house containing hard wood chips for bedding at 25±2 C˚ ,4/10 hour's light/dark cycle in Biotechnology Research Center, Al-Nahrain University.

2.4 Liquid diet with ethanol

The composition of the modified liquid diet with ethanol was: cows' milk 925 ml, ethanol 25-75 ml, vitamin A 5000 IU and sucrose 17 g[7]. This mixture supplies 1000.7 kcal/l.

2.5 Experimental layout (in vivo)

One hundred adult of female and fifty adults of male mice were purchased from the National Center for Drug Control and Research, Ministry of Health, Iraq were used, their age was ranged (8-12) weeks and weighting (22-28) g. They were housed in plastic cages containing hard wood chips for

bedding in controlled animal house at 25±2 C˚ ,4/10 hour's light/dark cycle in Biotechnology Research Center, Al-Nahrain University.

Group 1: - Not treated animals (control).

Group 2: - Animals were fed of Ethanol liquid diet for 25 days.

Group 3: - Animals were fed of Ethanol liquid diet and administrated orally with methanolic extract of M. communis at a concentration 0.7 g/kg of body weight according to for 24 hrs[8].

Group 4: - Animals were fed of Ethanol liquid diet and administrated orally with aqueous extract of M. communis at a concentration 0.4 g/kg of body weight according to for 24 hrs[8].

Group 5: - Animals were fed of Ethanol liquid diet and administrated orally with methanolic extract of M. piperita at concentration 4 g/kg of body weight according to for 24 hrs[9].

Group 6: - Animals were fed of Ethanol liquid diet and administrated orally with aqueous extract of M. piperita at a concentration 4 g/kg of body weight according to for 24 hrs[9].

Group 7: - Animals were fed of Ethanol liquid diet and administrated orally with methanolic extract of O. bacilicum at a concentration 1.5 g/kg of body weight according to for 24 hrs[10].

Group 8: - Animals were fed of Ethanol liquid diet and administrated orally with aqueous extract of O. bacilicum at a concentration 1.5 g/kg of body weight according to for 24 hrs[10].

2.6 Measurement of Serum Glutamic Pyruvic Transaminse

Serum alanine aminotransferase (ALT) activities were measured colorimetrically according to the method of Reitman and Frankel using a commercial assay kit[11].

2.7 Measurement of Serum Glutamic Oxaloacetic Transaminse

Aspartate aminotransferase (AST) activities were measured colorimetrically according to the method of Reitman and Frankel using a commercial assay kit[11].

2.8 Measurement of Serum Alkaline Phosphatase

Data are analyzed using statistical software IBM (SPSS version 18). The values of the parameters investigated were given in terms of mean ± standard error and the variance analysis (ANOVA) was used to make variations between the means of all the parameters. Differences were considered statistically significant at $p<0.05$.

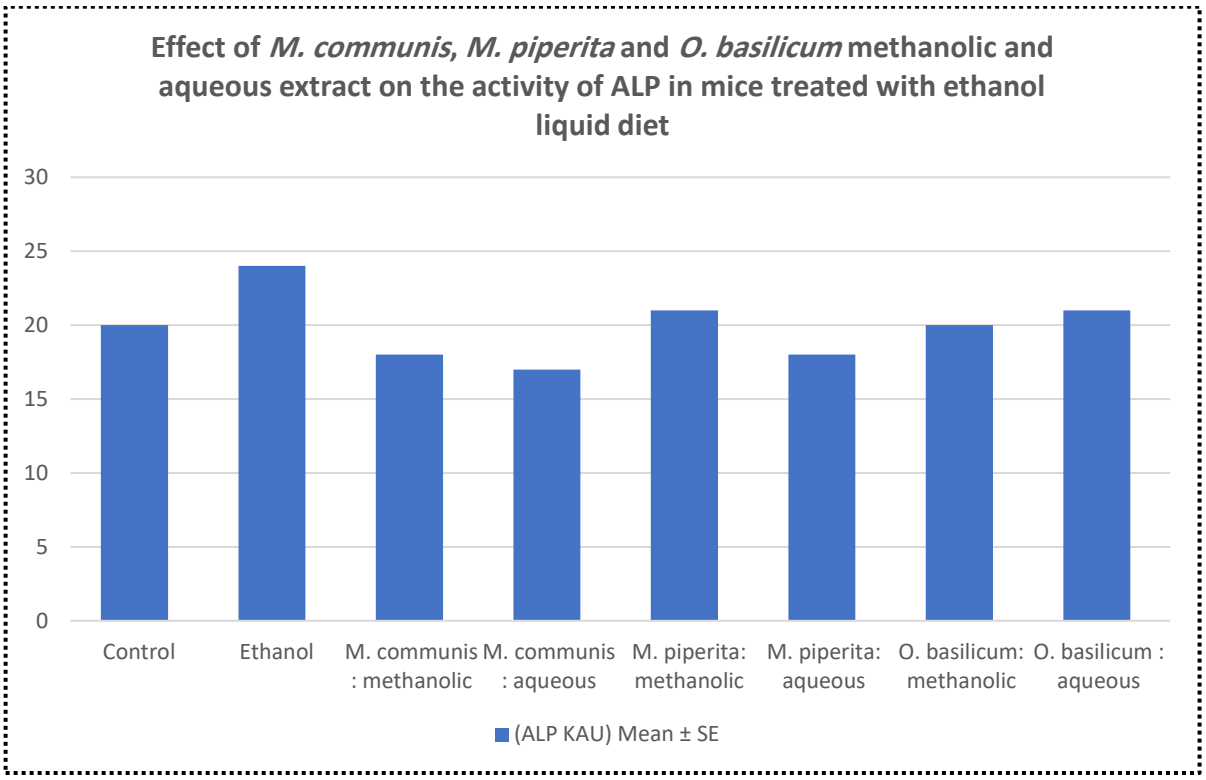
2.9 Statistical Analysis

One hundred adult of female and fifty adults of male mice were purchased from the National Center for Drug Control and Research, Ministry of Health, Iraq were used, their age was ranged (8-12) weeks and weighting (22-28) g. They were housed in plastic cages containing hard wood chips for bedding in controlled animal house at 25±2 C˚ ,4/10 hour's light/dark cycle in Biotechnology Research Center, Al-Nahrain University.

3. Results and Discussion

3.1 Effect of Crude Plant Extracts on Serum Alkaline Phosphatase (ALP) Activity

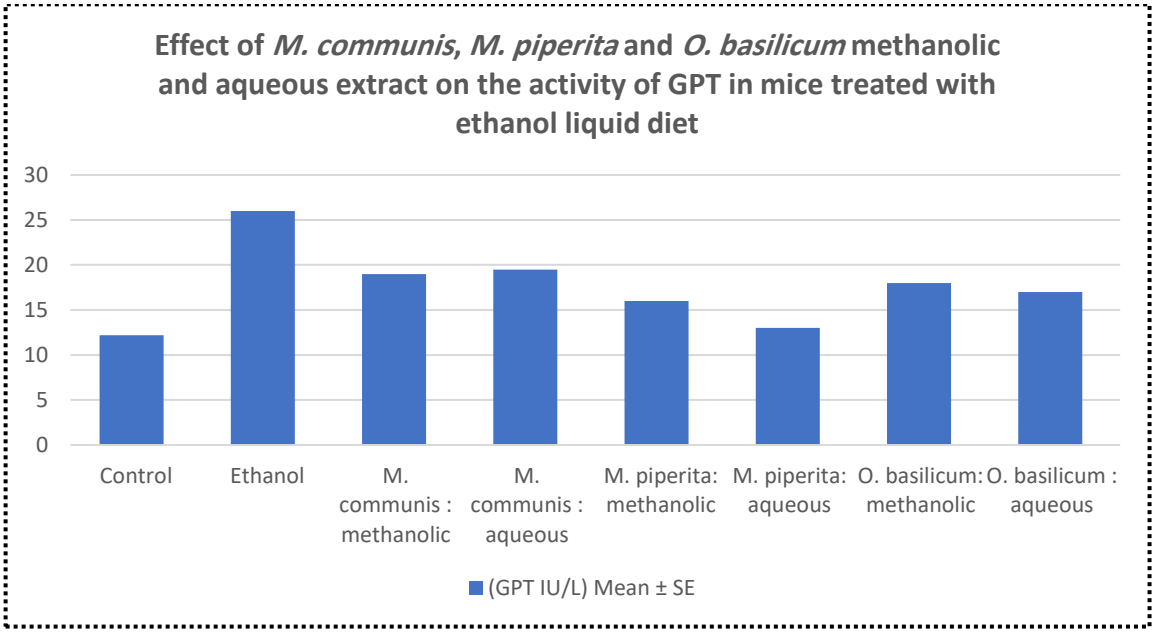
Results showed that the level of ALP in animals treated of ethanol liquid diet for 25 days that increase (24 ± 0.866) KAU in comparison with control group (20 ± 0.635) KAU, and When treated animal with methanolic and aqueous extracts led to decrease the level of ALP, and show no any effect of these extracts in positive control group except the aqueous extract of M. communis that increased.



The ethanol has a negative effect on liver because the metabolism of ethanol leads to the production of acetaldehyde material by the enzyme alcohol dehydrogenase which plays a main role as a toxic material and with harmful effects on the cellular composition of the liver cells and the hepatic damage occur[13], and when hepatic damage occur the level of ALT, AST, ALP and cholesterol were elevated in serum[14]. The hepatic damage is usually associated with elevated serum ALT, AST, ALP and cholesterol level[15].

3.2 Effect of Crude Plant Extracts on Serum Glutamic Pyruvic Transaminse (GPT) Activity

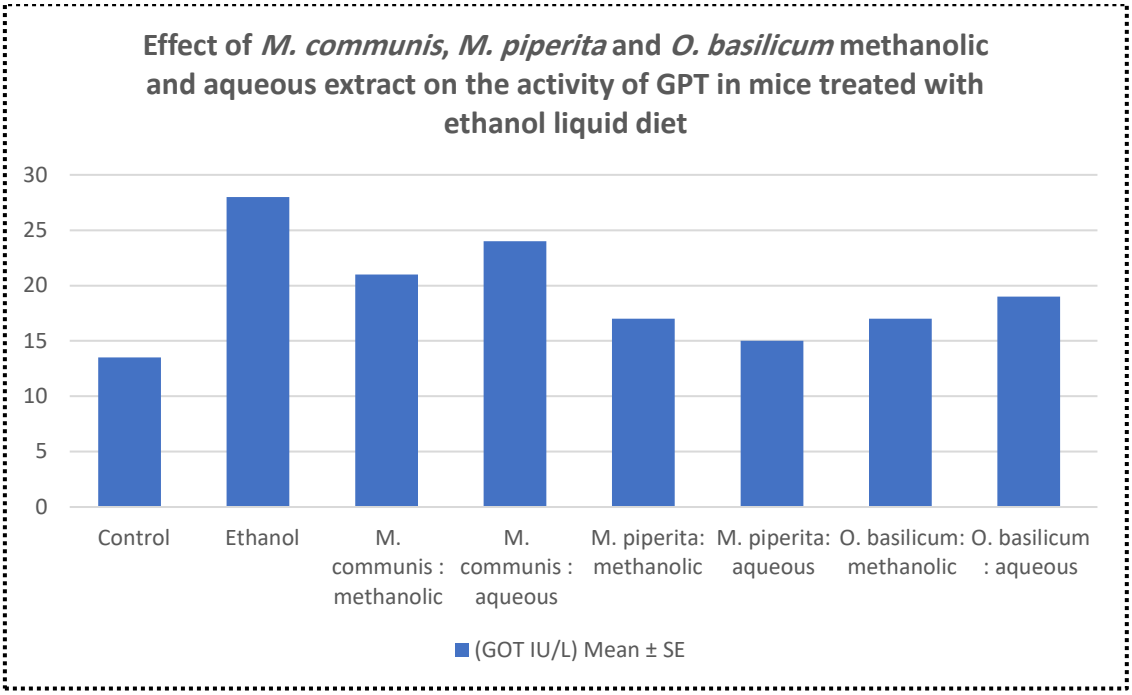
Results showed that animals treated with methanolic and aqueous extracts caused reduction in GPT, since it increased after treatment with ethanol for 25 days, and decreased when treated animals with the aqueous extract of M. piperita to normal level in comparison with control group. But in the positive control group, the GPT level was not affected after treatment with aqueous and methanolic extracts and this reflects the safety use of these extracts. At the same time, results showed no difference between the methanolic and aqueous extracts regarding GPT level in serum, indicating the presence of active compounds in both aqueous and methanolic extract.



The results above showed that animals treated with ethanol increased GPT and this consistent with the results of Flora et al. 16 who reported that some chemicals such as ethanol elevate blood SGOT and SGPT in rats it generates free radicals during metabolism in the liver cells leading to leakage of liver enzymes (GPT and GOT) to serum[17].

3.3 Effect of Crude Plant Extracts on Serum Glutamic Oxaloacetic Transaminase (GOT) Activity

Table (3-9) shows the level of GOT in animals treated with ethanol after 25 days which increased (28 ± 0.461) IU/L in comparison with control group (13.5 ± 0.288) IU/L. Results are in line with Flora et al., [17] who reported that some chemicals such as ethanol elevates blood SGOT and SGPT in rats it generates free radicals during metabolism in the liver cells leading to leakage of liver enzymes (GPT and GOT) to serum 17. When treated animal with methanolic and aqueous extract led to decrease the level of GOT, and show no any effect of these extracts in positive control group and this reflects the safety used.



6. Conclusions

The present study showed that Alcoholic and aqueous leaf extracts of M. communis , M. piperita and O. basilicum have significantly decreased Activity of ALP, GOT and GPT, as compared with previous mice fed of ethanol liquid diet

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