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This journal is dedicated to publishing targeted and applied scientific research and studies that are beneficial in all areas of contemporary medical sciences

It publishes peer-reviewed scientific work in a wide range of medical specialties, including general medicine, surgery, dentistry, pharmacy, microbiology, pathology, molecular biology, toxicology, ophthalmology, otolaryngology (ENT), oral and maxillofacial surgery, chronic diseases, plastic surgery, pediatrics, family and community medicine, primary health care, internal medicine, reproductive health, urology, dermatology, obstetrics and gynecology, as well as all other medical disciplines. It also covers medical laboratory sciences, radiology, ultrasound, nursing, therapeutic nutrition, and public health

The journal aims to contribute to the advancement of medical sciences by promoting the dissemination of new scientific knowledge, innovative ideas, modern experiences, and technological achievements, including nanotechnology and the use of advanced devices. It encourages researchers in the medical field to conduct impactful and beneficial scientific studies

In addition, the journal focuses on publishing experimental research, as well as innovative and advanced studies prepared by researchers in medical sciences, with the goal of enriching and developing high-quality scientific research.

The journal promotes scientific communication, intellectual exchange, and the cross-pollination of ideas, aiming to connect researchers from across the Arab world in a modern, purposeful framework for sharing information and practical scientific experiences

Editor-in-chief

Journal des Sciences Médicales Progressistes

Journal of Progressive Medical Sciences Revue internationale périodique publiée par le #Democratic_Arabic_Center – Berlin, Allemagne

Cette revue est dédiée à la publication de recherches et d'études scientifiques ciblées et appliquées, utiles dans tous les domaines des sciences médicales contemporaines.

Elle publie des travaux scientifiques évalués par des pairs dans un large éventail de spécialités médicales, notamment la médecine générale, la chirurgie, la dentisterie, la pharmacie, la microbiologie, la pathologie, la biologie moléculaire, la toxicologie, l'ophtalmologie, l'oto-rhino-laryngologie (ORL), la chirurgie buccale et maxillo-faciale, les maladies chroniques, la chirurgie plastique, la pédiatrie, la médecine familiale et communautaire, les soins de santé primaires, la médecine interne, la santé reproductive, l'urologie, la dermatologie, l'obstétrique et la gynécologie, ainsi que toutes les autres disciplines médicales. Elle couvre également les sciences de laboratoire médical, la radiologie, l'échographie, les soins infirmiers, la nutrition thérapeutique et la santé publique.

La revue vise à contribuer au progrès des sciences médicales en favorisant la diffusion de nouvelles connaissances scientifiques, d'idées innovantes, d'expériences modernes et de avancées technologiques, notamment les nanotechnologies et l'utilisation de dispositifs de pointe. Elle encourage les chercheurs du domaine médical à mener des études scientifiques percutantes et utiles.

Par ailleurs, la revue se concentre sur la publication de recherches expérimentales, ainsi que d'études innovantes et avancées réalisées par des chercheurs en sciences médicales, dans le but d'enrichir et de développer une recherche scientifique de haute qualité.

La revue favorise la communication scientifique, les échanges intellectuels et le brassage d'idées, en mettant en relation les chercheurs du monde arabe dans un cadre moderne et constructif de partage d'informations et d'expériences scientifiques concrètes.

Rédacteur en chef

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Detection of some mutations in some Mitotic Checkpoint Genes and their Association to Tissue Abnormalities in patients with Breast Cancer

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Abstract

Background: Breast cancer is a multifactorial disease, and several genetic and non-genetic factors contribute to its malignancy. The checkpoint genes Bub1, Bub1B, and MAD2L1 have been shown to be relatively uncommon mutations in cancer. This study aimed to screen for some mutations in some of the mitotic checkpoint genes, including MAD1L1, BUB1, and BUB1B.

Materials and Methods: Thirty breast cancer biopsy specimens or formalin-fixed, paraffin-embedded (FFPE) tissue were isolated. Then, the patient information of the breast cancer patient was recorded. Histological examination and then genomic DNA extraction were performed for all samples. In the next step, DNA purity and concentration were estimated. For MAD1L1, BUB1 and BUB1B genes, initial design was performed and polymerase chain reaction (PCR) and then gel electrophoresis were performed for PCR products. Electrophoresis bands were cleaned and DNA was sequenced and finally statistical analysis was performed.

Results: Histological examination showed differences in shape, orientation, and echo pattern between the invasive cancers and DCIS. An irregular shape (72% vs. 35%), a not parallel orientation (42% vs. 9%), and a hypoechoic or complex echo pattern (92% vs. 6%) were more frequent in invasive cancers when compared with the DCIS cases. The results of matching the sequence obtained according to Sanger method with Chromas version 2.6.6 software showed there were multiple mutations in study genes.

Conclusion: In the present study, after histological examining and PCR product sequencing of all samples, it was determined that all the samples studied had altered genotypes in terms and this alteration associated to histological abnormalities

Keywords: Breast Cancer, Mitotic Checkpoint Genes, PCR

Introduction:

The latest WHO estimates show that breast cancer is the most common malignancy worldwide in 154 of 185 countries and is the leading cause of cancer-related deaths in more than 100 countries. Globally, this cancer is the most common cancer among women, accounting for 25% of all recorded cancers in women (1,2). In Iraq, breast cancer ranks first among the top ten malignant neoplasms in society, including 19.5% of all cases and 34.3% of women's cancers cases. During

2016, 897 women died from this disease, which is the first cause of cancer deaths among Iraqi women (23.6%) and the second overall cause among men and women (12.1%) after bronchogenic cancer (3). The exact cause of breast cancer has not been identified so far and this disease is considered a multifactorial disease (4). Apart from genetic predisposition, many other factors such as demographic characteristics, clinical characteristics, fertility and environment can affect the incidence of breast cancer in women. Increased risk is associated with advanced age, positive family history, socioeconomic status, diet, endogenous or exogenous hormones, unusual breast diseases, benign tumors, oncogenic viruses, and exposure to carcinogens (5).

Early detection and diagnosis of this disease can be very effective in its treatment. Classification of women based on breast cancer risk factors can be effective in improving risk-free methods and designing targeted breast cancer screening programs (6,7). Breast tumors usually start from ductal proliferation and turn into benign tumors or even metastatic carcinomas after continuous stimulation by various carcinogenic agents (8). There are two hypothetical theories for the initiation and progression of breast cancer: the theory of cancer stem cells and the random theory (9). Cancer stem cell theory states that all tumor subtypes are derived from the same stem cells. Acquired genetic and epigenetic mutations in stem cells or progenitor cells lead to different tumor phenotypes. Random mutations can gradually accumulate in each breast cell (13). BUB1 gene is coding for protein kinase that binds to kinetochore and is involved in the regulation of cyclin-B levels. The gene coding for this protein is located on (2q13) with a length of 40,580 bp and contains 25 exons and 14 transcripts, and the highest expression of this gene has been observed in testicular tissue and lymph nodes. BUB1B gene also coding for protein kinase that plays a key role in SAC activity. The location of the gene encoding this protein is located on (15q15.1), it has 23 exons and span along 60,128 bp, this gene is most expressed in testicular tissue, lymph nodes, and bone marrow (21). MAD1L1 gene is a checkpoint component of the mitotic spindle and prevents the onset of anaphase until all chromosomes are properly positioned at the metaphase plate and located in 7p22.3, it has 21 exons, and a length of 8379 bp. MAD1L1 functions as a homodimer and interacts with MAD2L1. MAD1L1 may also play a role in cell cycle control and tumor suppression. This gene is expressed in testis, spleen and 25 other tissues (21). This study was aimed to screening of some mutations in some mitotic varicella genes (MAD1L1, BUB1 and BUB1B) and determining the effect of mutation in the pathological tissue.

Materials and methods:

DNA Extraction: Tissue samples were taken from 30 breast cancer patients and the DNA extraction was carried out by Quick-DNA™ FFPE Kit depending on the manufacturing procedure.

Polymerase Chain Reaction: In order to amplify the desired fragment for each SNP, PCR reaction was performed on the extracted DNA of all samples. For this purpose, the primer sequences were first selected. Then, the specific primers of each SNP were blasted on the NCBI site, and thus the sequence of the primers was determined (Table 4-2).

Table (1): Specific primers for BUB1, BUB1B and MAD1L1 genes

Gene	Sequence (5′→3′ direction)	Cg%	Tm (°C)	Product Size(bp)
BUB1	GCCTGGCTTTGTTTGTGTTT	55%	58	192
	GCCTGGCTTTGTTTGTGTTT	57%	58	
BUB1B	TGAGGCCACAGTGTCTGTTC	60%	62	174
	CTGAGGCAGCAATCTGTGAG	58%	62	
MAD1L1	ATGCCTGCTCTCCTCACTGT	62%	60	242
	GCTTCTTTCCCCAATTAGCC	60%	62	

Maxime PCR PreMix kit (i-Taq) 20µlrxn: Is a product that contains a mixture of the following components: i-Taq DNA Polymerase, dNTP mix, reaction buffer. The first reason is that it has all the components of PCR, so we can perform PCR by just adding template DNA, primer set and D.W.

PCR Reaction and steps: The materials required for PCR reaction to BUB1, BUB1B and MAD1L1 genes was carried out by mixing:5 µl Taq PCR PreMix, 10 picomols (1µl) Forward and Reverse primer, DNA templet 2 µl and 16 µl D.W. The PCR steps are shown in Tables (2).

Table (2): PCR Reaction Temperature cycle for BUB1, BUB1B and MAD1L1 genes

Steps	Temperature	Time	No. Of cycles
Initial denaturation	95 C°	5 min	1 cycle
Denaturation	95C°	45sec	35cycles
Annealing of BUB1	58 C°	45sec	
Annealing of BUB1B	60 C°		
Annealing of MAD1L1	62 C°		
Extension	72C°	45 sec	
Final extension	72C°	5 min	1 cycle
Holding	4 C°	10 min	1 cycle

Gel electrophoresis: Electrophoresis was used to confirm the target size. PCR products were separated on 2% agarose gel electrophoresis and observed by exposure to ultraviolet light (302 nm) after staining with ethidium bromide.

Gene Sequencing: was performed by Macrogen Korea, Homology search using Basic Local Alignment Search Tool (BLAST) program available at National Center for Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm. nih.gov) is available and the BioEdit program was performed. PCR products were purified and sequenced using BigDye Terminator v3.1 Cycle Sequencing kit on ABI 3130 Genetic Analyzer.

Sequences Analysis: The analysis of nucleotide databases using NCBI's Basic Local Alignment Search Tool Bio ID program for sample identification and submitted to GenBank (ID). Sample sequences were obtained from the NCBI nucleotide database (www.ncbi.nlm.gov/nucleotide) and included in a multiple alignment using the Bio ID program.

Histological Examination: The breast specimens were formalin-fixed, paraffin-embedded tissue blocks subsequently stained with hematoxylin and eosin. Histological tumor types were divided into invasive cancers and ductal carci-noma in situ (DCIS). Invasive cancer was graded as grade 1 (well differentiated), grade 2 (moderately differentiated), or grade 3 (poorly differentiated) according to the Scarff-Bloom-Richardson System (1957). DCIS cases were classified as group 1 (nonhigh grade DCIS without comedo-type necrosis), group 2(nonhigh grade DCIS with comedo-type necrosis), or group 3 (high grade DCIS with or without comedo-type necrosis) according to the Van Nuys Classification (1995)

Statistical Analysis: In order to perform statistical analysis, first all the data were entered in an excel file. Then, using spss software version 22, the variables were compared based on the chi-square test, and P value less than 0.05 was considered significant.

Results and discussion:

Obtained from DNA extraction After performing PCR, we examined the resulting fragments on the gel. The primary results are given in Table (1-3). In this table, the sign + and - means getting or not getting the result. After DNA extraction, the extracted samples were electrophoresed on a 2% agarose gel and observed with a gel dock device. The results of the extracted samples on 2% agarose gel are shown in figures (1,2,3)

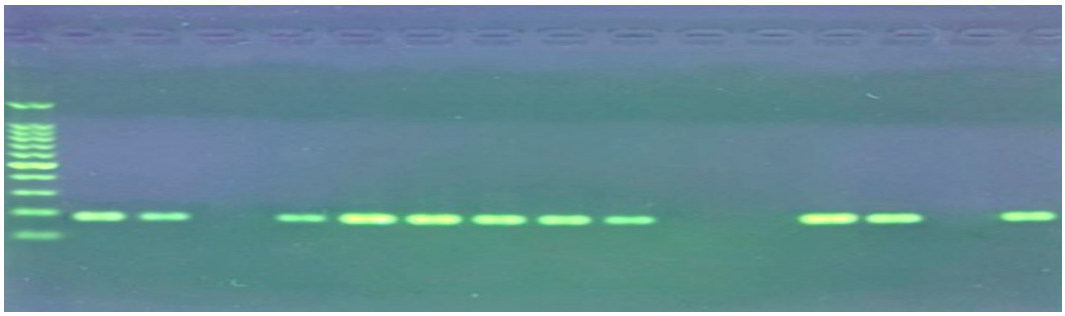

Figure (1): BUB1 gene PCR product. The band size is 192 bp. The product was electrophoresed on 2% agarose with a voltage of 25 volts/cm.

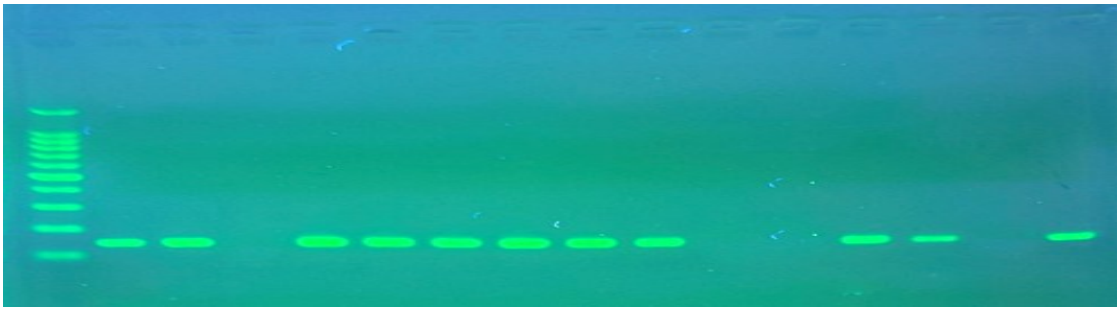

Figure (2): PCR product of BUB1B gene. The band size is 174 bp. The product was electrophoresed on 2% agarose with a voltage of 5 volts/cm2.

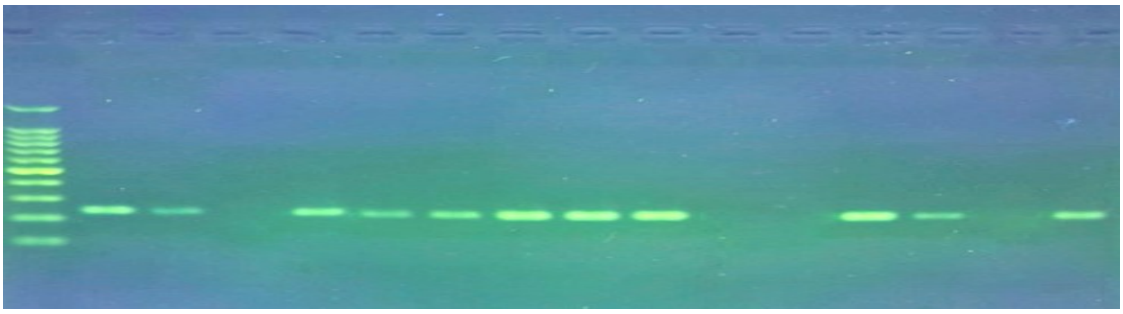

Figure (3): MAD1L1 gene PCR product. The band size is 242 bp. The product was electrophoresed on 2% agarose with a voltage of 5 volts/cm2.

Results obtained from trench sequencing:

PCR products confirmed by gel electrophoresis were sequenced by the Sanger method with Chromas version 2.6.6 software, which are shown in Figure (3-4).

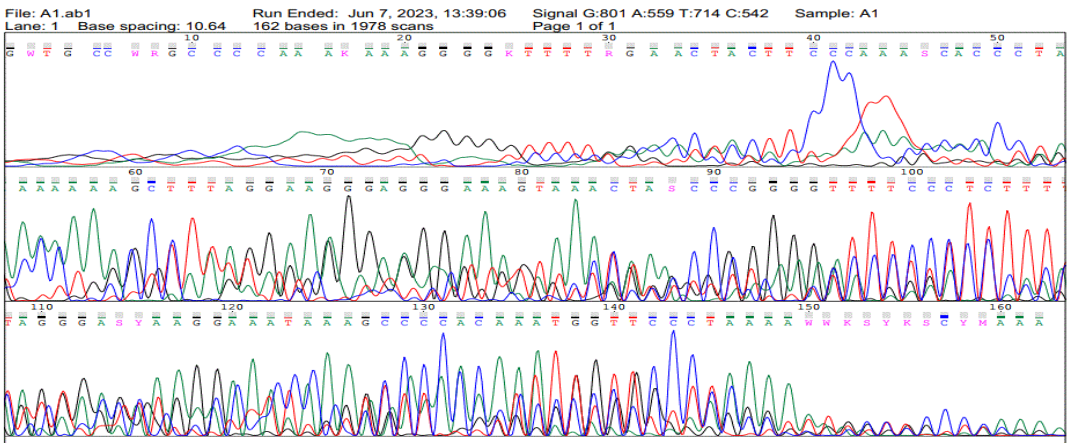

Figure (4): Diagram resulting from sequencing with Chromas software

Table (4): The results of matching the sequence according to Chromas version 2.6.6 software

Gene	W.T	Mutant	Site	Name of mutation	a.a change	Type of Mutation
BUB1	CCA	CTT	2641	Sub:CA>TT	Q > L	Substitution
	ATAA	----	2730	Del: ATAA	---	Frameshift
	TCCGT	-----	3030	Del:TCCGT	---	Frameshift
BUB1B	CAT	CGG	2778	Sub:AT>GG	H>R	Substitution
	AAA	TAA	2962	Sub:A>T)	N>stop	Substitution
	TCT	TAT	3001	Sub:C>A	S > R	Substitution
MAD1L1	CCA	CTT	2641	Sub:CA>TT	Q > L	Substitution
	TAT	TTT	2823	Sub:A>T	R>P	Substitution
	C--	CAA	3069	Ins:AA	---	Frameshift
	CTA	CAA	3078	Sub:T>A	L> Q	Substitution

After examining the sequence of all the samples, it was found that all the examined samples had altered genotypes in terms of the variants in these regions and in their correspondence with the reference sequence, genetic changes were observed in the variants in question and the upstream and downstream regions that were sequenced.

Histological Examination Results:

Biological markers correlated with the histological grade in invasive cancers. ER negativity, PR negativity, and HER-2/neu positivity were more frequent in grade 3 invasive cancers than in grade2/grade 1 invasive cancers (pB0.0001). There was no significant difference between invasive cancers and DCIS for the presence of the biological markers (p0.05). Results of the univariate and multivariate regression models comparing the ultrasound findings of the 30 breast cancers. Differences were seen in shape, orientation, and echo pattern between the invasive cancers and DCIS. An irregular shape (72% vs. 35%), a not parallel orientation (42% vs. 9%), and a hypoechoic or complex echo pattern (92% vs. 6%) were more frequent in invasive cancers when compared with the DCIS cases.

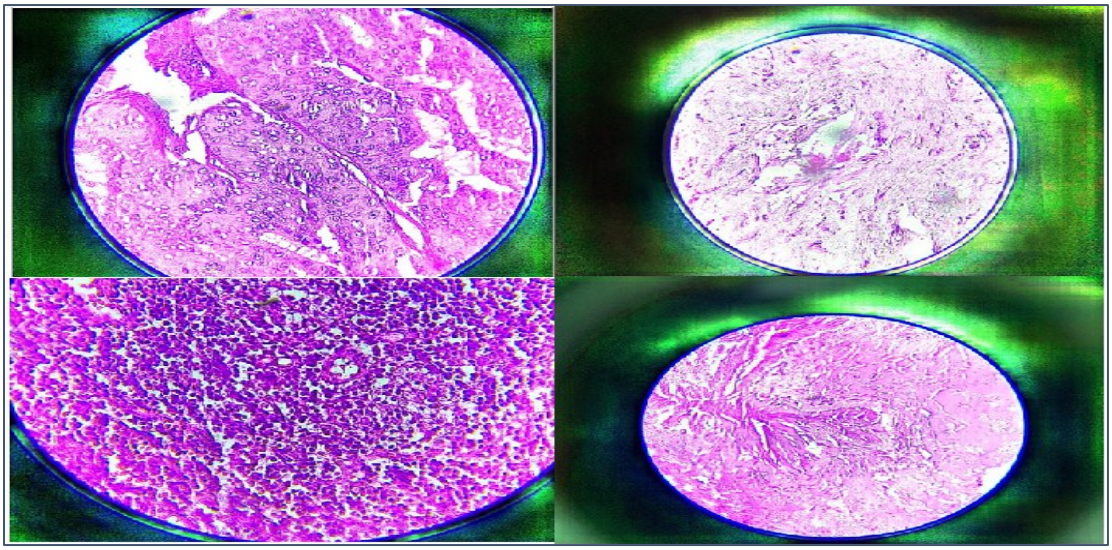

Figure (5): Images of breast Cancer tissues in patients

Statistical Review of patients Information:

The total number of patients was 30, the youngest of whom was 20 years old and the oldest was 68 years old. The average age of the patients was 45.6 years. Out of this number, 7 people (23.33%) were men, the rest (76.66%) were women. The distribution of patients in age groups is given in Table (5).

Table (5): Age range of patients

Age	Number	Percentage
20-30	3	10%
31-40	9	30.0%
41-50	7	23.33%
61-60	7	23.33%
61-70	4	13.33%

According to table (6) and based on the results of Chi-square statistical test, there is a significant difference between age in both male and female groups.

Table (6): Chi-square test results between male and female groups

Variable	X^2	Df	P value
Age	20/417	4	0/0000
Number	30		

Discussion:

Cancer is one of the most common diseases worldwide and the second cause of death after cardiovascular diseases. Breast cancer is the most common type of cancer among Iraqi women, because it accounted for the highest percentage of malignant tumors in women until 2018 (34). Breast cancer involves the patient, family and society and wastes many financial and spiritual resources. Although the primary cause of breast carcinoma is unclear, many risk factors have been documented. It lists "smoking, alcohol consumption, and diet" as factors that can vary and depend on lifestyle. On the other hand, other factors have been documented "age, race, gender and family history". In addition, hormones play an important role in some forms of breast carcinogenesis. It can be said here: "Aging, a history of breast cancer in the family, specific changes in the breasts, genetic changes, history of pregnancy and menopause, physical inactivity, alcohol consumption, diet and nutrition, race and radiation therapy to the chest are risk factors for breast tumorigenesis. (35). Several studies have shown that low expression levels of key mitotic spindle checkpoint genes can help control defective spindle checkpoints in cancer. For example, according to reports, MAD2L1 is less expressed in some breast and ovarian cancers (36). Apparently, such reduced expression has functional significance, because deletion of the MAD2L2 allele leads to a defective mitotic checkpoint in human cancer cells and early embryonic fibroblasts (37) and haploid insufficiency of BUB1B in mice leads to defective mitotic arrest as well as tumors. It becomes (38).

In this study, 30 breast cancer patients were sampled. The average age of these patients was 45.6 years, the youngest was 20 years old and the oldest was 68 years old. Of these, 7 were men and 23 were women. The results of SPSS analysis showed that there is a significant relationship between the age of disease and gender (P=0.000). This work showed 30 affected patients were sampled to investigate the relationship between mitosis checkpoint gene mutations and breast cancer tissue abnormalities. DNA was extracted from the tissues and then PCR. Sequencing was done by Sanger method. In the next step, we compared the obtained sequence with NCBI to see if the sequence we obtained is the same with the sequence available in NCBI.

Hempen et al. (2003) sequenced the entire coding regions of the BUB1 and BUB1B genes in pancreatic cancer cell lines and xenografts to determine sequence alterations of the BUB1 and BUB1B genes in pancreatic cancer. Although only polymorphic changes were found in the BUB1B gene, the aneuploid pancreatic cell line Hs766T had two novel missense variants (p.[Y259C; H265N]) in the BUB1 gene. These mutations were on the same allele associated with a wild-type BUB1 allele. This change was not found in other samples, articles, or 110 additional chromosomes from a reference population. Compared to two cell lines with microsatellite instability (MIN), the wild-type pancreatic cell line TP53 Hs766T had a defective mitotic spindle checkpoint, indicating a cell line with chromosomal instability (CIN) (39). The results of this study are consistent with our results.

Langerød et al (2003) selected 20 cases with genomic instability by comparative genomic hybridization (CGH), and without somatic TP53 (p53) mutations, and sequenced the entire coding

region of the BUB1 gene. The results showed that genomic instability as copy number changes by CGH in TP53 wild-type breast carcinomas is not caused by somatic mutations in the BUB1 gene (40). The results of this study were not consistent with our study.

Mafalda et al. (2008) determined the mRNA expression levels of major mitotic checkpoint genes that were not inhibited by the benzimidazole family (BUB1, BUBR1, BUB3) and the MAD gene family (MAD1, MAD2L1, MAD2L2) by quantitative PCR in 39 cc and in 36 samples. The normal kidney tissue was analyzed. analyzed these tumors by comparative genomic hybridization (CGH) to assess the relationship between mitotic checkpoint defects and the pattern of chromosomal alterations in this subset of RCC. BUB1, BUBR1, MAD1 and MAD2L1 showed significant differences in tumor tissue compared to the control group (BUB1, BUBR1 and MAD2L1 were overexpressed, while MAD1 was under expressed). Overexpression of BUB1 and BUBR1 was significantly associated with the number of genomic copy number changes ($p<0.001$ for both genes) (41). This study was consistent with the results of our study.

M. de Voer et al. (2013) performed genome-wide analysis of copy number and genetic mutations of 208 patients with familial or early-stage (40 years or younger) colorectal cancer. Heterozygous or haploid insufficiency mutations in BUB1 and BUB3 genes of spindle assembly were identified in 2.9% of them. In addition to colorectal cancer, these patients had various aneuploidies in multiple tissues and variable malformation features. The results showed that mutations in BUB1 and BUB3 cause mosaic aneuploidy and increase the risk of developing colorectal cancer at a young age (42). This study was consistent with the results of our study.

Germline mutations in BUB1 and BUB3 have been reported to increase the risk of developing colorectal cancer (CRC) at a young age, in the presence of variegated aneuploidy and malformation features reminiscent of mosaic aneuploidy syndrome. We performed a mutational analysis of BUB1 and BUB3 in 456 hereditary non-polyposis CRC families and 88 polyposis cases. Four novel or rare germline variants, one splice site and three missense variants, were identified in four families. Neither diverse aneuploidy nor malformation traits were observed in the carriers. Obvious functional effects were observed in the heterozygous form for c.1965-1G>A, but not for c.2296G>A (p.E766K), despite common positive segregation in the family. BUB1 c.2473C>T (p.P825S) and BUB3 c.77C>T (p.T26I) remained uncharacterized significant variants. As of today, the rarity of gain-of-function mutations identified in familial series and/or early onset does not support the inclusion of BUB1 and BUB3 testing in routine genetic diagnostics of familial CRC.

Conclusion:

Many genetic and environmental factors including demographic characteristics, clinical, reproductive and environmental characteristics, advanced age, positive family history, socioeconomic status, diet, endogenous or exogenous hormones, unusual breast diseases, benign tumors, oncogenic viruses and exposure Carcinogens cause breast cancer.In the present study, after examining the sequence of all the samples, it was found that all the investigated samples had altered genotypes in terms of the variants in these regions, and in their correspondence with the reference

sequence, genetic changes were observed in the variants in question and the sequenced upstream and downstream regions. ; Therefore, further study in this field is also recommended to examine the resulting changes in the sequence of genes involved in mitotic checkpoints.

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Evaluation of the prevalence of oral parasites and some variables affecting them among students of Al-Furat Al-Awsat Technical University

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Abstract

The present study was conducted for the period from September 2024 to March 2025 to investigate the infection of oral parasites Trichomonas tenax and Entamoeba gingivalis among students of the Al-Furat Al-Awsat Technical University in Kufa. The number of samples examined 150 saliva samples and gum swabs were collected from the students of the Technical Institute Kufa and the Medical Technical College Kufa; the samples were examined by direct smear and the preparation of wet slides in the laboratories of the Pathological Analysis Department in the Technical Institute Kufa. The prevalence of the E. gingivalis parasite was 10%, and the T. tenax parasite was 18%. The infection rate was higher in males, reaching 20%, than in females, which reached 8%. The incidence rate among students living in rural areas is 60.4%, while 39.6% of infected students are urban residents.

Key word: oral parasite, university students in Kufa.

INTRODUCTION

The parasite T. tenax is found in the oral cavity of humans and is anaerobic. It is most common among people with an unhealthy oral environment and people with dental and gum disease [1]. The parasite is usually transmitted by kissing and flying mist or using contaminated food and toothbrushes or through drinking. The parasite is resistant to temperature changes and can survive in drinking water from hours to many days [2]. The polycystic phase is not present in the life cycle of this parasite, so reproduction is carried out by the cellular division of the active phase [3]. The nucleus is divided into 2–8 nuclei, followed by cytoplasmic division, and eventually the parasite becomes 2–8 individuals [4]. The oral cavity of humans is home to many microorganisms and has a number of characteristics that make it a unique microbiological environment [5, 6]. E. gingivalis is a parasite that exists in the pits of teeth and tissue gums, causing suppuration and digging tonsillitis. Many studies suggest that there is a relationship between its presence and periodontitis infections [7]. E. gingivalis belongs to the Entamoebaidae family and the Sarcodina Division [8]. Some researchers have noted that this parasite is opportunistic because it is present in the oral cavity of healthy people but has the ability to reproduce in the oral environment with periodontal disease [9]. noted the researcher

[10] in his study that the infection parasites of oral are common among people with infections, gum Periodontal disease, the parasite transmission from one person to another by kissing, spray mist and saliva, or in combination with tools eating, and 95% of individuals with unhealthy mouth infected have *E. coli, E. gingivalis* [11]. Due to the large number of reviewers who complain of infection of teeth, gums and tonsils to hospitals, health centers and private clinics on the one hand and due to the lack of studies concerning the infection of these parasites *T. tenax* and *E. gingivalis* and If any, they are very few, so the aim of this study was to determine the incidence of the above parasites among young people represented by the students of the Technical Institute and the Technical College in Kufa and the extent of the incidence of sex.

Materials and methods

During the period from September 2024 to March 2025, 150 samples of saliva and sputum from both sexes were collected from students of Al-Furat Al-Awsat Technical University in Kufa using an information form containing the patient's name, sex, and area of residence in an attempt to study the epidemiology of oral parasites E. gingivalis and T. tenax among students of Al-Furat Al-Awsat Technical University in Kufa.

Methods of examination:

A- Microscopic examination: It is divided into- :

1-Direct Smear Method

Samples are taken from the mouth and then fixed on the glass slide and then examined under a light microscope under the forces of minor and major (X10, X40, X100), under which we can observe the movement of spiral parasites, which is one of the characteristics of the parasites, which we can distinguish from the other [12].

2-Wet Preparation Method

Put the submerged saliva sample taken from the student's mouth on a clean glass slide and sterilize and mix well with saliva, and then place the cover of the slide on it and examine under the major powers of the microscope magnification force X400 to detect the parasites of *E. gingivalis* and *T. tenax.* In wet specimens, the *T. tenax* parasite is a moving wave, and the anterior tuft of the capillaries and the lateral membrane ripple can be distinguished. If the characteristics of the parasite are present in the sample, the result is positive in terms of size, shape, nucleus shape, and number of hairs [13].

3-Staining method

This method was done by taking a swab from the mouth and putting the swab on a glass slide, then fixing it by flame, and then putting a type of dye, such as Giemsa stain, on it. This dye works on discrimination.

The flagella and cytoplasm of the parasite were then washed with distilled water to dilute the dye and then examined under a microscope. There are many dyes used, including Giemsa staining, methylene blue, and Gram stain [14].

Statistical analysis: Statistical analysis was carried out using chi-square under a significant level (0.05) [15].

Results

1. Parasite infection rate, *Entamoeba gingivalis* and *Trichomonas tenax*:

The results of the present study showed that the total infection rate was 28% for both parasites, The infection of *Trichomonas tenax*, was 18% while the infection rate of *Entamoeba gingivalis* was10% as in Table (1) and Figure (1).

Table 1: Parasitic infection rates, *Entamoeba gingivalis*, *Trichomonas tenax*

Parasite Type	Number of Infected	Percentage
Entamoeba gingivalis	15	10%
Trichomonas tenax	27	18%
Total	42	%28

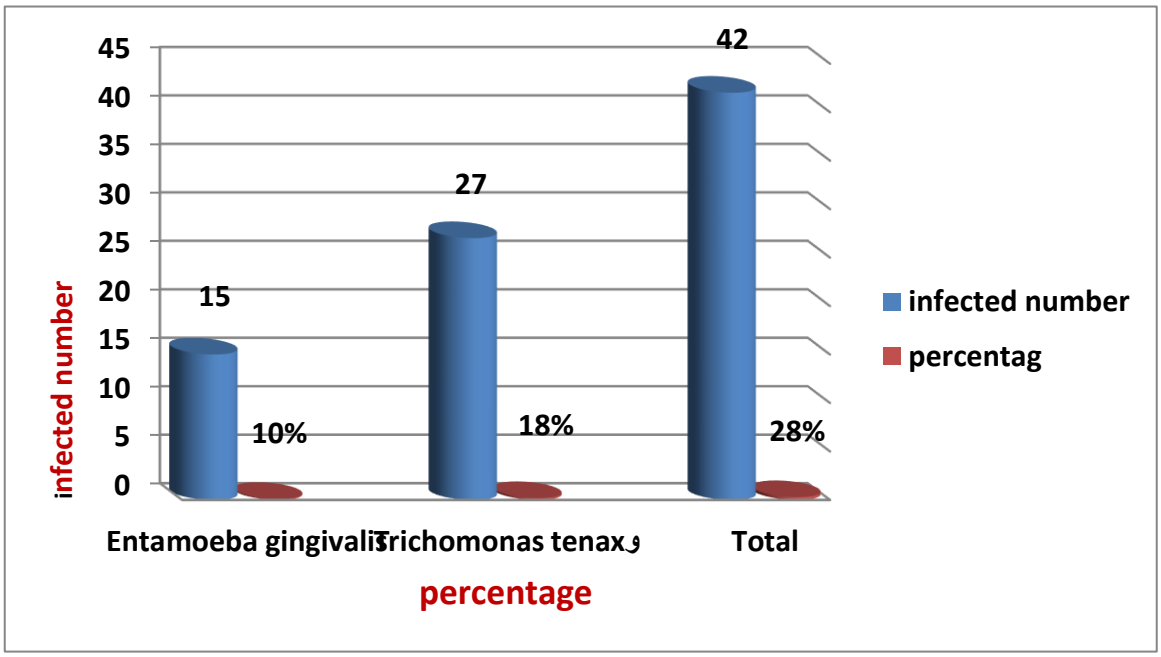

Figure (1): Parasitic infection rates, *Entamoeba gingivalis*, *Trichomonas tenax*

2. The relationship between infection rate and sex of the infected:

The results of the current study show the high proportion of males with mouth infection parasites as 18% compared with females' infection, which accounted for 10% of the total proportion of the infection (Table 2) and Figure (2).

Table (2): The relationship between the incidence of parasites of the mouth and the sex of the patient.

Sex	Infected number	Checked number	Percentage
Male	27	75	18%
Female	15	75	10%
Total	41	150	28%

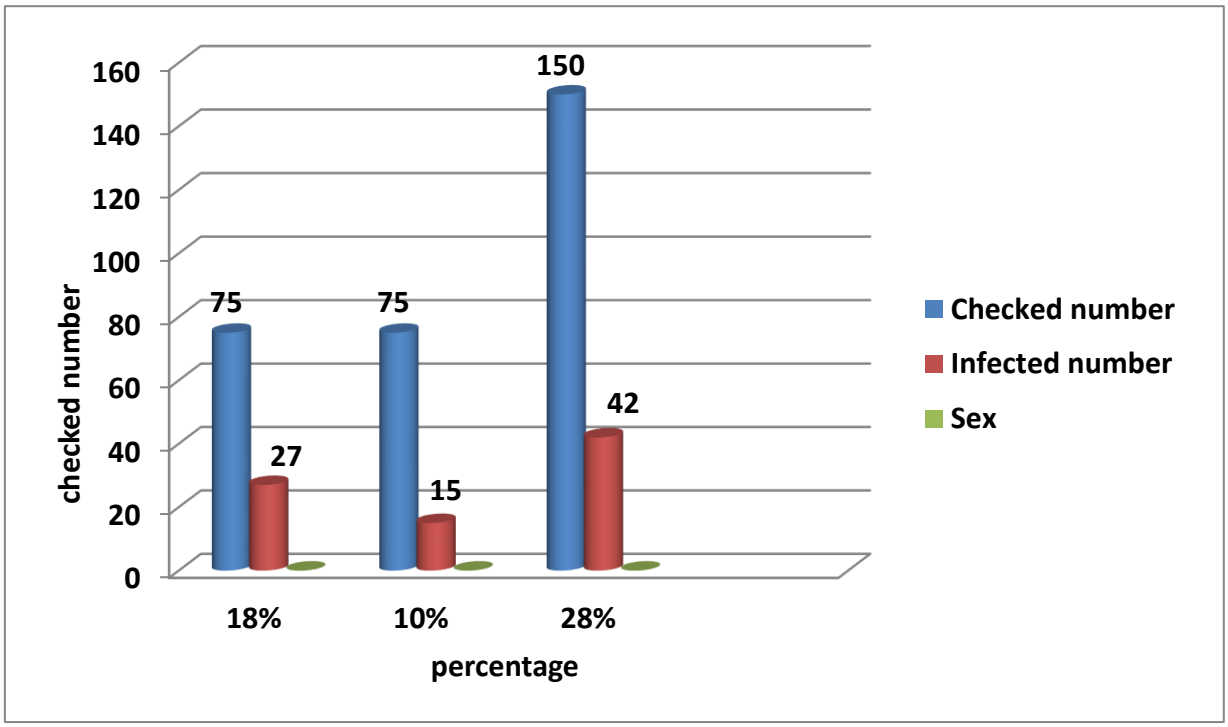

Figure (2): The relationship between the incidence of parasites of the mouth and the sex of the patient.

3 - The relationship between the proportion of infection and housing area of the infected:

The results of the study showed that there were significant differences in the incidence rates among the infected students according to their areas of residence. The incidence rate was high among students with rural incidence, where it was 59.6%, compared with the proportion

of injury among students living in cities, where it was 40.4%, as shown in Table (3) and Figure (3).

Table (3): The relationship between infection rate and housing area for the infected.

Housing Area	Number of Infected	Number of Examiners	Percentage
Rural	25	75	59,6%
Town	17	75	40,4%
Total	42	150	100%

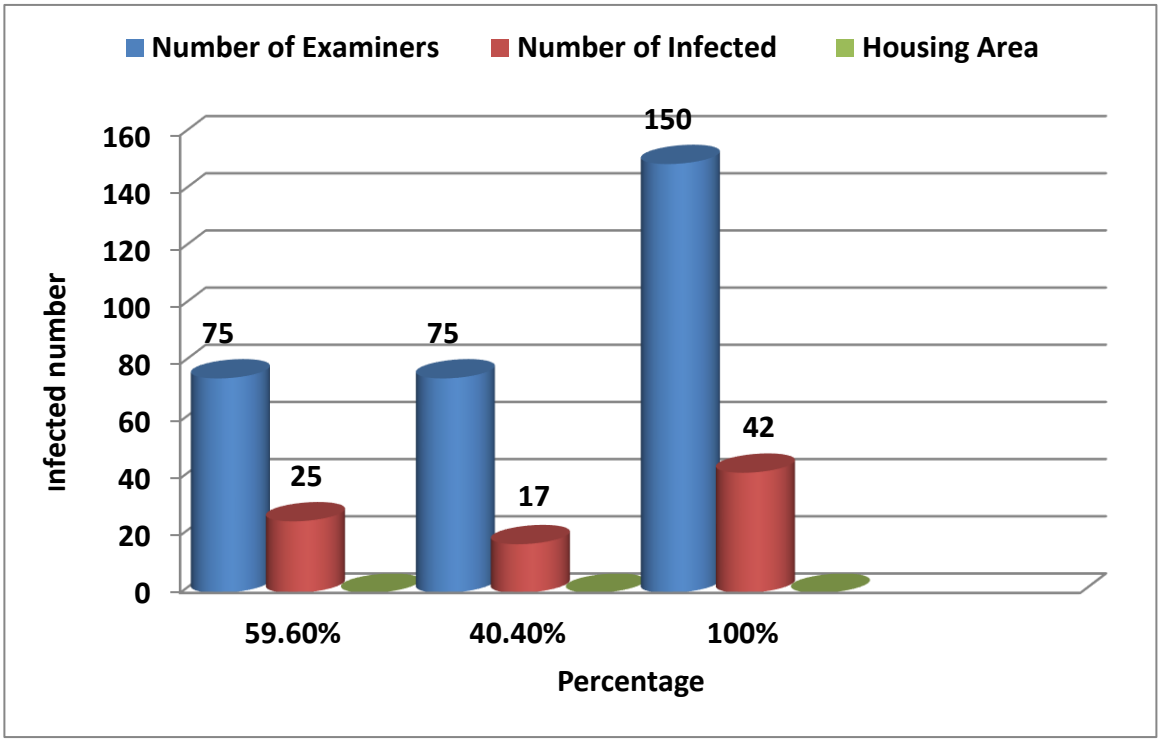

Figure (3): The relationship between infection rate and housing area for the infected.

Discussion

The presence of oral parasites E. gingivalis and T. tenax in the oral cavity is a sign of a lack of attention to oral hygiene, dental care, and gum disease, and the method of spreading is similar in both parasites through kissing and volatile spray or the common use of eating and drinking tools [16]. Previously, people believed these parasites had no obvious pathological symptoms, but recent studies confirm that the presence of these parasites is associated with a poor oral environment as well as supportive tissue disease and gum bleeding [17].

The results of this study showed that the total infection rate of oral parasites was 28%, distributed to 10% for E. gingivalis and 18% for T. tenax. These rates are comparable to several studies [18, 19], which recorded ratios ranging from 12% to 32%.The results were not identical to the study [20], which recorded an infection rate of 4.9% for T. tenax and 11.3% for E. gingivalis, and [21], which had an infection rate of 41.7% for E. gingivalis and 9.2% for T. tenax, as well as a study [22] in which the infection rate of E. gingivalis was 31.37%, higher than 22.53% for T. tenax, and in a study [23] the prevalence of E. gingivalis was 10%, while T. tenax was 2%.

The reason is due to neglect of oral hygiene and teeth and its impact on many diseases, including oral infection of parasites. It is mentioned that there is increasing lack of hygiene and vice versa, but excessive hygiene has a negative impact on many diseases due to lack of immunity because of the inefficiency of the immune system and the lack of exposure to such parasites previously [24]. The results of the present study showed that there is a relationship and influence of the sex factor in the incidence of both parasites, where the total infection rate in males was 18%, which is higher than the infection rate in females recorded at 10%, and the total infection rate for females was 7.63% for T. tenax, less than the total For males, 8.07%; these results were consistent with several studies, including [25], which found that the prevalence of E. gingivalis in females is lower, probably due to more dental care in females than in males, and the study of [26] found that infection with E. gingivalis is more prevalent in males, and the lower infection rate in females is attributed to being more concerned with oral health than males.

This is identical to the results of the current study and may be the reason for this decrease in the infection rate of both parasites in females due to health awareness and much care of the mouth and teeth as a cleaner and elegance and the availability of the factor of time and comfort and presence in the home when compared to males. On the other hand, the reason may be attributed to it considering itself sex. Gentle, therefore, take care of her oral hygiene more than males, and also, high immunity in women against the incidence of many diseases is more than in men.

If we take this study from another perspective, we find it different and far from the results of many studies, and this characteristic is contained in the results of scientific research, where the reason for this is due to the time, conditions, and samples of research taken with the nature and level of social, cultural, and economic factors of society, including the study of [26], which reached The sex factor was not significant in the infection rate of both parasites, and the differences in infection rate between males and females were not statistically significant in all studied cases.

In this study, data were collected from the students, taking into account the environmental degradation of these students and the extent of its impact on the incidence of oral parasites *E. gingivalis* and *T. tenax*, where it was found that the overall incidence of oral parasites among

students with rural environmental decline was the highest at 60.4% of the rates of injury among students from the city's residents and from various Iraqi provinces, which recorded 39.6%. These ratios are comparable to several studies [10, 20, 21]. Perhaps the reason for the lower rate of infection in the city center than in the villages is the increased health awareness of people and their knowledge of the importance of oral hygiene and dental health and gum health, the quality of water used, and the lack of these in the villages and the impact of educational level on the infection rate of parasites in the study. In other words, there is an inverse relationship between educational level and infection rates; they are high among non-educated people.

This is attributed to the cultural and scientific awareness and the correct health guidance followed in the advanced stages of the study and to the recognition of the importance of oral care and teeth and serious consideration of oral diseases spread among persons, while we note the opposite in people from the initial stages of education and the type of work they do and the lack of adequate health guidance to them. And oral hygiene and bad habits led to the infection and spread of oral parasites, and this was evident in the results of our study.

Conclusions:

The decrease in the infection rate of oral parasites in this study is attributed to the cultural and scientific awareness and the correct health guidance followed in the advanced stages of study (university) and to the recognition of the importance of oral care and teeth and the serious consideration of the prevalence of oral diseases among people, while we note the opposite in people in the early stages of education and lack of access to adequate health guidance about oral hygiene and bad habits led to the infection and spread of oral parasites, and this was evident in the results of our study.

This may also be attributed to the critical conditions that Iraqi society has been going through, such as the deterioration of health services, the lack of medicines, and the high cost and lack of availability in health centers and hospitals, especially those related to dental centers. Therefore, it is difficult for people with low living standards to consult private dentist clinics because of the high cost. Therefore, these conditions combined led to an increase in the infection rate of oral parasites, which led to the deterioration of their health, while the low infection in people with a high level of living is due to the availability of good conditions that enable them to visit private clinics with high health care, which is rarely available in some centers of Health and Hospitals.

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Prevalence of *Entamoeba histolytica* in Diarrheal Patients in Al- Najaf Province\ Iraq

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Abstract

The study was done in Al- Najaf Teaching Hospital. The total number of examined patients were (1061) for both sexes (males and females) in different ages (children's and adults). The infection with Entamoeba histolytica was diagnosed in 241 stool samples from the total number examined, percentage Infection with intestinal protozoa according to the months of the year January was relatively high (27%), then it began to gradually to decline in the month of April, reaching (6%). The highest incidence was recorded in aged (15-44) year (29%) while the lowest was in children aged 1 years (6%). The results showed Distribution according to sex, males (46%) and females (54%) in tested with parasites. The samples were recorded the average percentage in the cyst stages of E. histolytica at a higher rate, where it reached (80%) than the trophozoite stage of E. histolytica, where it reached (20%) .

Key words: E. histolytica, diarrhea, Cyst, Trophozoite, parasite.

INTRODUCTION

Most tropical and subtropical developing nations are endemic for parasitic diseases (1). It is estimated that 3.5 billion individuals worldwide are infected with intestinal parasites. Intestinal protozoa and helminthes parasites, pathogenic bacteria, and viruses are the most common causes of diarrhea in children in underdeveloped countries (2,3). *Entamoeba histolytica*, is one of species which parasites the human intestinal tract and this is the only species of amoeba that found to be associated with intestinal disease (4), causes asymptomatic infections in around 90% of infected persons and plays an important role in parasite dissemination. Asymptomatic infection can progress to invasive amoebiasis, which can cause bloody diarrhea, abdominal pain, flatulence, nausea, and vomiting. In some situations, amebae can travel from the gastrointestinal tract to the liver, causing ulcerations and abscesses and ultimately resulting in amoebic liver abscesses (5). Young children are reported to be affected by IPIs (Intestinal parasitic infections) compared to adults due to their increased nutritional and less developed immune systems (6). Some researches concentrated on gender and occupation-related prevalence (7), others concentrated on the link between anemia and parasitic infections of the gastrointestinal tract (8).

MATERIALS & METHODS

Stool Samples Collection

One thousand sixty-one stool samples were collected using clean plastic containers with a cover from diarrheal patients and some other intestinal disorders attending AL- Najaf Teaching Hospital, their ages ranged from less than 1 yr to more than 45 yr. for a period from Jan.2024 to June 2024. Information have been recorded according to a special form prepared for this purpose.

Statistical Analysis

The data in this study were represented by numbers and percentages. Chi-Square Test (χ^2) was used to test the differences (9). The significance was detected at $p<0.05$.

The analysis was performed by SPSS (Version 28), and Microsoft Software Excel 2019 for graphics.

RESULTS & DISCUSSION

Table (1): Distribution of diarrhea cases and percentages of their occurrence according to the study months.

Months	Examined	Infected	%	Statistical value
January	254	66	27	χ^2= 35.716 p-value=0.0001 *
February	198	63	26	
March	125	26	11	
April	172	15	6	
May	138	32	13	
June	174	39	16	
Total	1061	241	100	

***Significant differences at p-value <0.05.**

Table (1) shows that there were significant differences Infection intestinal protozoa according to the months of the year, as the infection rate in January was relatively high (27%), then it began to gradually to decline in the month of April, reaching (6%).

Differences in infection depending on the months of the year may be due to weather fluctuations, where temperatures rise during summer, then gradually decrease with the advent of the fall and winter. Or as a result of the proliferation and spread of disease-carrying insects, such as house flies, which are mechanical carriers of intestinal protozoan cysts and worm eggs, in addition to Frequent consumption of soft drinks and cold juices from street vendors, which can be a suitable medium for the spread Parasites.

Table (2): Distribution of diarrhea cases and percentages of their occurrence according to age.

Age (yr.)	Examined	Infected	%	
Less than 1	98	14	6	
1-4	131	28	12	
5-14	212	62	26	Statistical value
15-44	305	71	29	χ^2= 9.863
More than 45	315	66	27	p-value=0.043 *
Total	1061	241	100	

***Significant differences at p-value <0.05.**

Table (2) shows that there were significant differences infection was prevalent in all age groups that involved in this study. The highest incidence was recorded in aged (15-44) year (29%) while the lowest was in children aged 1 years (6%). Children are more likely to be infected with E. *histolytica* than adults. They are more susceptible to water-borne and foodborne diseases, as their playing and hygiene routines predispose them to infection than older age groups. Furthermore, their immune systems are not fully developed, even their low level of health care, may elevate parasitic infection rates.

The incidence of intestinal parasites did not differ by age group. This study is agreeing with (10) who reported a prevalence of (36.7%), In this investigation, E. *histolytica* was found to be strongly linked with diarrheal situations. Children with diarrhea had a substantially higher incidence (63.92%) than those without (17.58%). These results coincide with (11) who found the prevalence for children with diarrhea (47.3%) and without diarrhea (31.5%). Diarrhea is often regarded as leading cause of childhood mortality and morbidity in underdeveloped countries. Mortality due to diarrhea estimates 2.5 million people each year (12).

Table (3): Distribution of E. *histolytica* according to sex.

Sex	Male	Female	Total
Examined	330	731	1061
+ ve No.	112	129	241
%	46	54	100
Statistical value. χ^2= 34.37, p-value=0.0004 *			

***Significant differences at p-value <0.05.**

Table (3) shows that there were significant differences in the patients with *E. histolytica* parasites concerning gender distribution for both males and females. The highest incidence was recorded in female (54%) while the lowest was in male (46%).

This result may due to social customs or related to the abundance of male activity, which it increases their chance of being exposed to sources of infection more than females, and this result agree with the findings of (13).

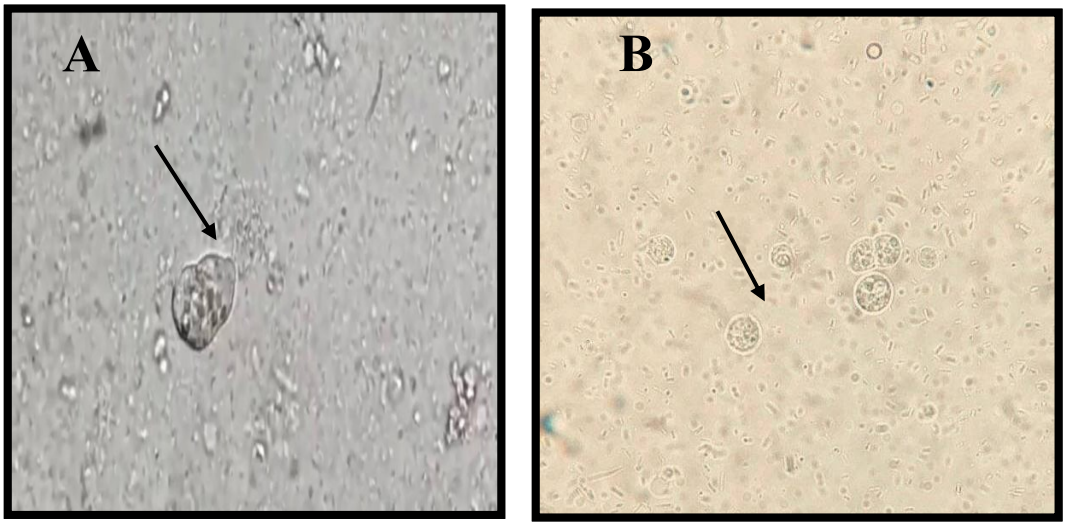

Figure (1) :(A) *E. histolytica* trophozoite (B) *E. histolytica* cyst examined by light microscope (40X).

Table (4): Distribution of E. *histolytica* stages.

Stages	Trophozoite	Cyst	Total
+ ve No.	49	192	241
%	20	80	100
Statistical value χ^2= 84.85, p-value=0.0003 *			

***Significant differences at p-value <0.05.**

Table (4) shows that there were significant differences between the cyst and trophozoite stages; the highest percentage was reported in the cyst stage, reaching around (80%) While in the trophozoite stage, it reached about (20%), were observed trophozoite stage and cyst stage as in Figure (1).

The study found that the cyst stage of E. *histolytica* parasite had the highest prevalence of parasitic infection compared to the trophozoite stage. This could be owing to the parasite's motility, as *Entamoeba* is immobile in cyst stage but can tolerate extreme circumstances such stomach acidity (14,15,16).

Conclusions:

Entamoeba which can live outside the body, can spread infections between individuals. The source of the high infection may be owing to the use of river water or liquefied water, which may not be sufficiently sanitized, or due to chlorine supplementation results, according to various research.

Recommendations:

1- More public health awareness programs should be pushed to improve understanding of the source of amoebiasis, especially in children.

2- Improve sanitation, provide safe drinking water, and promote good toiletry habits.

3- Proper and effective diagnostic techniques, such as using sensitive and specific assays like ELISA and PCR.

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Association of MTHFR gene polymorphism (rs1801133) with Type 2 Diabetic Iraqi population

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Abstract

Background: Type 2 Diabetes Mellitus is a complex of endocrine metabolic disorder. It is believed that polymorphism of Methylenetetrahydrofolate reductase (MTHFR) C677T related to type 2 diabetes mellitus. However, results are conflicted from different ethnic and races. This study aimed to evaluate the relationship between MTHFR (rs1801133) gene polymorphism and type 2 diabetes mellitus.

Methods: **This** case-control study was included 100 patients were diagnosed with type 2 diabetes mellitus (T2DM) cases and 100 healthy control individuals. The blood sample was collected for estimation of biochemical parameters for analysis among the T2DM cases and healthy control groups. DNA extraction from whole blood was done to study the MTHFR gene polymorphism by RFLP-PCR method.

Results: There were significant difference in genotype distribution among Type2 diabetic patients and control group. Compared with CC wild genotype, CT heterozygous genotype (OR= 5.7, 95% Cl= 3.0-11.0 and P=0.0001) and TT homozygous genotype (OR=4.3, CI=1.5-11.9 and P=0.0005). The T allele frequency increased the risk in diabetic patients by three folds when compared with C allele (OR= 3.6, C. I=2.2-5.5, P= 0.0001), suggested that the effect of MTHFR point mutation on type 2 diabetes mellitus implicated with increased risk of disease.

Conclusion; Our results indicate that polymorphism in MTHFR C677T plays significant role in type II diabetes mellitus risk for Iraqi populations

Key words: MTHFR, gene, C677T, polymorphism, T2DM.

Introduction:

Type 2 diabetes mellitus (T2DM), is a polygenic and multifactorial disease that is considered a major life-threatening health problem throughout the world (1)**.** Diabetes is a type of glucose metabolic disorder which is one of the major health related global problems (2)**.** According to WHO, approximately 422 million people affected with diabetic conditions globally. In Iraq more than 13.9% of adults live with diabetes (3,4). The alteration in genetic material such as changes in

nucleotide sequences may lead to alteration in protein ultimately which directly affect the signaling process could be the determining factor for complication of diabetic (4).

Evidences suggest that gene polymorphisms involved in folate metabolism play a critical role in the etiology of diabetes and diabetic complications (5). Folate metabolism related disorders can be caused by genetic or environmental factors that include an individual's genetic variability and diet (6). Many of the genes involved in folate metabolism are polymorphic. Methylenetetrahydrofolate reductase (MTHFR) is one of the important enzymes in the first step of folate metabolism and converts dietary folate to 5- methyltetrahydrofolate, the methyl group donor required for the remethylation of homocysteine (Hcy) to methionine (7, 8). Methionine is the substrate for S-adenosyl methionine (SAM), a major cofactor and a methyl group donor for numerous methylation reactions (9). MTHFR regulates the metabolism of folate and it is an important factor in DNA methylation and synthesis (10). Low MTHFR activity reduces DNA methylation but may enhance de novo thymidylate biosynthesis (11,12).

Patients and methods:

The present case-control study was done in Diabetic Centre of Al- Sadder medical city hospital in Najaf/ Iraq. The study population was composed of one hundred patients with type2 diabetes mellitus (50 males and 50 females) their age range (30-70 years), were chosen compared with age and sex matched 100 healthy control individuals (50 males and 50 females), between the period of 2023 to 2024. In this study the lipid profile parameters were evaluated for each participant. The medical examinations for patients were carried out by experienced physician, they were identified with T2DM based on WHO classification and diagnostic criteria (4). Anthropometric and clinical parameters, which include age; gender; BMI, blood sugar, glycosylated hemoglobin (HbAIc), homocysteine, blood urea and serum creatinine were determined by standard enzymatic technique and colorimetric method applied to evaluation of blood urea and serum creatinine by using RANDOX kits (United Kingdom BT 29 4QY) with standard procedures. Total plasma homocysteine was analyzed according to the manufacturer procedure for determination by using microplate enzyme immunoassay ELISA kit method of Biorad laboratories.

From all participants a total of 5 milliliter venous blood samples were collected after obtained informed consent. One ml of blood transferred to EDTA tube for DNA extraction, another 4 ml centrifugated in 2000xg for serum separation, and serum were stored at -17°C until the assayed to be performed. DNA extracted by method that has been published previously (13). The Single nucleotide polymorphism was done by polymerase chain reaction (PCR) using primers mentioned by Alkhafaji S.M. (14). The primers that used for PCR–RFLP were 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ forward and 5′-AGG ACG GTG CGG TGA GAG TG-3′ reverse that resulting of 198bp PCR product. The polymorphism was detected by enzymatic digestion of the initial polymerase chain reaction product with HinfI (Promega.USA) at 37˚C for 4 hrs.

The resulting of DNA fragments was separated on 3% agarose gel stained with ethidium bromide, and photographed under UV light. Accordingly, Samples who lack the mutation appeared one 198bp fragment, sample with heterozygous for the mutation revealed both 198bp and 175bp fragments, and homozygous sample revealed one 175bp fragment. The resulting data were collected and analyzed on SPSS software package (revision 20 Inc., Chicago, USA), the appropriate tests such as Chi-square, t-tests and ANOVA were used. Also diabetic risk was tested and estimated by the use of odds ratios (ORs) and 95% confidence intervals (95% CI). Values of $P < 0.05$ were considered statistically significant.

Results:

Table (1): General and clinical characteristics of diabetic and non diabetic groups

Variables	*DM Patients No. =100, Mean ±SD*	*NDM Patients No. =100 ,Mean ±SD*	*P – value*
Gender: Male/Female	50/50	50 /50	
Age at study (years)	47±4	46 ±3.4	0.058
Blood glucose(mg/dl)	183± 12	92 ± 8	0.0001
HbA1C (%)	9 ± 1.9	5.5 ± 0.7	0.0001
BMI (kg/m^2)	29 ± 5.0	21 ± 2.7	0.0001
Blood Urea(mg/dl)	37±1.0	34 ± 1.6	0.073
Creatinine(mg/dl)	0.8± 0.3	0.5± 0.2	0.085
Homocysteine (µM)	19.7±2.1	6.0±0.9	<0.0001

P<0.05 statistically significant; BMI: body mass index; HbA1c: glycated hemoglobin.

Table 2: Genotype frequency of MTHFR (C→A) gene polymorphism in diabetic patients and control groups.

Genotype and allele frequency	*DM No.=100*	*NDM No.=100*	*OR*	*(95% C.I)*	*P- Value*
CC	30 (30%)	70(70%)	1	Ref.	-
CT	57 (57%)	23 (23%)	5.7	3.0-11.0	0.0001
TT	13 (13%)	7 (7%)	4.3	1.5-11.9	0.0005
C	117(38.6%)	186 (61.4%)	1	Ref.	-
T	83(69.2%)	37 (30.8%)	3.6	2.2-5.5	0.0001

No: number; OR: odds ratio, 95%C.I, confidence interval, P<0.05 statistically significant

Results:

Characteristic feature of patients and control groups.

The general and clinical features of type 2 diabetic patients with normal control groups are showed in Table1. The studied groups were matched for gender and age. Blood urea, serum creatinine showed no significant differences, while blood sugar, HbA1c, body mass index (BMI), and the levels homocysteine were considerably increased in diabetics patients when compared with healthy control group as revealed in table (1). The alleles frequency of MTHFR gene is studied using PCR-RFLP technique. The distributions of genotype and allele frequencies were compared between type2 diabetic patients with normal control group (Table 2). The allele frequency and genotype of SNP of the MTHFR gene in DM patients 30% for CC, 57% for CT and 13.0% for TT respectively, whereas in control group individuals 70%, 23% and 7% respectively. The allele frequency obtained in the DM patients for C was (38.6%) and for T was (69.2%), whereas in the control group for C was (61.4%) and for T was (30.8%). The genotypes frequency of C677T in TT variant 13 (13.0%) which was significantly increased the risk of diabetic's patients by four folds in homozygous genotype of DM patients when compared with wild genotype (OR 4.03, 95% CI= 1.5-11.9, p=0.0005). The T allele frequency increased the risk in diabetic patients by four folds when compared with C allele (OR= 3.6, C. I=2.2-5.5, P= 0.0001)

Discussion

Diabetes mellitus type II is a complex metabolic and endocrine interaction between multiple genetic and environmental factors cause a progressive, various disorder and dysfunction of pancreatic beta cells and is associated with changes in biochemical, physiological and pathological liver diseases (15). The biochemical characteristics of diabetic patients and control groups are revealed that T2DM patients showed significantly higher HbA1C, blood sugar and homosystiene, same finding was also observed in a study by Sherwani SI et al which were presented the higher HbA1C in T2DM patients (16-18). In our study we tried to evaluated a possible role of the point mutation C677T on gene coding for MTHFR enzyme as a risk factor that increasing in diabetic patients so we summarized possible association of mutation polymorphism of MTHFR (677 C>T) gene in diabetic patients in contrast to healthy individuals in Iraqi population.

According to the relation between T2DM and C677T point mutation on gene coding for MTHFR enzyme, our results showed that homozygous mutated TT genotypes of C677T and T allele was higher in study group of DM patients compared to Control group NDM. Indeed, homozygous mutation for the C677T in MTHFR gene, causing decrease production of 5-methyltetrahydrofolate, the main source of methyl donor in alteration of homocysteine to methionine which lead to rise of homocysteine in plasma Di et al. [19]. A 677 C→ T mutation is responsible for reduced MTHFR activity, and it is found significantly effective only in recessive homozygous state Paul and Sreyoshi (20). Furthermore, the association between recessive homozygous 677C→T in MTHFR gene and DM, and the presence of higher 677C→T mutations in MTHFR gene among patients with DM compared to normal individuals, the TT genotype and

T allele frequencies were increased and they were significantly increased in diabetic patients than in those without DM since our normal individuals had a higher frequency of the C allele than those diabetic patients. Several investigations supported our findings, including one by Abd Raboh et al., who used RFLP techniques to investigate the effects of A1298C and C677T polymorphisms in Egyptian patients with type II diabetes mellitus. According to their findings, polymorphisms in the MTHFR gene increase the incidence of type II diabetes (OR: 2.2, 95% CI = 0.7-6.9, P = 0.004) Abd Raboh et al. (21). The MTHFR C677T polymorphism also suggests that the T allele confers a considerable genetic risk in subacute combined degeneration illness, according to Zhang X et al. in 2019 (22), the MTHFR C677T mutation was proposed as a reliable biomarker for type 2 diabetes in the Chinese population. MTHFR C677T mutation in Chinese population was suggested to the predictable biomarker among T2DM as found by Sun et al. (23)**.** MTHFR polymorphism C677T, CC genotype suggested to have protective role in T2DM while TT genotype increases the risk of diabetics (24).

Conclusions:

We concluded from this study that the polymorphism in MTHFR C677T plays significant role in type II diabetes risk and MTHFR C677T gene polymorphism may confers toT2DM, especially in Iraqi populations**.**

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ROGNOSTIC SIGNIFICANCE OF STRUMAL CALLA EXPRESSION IN PRIMARY BREAST CARCINOMA IN RELATION TO PATHOLOGICAL RSPONCE AFTER NEOADJUVANT CHEMOTHERAPY

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Abstract:

Background: In this study we aimed to assess the IHC expression of stromal CALLA in invasive breast carcinomas as prognostic factor and correlate its relationship with six clinico-pathological parametrs by assessing the pathological response to neoadjuvant chemotherapy

Method: Study design is cross-sectional, it conducted on 50 females with breast invasive ductal carcinoma, FFPE tissue blocks collected from the archive of laboratory of Al-Sader medical city - Al Najaf governorate/ Iraq, during the period from September 2023 to September 2024. CALLA immunohistochemistry expression detected by labeled polymers and enhanced polymer system (Dako En-Vision™ Flex) Dako-protocol. it was correlated with age of patients, tumor grade, hormonal expression, her-2/neo expression, Ki-67 expression and molecular profile.

Result: Stromal CALLA immunohistochemistry was expressed in 48%. There is statistically significant positive correlations between stromal expression and response to neoadjuvant chemotherapy ($P = 0.011$), most cases with positive CALLA expression either nonresponding to NAC or achieved partial response while most of CALLA negative cases achieved complete pathological response the same for her-2 positive cases, though statistically non-significant but most CALLA expressed cases were Grade III, her-2 enriched and triple negative molecular profile most of them had partial pathological response (not complete)

Conclusions: Stromal CALLA had been expressed in 48% of invasive ductal carcinoma and there is positive correlation between stromal CALLA expressions and higher tumor grades, her-2 expression and triple negative profile suggesting the effects of stromal CALLA expression on aggressive behavior of breast ductal carcinoma with decreasing a chance of complete pathological response after neoadjuvant chemotherapy

Key words: CALLA immunohistochemistry, CD-10, breast carcinoma, prognostic factor, neoadjuvant chemotherapy, molecular profile.

Introduction:

Breast carcinoma regarded as the 2nd most prevalent cause of cancer mortality in female also most frequent malignancy in women. In 2020, there were over 2.3 million newly reported illnesses and almost 685,000 fatalities, it is regarded as most prevalent malignant tumor in women [(1)]. Thirty percent of females with early-stage BC still develop recurrent disease despite improvements in early identification and thorough therapy. It has a poor prognosis due to metastases and localized recurrences, that are the primary causes of unsuccessful therapy. This issue may be attributed to the biological characteristics and nature of the malignant cells [(2)]. It is a diverse illness that has unique inherent subgroups. Utilising the three distinct

immunohistochemistry markers estrogenic-receptor (ER), progesterone-receptor (PR), and human-epidermal-growth-factor-receptor 2 (HER-2/Neu), Breast tumors diagnosed clinically are classified into one of four primary subtypes, which are treatable using targeted therapies: luminal A, luminal B, HER2-enriched and triple negative breast cancer (TNBC) [3].

The tumor-node-metastasis (TNM) staging system is another extensively utilized classification criterion for identifying clinical aggressiveness of a cancer, predicting prognosis, and directing therapeutic approaches.[4]. Nevertheless, it is noteworthy to consider tumor malignant behaviors are affected not only by cancer cells but also by the tumor microenvironments around them [5]. Tumor micro-environments (TMEs) refer to the reciprocal interactions between stromal cells, tumor cells, and cellular components. Recent studies have demonstrated that TMEs not only influence the growth and spread of tumors, but also affect the biological characteristics of cancer cells [6]. The production of numerous nutrients, growth factors, chemokines, and cytokines by tumor stroma, a significant component of TME, aids in the development of tumors [7].

Numerous cell types, involving fibroblasts, lymphocytes, epithelial, and mesenchymal stem cells, are present in these microenvironments. In this situation, many biological markers had been identified to divide patients into various subgroups depending on the traits of their tumor stroma. Various subsets of mononuclear-inflammatory-cells and cancer-associated fibroblasts have been distinguished by their production of matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases [8]. Common acute lymphoblastic leukemia antigen (CALLA) is the name given to CD10, a zinc-dependent metalloprotease with a cell surface molecular mass of 90–110 kDa. The epithelial cells of several organs, like the prostate, colon, liver, and stomach, usually express this enzyme. By reducing the local quantities of peptide substrates accessible for receptor binding, It is well-established that this protease controls the biological functions of numerous peptide substrates. Much research shows that the level of CD10 expression in the stroma is related to how aggressively different epithelial cancers grow [9].

Numerous studies have revealed a substantial correlation between dysregulation of CALLA expression and tumour growth and aggressiveness in a diverse spectrum of malignancies, including melanoma [10], colorectal cancer [11], and nasopharyngeal cancer [12]. although it is still not obvious how CD10 is used for diagnosis or prognosis [13]. In vitro research has identified CD10 as the marker of breast stem-like or bipotent progenitor cells [14].

The aim:

- To correlate CALLA expression with various clinicopathological parameters including age, grade, hormone expression, Her-2/neo, ki-67 and molecular profile.
- To assess the response to neoadjuvant chemotherapy in CALLA positive patient which gives hint about prognostic significance

Material and Method:

The study was design was a prospective cross-sectional study include Iraqi female patients with primary breast invasive ductal carcinoma. All data were kept private. No information was divulged and used for research only. Ethical committee for clinical studies with approval code MEC-198 authorized permission to conduct the study and it followed the tenets of the Helsinki Declaration.

The study was done in the Najaf governorate, department of pathology and Forensic Medicine / Faculty of Medicine - University of kufa

Selection of Cases

After applying inclusion criteria, 50 FFPE tissue blocks and slides were collected from laboratory of Al-Sader medical city - Al Najaf in a convenient way. All slides had been re-examined by two pathologists to confirm the diagnosis on H&E-stained slides, then confirm the ER, PR and HER-2 scoring on the tru-cut biopsy then reviewed the slides of mastectomy or breast conserved surgery after completing neoadjuvant chemotherapy 6-8 cycles

Inclusion criteria

- Core biopsy samples positive for invasive ductal carcinoma NST
- Complete biological profile ER, PR and HER2 with or without Ki67 on the tru-cut biopsy
- Patient scheduled for neoadjuvant chemotherapy.
- Resection after 6-8 cycles neoadjuvant chemotherapy to assess the pathological response

Exclusion criteria

- In adequate material (depleted paraffin blocks)
- with incomplete postoperative information
- Local recurrent tumors.
- Male gender.
- ILC
- Unknown molecular subtype (biological profile).

Slide Preparation:

One slide was prepared for each case on the treatment naive tru-cut biopsy, for staining with primary antibody CALLA immunohistochemistry. (FLEX Monoclonal Mouse Anti-Human CD 10 (Agilent Technologies Singapore International, Denmark))

Procedure of Immunohistochemistry by Envision Antibody Complex

The formalin-fixed and paraffin-embedded blocks were 4 µm thick, cut by a microtome, and mounted on the positively charged slides. Deparaffinization and rehydration, then antigen retrieving using heat induced antigen retrieving, blocking by endogenous enzymes to ovoid background staining, primary antibody incubation for 30 minutes, polymer -bound secondary antibody also incubated for 30 minutes which enhance amplification and more chromogenic detection, finally counterstaining and mounting with DPX for visualization under microscope. to evaluate the expression of CALLA in breast carcinoma samples. (< 10% regarded as negative and equal or more than 10% of stromal membranous staining regarded as positive no matter the staining intensity).

Review of Immunohistochemical Scoring Systems

The already ER, PR and Her-2-stained slides were reviewed for scoring; hormone receptor status (ER and PR) is graded according to Allred scoring system while HER2 classification in breast cancer is determined by ASCO / CAP guideline criteria

Follow up data and response to neoadjuvant therapy.

All included patients were scheduled for neoadjuvant therapy. After course completion 6-8 cycles of chemotherapy, patients underwent breast conservative surgery or mastectomy and the pathological response was assessed following the Royal college guidelines {Ellis, 2016 #1507}.

1-Complete Pathological Responces, which could be a) No residual breast carcinoma. or b) No residual invasive malignancy but ductal carcinoma insitu present.

2-Partial Pathological Responses to therapy, which could be a) Minimal residual tumor/near total effects typically (e.g., <10% of tumor remaining in the tumour bed seen as foci of residual fibrosis demarcating the original tumor extents) or b)10%-50% of tumor clusters remaining or c) > 50 % of tumor cells remaining. Comparison with the previous core biopsy sample was helpful.

3-No evidence of response to therapy, most tumor cells still viable

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (IBM SPSS) software-25. data that observed were presented as frequency & percentages. The continuous variables were expressed as mean ± standard deviations (SD) or range according to data distribution. Statistical comparisons were done using Chi-square tests or Fisher's exact test to assess proportions of nominal/ ordinal variables in different groups. P value of less than 0.05 was regarded as statisticaly significant

Results:

1- Study group characteristics:

In this study there are 50 cases of female with invasive ductal carcinoma, with a mean age of 49.53 years, standard deviation was 11 years and median was 50.3 year. Age range was between 29 to 71 years, 28 (56%) of cases being 50 years and older. Most of the tumours 37 (74%) were grade II. In almost two third 31 (62 %) of the cases, the estrogen recepters (ER) and progesterone recepters (PR) were positive. While, membranous HER2/neu was positive in 15 cases (30%). A high mitotic index (> 14%) was seen in 5 cases (10 %), low mitotic index (≤14%) was seen in 13 cases (26 %) and it was unknown for 32 (64%) of the cases.

Regarding molecular subtype, luminal-A accounted for 5 (10%), Luminal-B accounted for 13 (26%) but there are 15 (30%) of luminal cases were not classified as A or B (no Ki-67). HER2-neu enriched cases accounted 10 (20%) of the cases. While triple negative cases were the least accounted for 7 (14%).

2-Response to neoadjuvant therapy

Complete response was observed in 31 (62%) of the cases. More than a quarter 18 (36%) had partial response and one case showed no response, as further illustrated in Figure 1

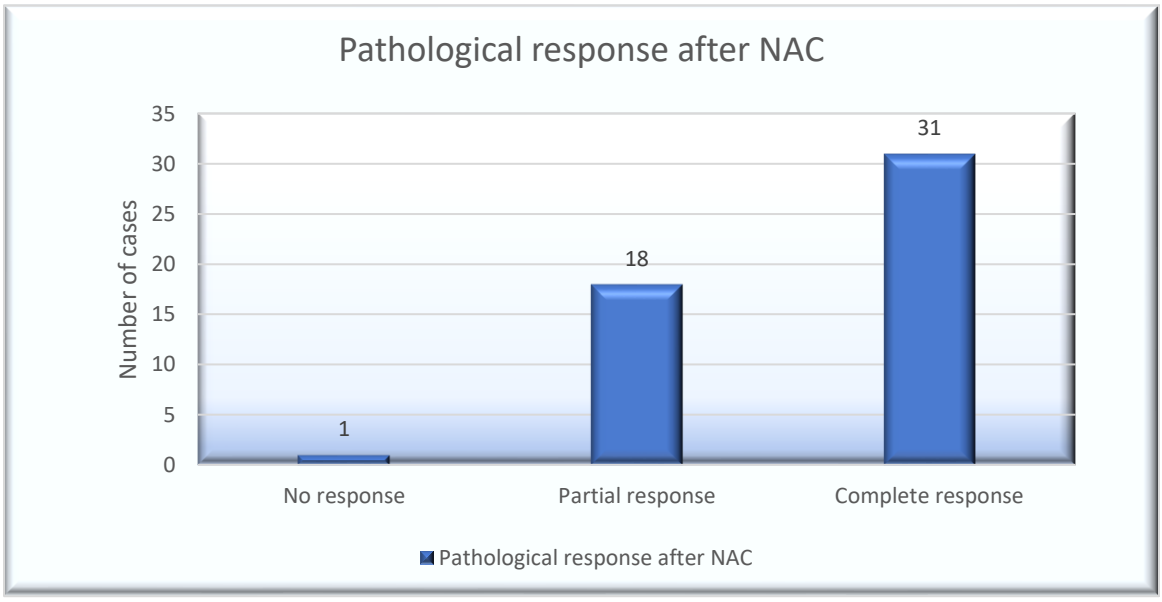


Figure 1: Response to neoadjuvant therapy.

Table 1 shows out of CALLA expressed tumors, 14 (77.8%) had partial pathological response compared to 4 (22.2%) of negative cases. Conversely, the majority of CALLA negative cases achieved complete pathological response 22 (70.97%) as compared to 9 (29.03%) of CALLA positive cases, P<0.011.

Table 1 The association between CALLA expression and response to therapy

Response to NAT	Total	CALLA IHC testing		P value
		CALLA negative No. (%)	CALLA positive No. (%)	
No response	1	0	1 (100)	<0.011
Partial	18	4 (22.2)	14 (77.8)	
Complete	31	22 (70.97)	9 (29.03)	

3-Correlation between CALLA expression, clinicopathological parameters and response to therapy

3-1- Correlation with age:

For young patients (< 50 years old), 13 (59.1%) of them were CALLA negative and 9 (40.9%) were positive CALLA positive with p value 0.003. Malignancy of older female (≥50 years old) were more frequently CALLA expressed 15 (53.6%). As in Figure 2, the difference was statistically not significant.

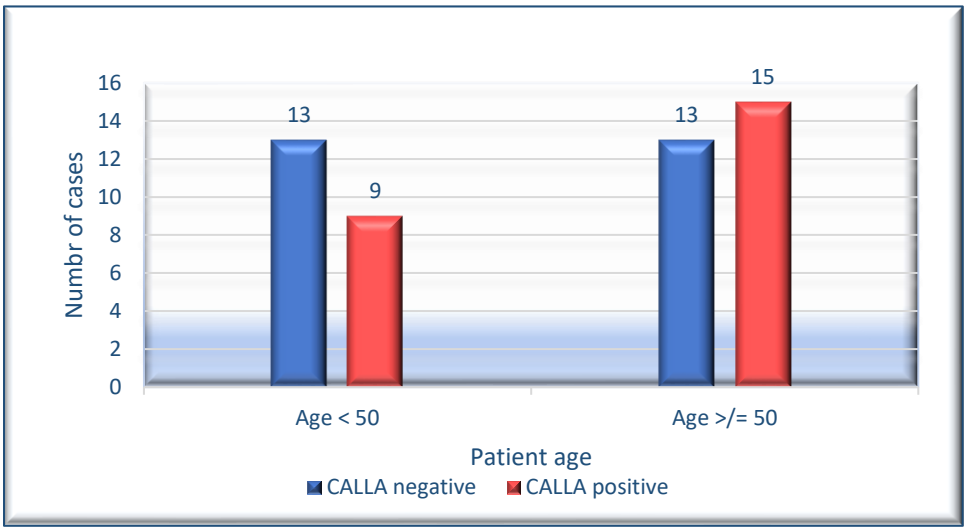


Figure 2: Correlation of patients age with CALLA expression

The correlation between responses to NAC therapy and CALLA expression in both age groups explained in Table 2. Young patients with CALLA positive malignancy revealed a significant correlation with partial response in 6 patients (85.7%) in comparison with those who are negative 12 (85.7%) of them achieved complete response, P=0.003.

Despite statistically not significant (p value 0.102) but older patient had a similar pattern. The majority of them who achieved partial responses 8 (72.7%) had positive CALLA staining while about two-third of those with complete pathological responses 10 cases (58.8%) had negative CALLA staining.

Table 2: The correlation between patient age and response to neoadjuvant chemotherapy with CALLA expression

Age	Response to NAT	Total	CALLA stromal expression		P value
			Not Expressed No. (%	Expressed No.(%)	
<50	No responses	1	0	1 (100)	0.003
	Partial responses	7	1(14.3)	6(85.7)	
	Complete	14	12 (85.7)	2 (14.3)	
≥50	Partial	11	3 (27.3)	8 (72.7)	0.102
	Complete	17	10 (58.8)	7 (41.2)	

3-2-Correlation with tumor grade:

Most of patients with grade III tumours, were CALLA positive 10 (83.3%), while 2 (16.7%) were CALLA negative. Out of the grade II tumors, 14 (37.8%) were CALLA positive, whereas the remaining 23 (62.2%) were negative. The single (100%) grade I case had negative staining. Grade III tumors had a significantly higher rate of CALLA expression, Figure 3.

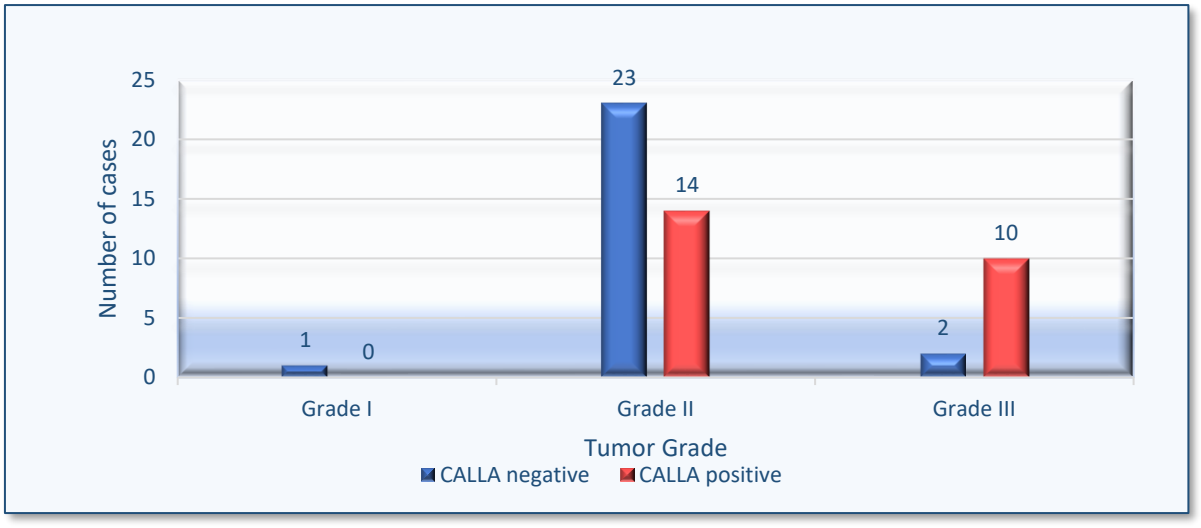


Figure 3: Correlation between tumour grade and CALLA expression

The correlation between response to neoadjuvant therapy and CALLA expression in each tumour grade was illustrated in Table 3. The single grade I patient who had a complete pathological response was CALLA negative. In GII patients, tumours with expressed CALLA had a lower rate of complet pathological responce in 8 (29.6%) in comparison to those with negative staining 19 (70.4%). In contrast, CALLA positive achieved partial response 6 (60%) in comparison to CALLA negative tumors 4 (40), P value= 0.190. Regarding Grade III although all cases with CALLA expression achieved only partial responces 8 (100 %) and only 2 cases (66.7) of CALLA negative reached to complete pathlogical responce, the difference did not reach statistical significance (may be due to small sample size).

Table 3: The correlation between tumour grade and pathological response to neo-adjuvant therapy with CALLA expression

Tumour grade	Responce to Neoadjuvant therapy	Total	CALLA expression		P value
			CALLA negative No (%)	CALLA positive No (%)	
I	Partial	0	0	0	-
	Complete	1	1 (100)	0	
II	Partial	10	4 (40)	6 (60)	0.190
	Complete	27	19 (70.4)	8 (29.6)	
III	No response	1	0	1 (100)	0.147
	Partial	8	0	8 (100)	
	Complete	3	2 (66.7)	1 (33.3)	

3-3- Correlation with Hormonal receptors, Her-2/neo and Ki-67:

The status was significantly different. Approximately two-thirds of CALLA expressed tumors 14 (82.4%) (77.8%) were hormonal negative (ER and PR negative). In contrast, most of CALLA negative patients 23 (69.7%) were Estrogen Receptor (ER) positive and 22 (68.8%) were Progesterone Receptor (PR) positive, P-value < 0.001. as in Figure 4 and Table 4.

Table 4 The correlation between ER, PR, Her-2/neo and Ki-67 with CALLA expression.

Hormone status		Total	CALLA expression		P value
			CALLA negative No (%)	CALLA positive No (%)	
ER status	Negative	17	3 (17.6)	14 (82.4)	<0.001

	Positive	33	23 (69.7)	10 (30.3)	
PR status	Negative	18	4 (22.2)	14 (77.8)	<0.001
	Positive	32	22 (68.8)	10 (31.2)	
Her2/neu	Negative	35	29 (82.8)	6 (17.2)	0.042
	Overexpressed	15	2 (13.3)	13 (86.7)	
Ki 67	≤14%	13	6 (46.2)	7 (53.8)	0.492
	>14%	5	4 (80.0)	1 (20.0)	
	Unknown	32	13 (40.6)	19 (59.4)	

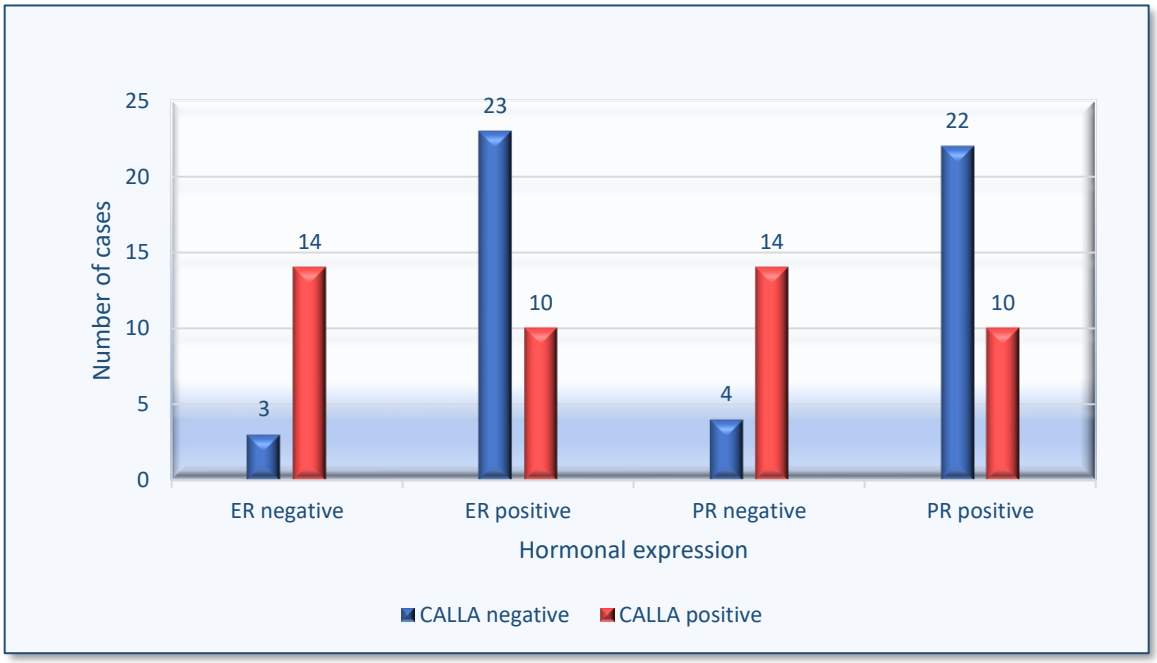


Figure 4: The correlation between hormone receptors ER and PR with CALLA expression, P-value < 0.001.

Regarding Correlation with Her2/neo; A statistically significant correlation was observed between HER2/neu expression and CALLA expression, as in Table 4 and Figure 5. Most patients with overexpressed Her-2/neo were CALLA positive 13 (86.7%); While paients with negative CALLA expression were significantly associated with HER2 negative expression 29 (82.8%), P=value = 0.042.

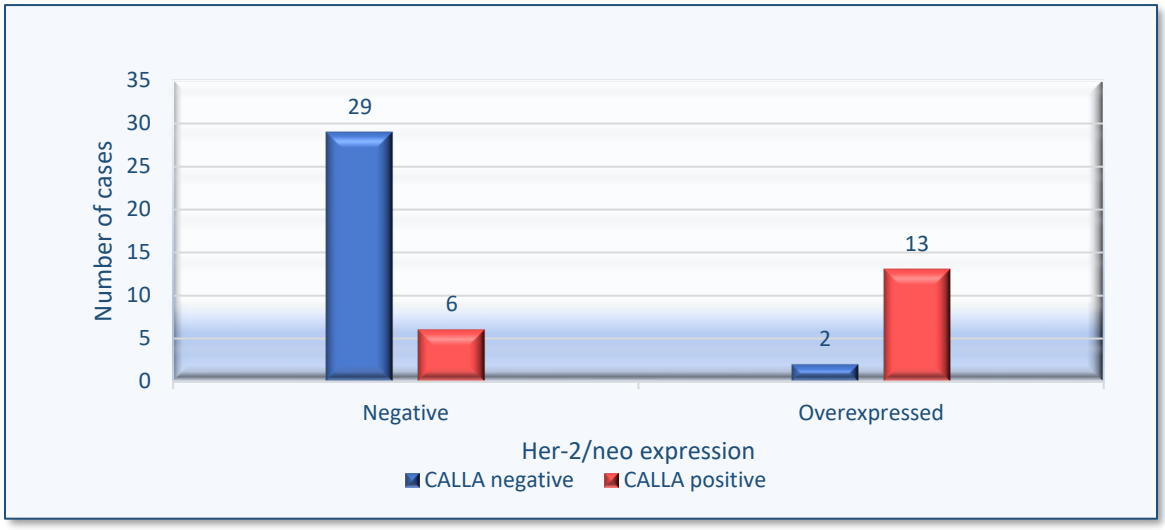


Figure 5: The association between HER2 neu expression and CALLA expression.

While with low Ki-67 index there were no significant differences in the mitotic index between CALLA positive and CALLA negative patients, however there were 32 cases (64%) tumours with unknown Ki-67 expression, 19 (59.4%) of them were CALLA positive. Almost the same proportions of CALLA positive cases 7 (53.8%) and negative cases 6 (46.2%) revealed low mitotic index < 14%. Despite CALLA negative cases showed a higher mitotic index 4 (80.0%) versus one case (20.0%) the difference was statistically not significant even after exclusion of tumors with unknown Ki-67 expresion. The details are shown in Table-4 and Figure-6.

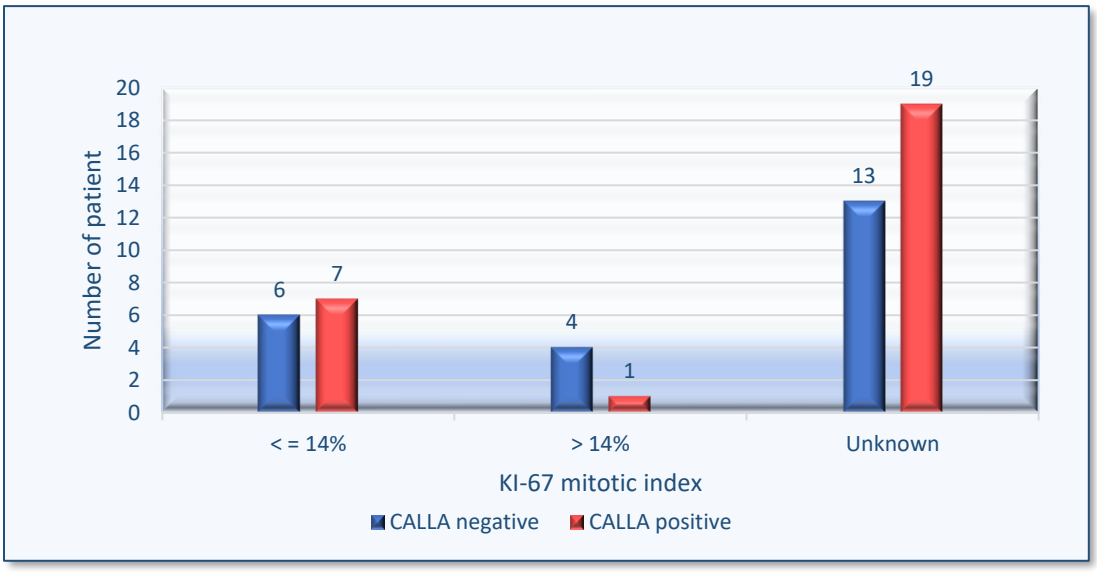


Figure 6: The correlation between Ki67 proliferation index and CALLA expression

3-4 Correlation with Molecular profiles:

A strong association was noted between both HER2/nue-enriched carcinomas and triple negative carcinomas with CALLA expression (P-value =0.016). Table 5 reveals 8 out of 10 HER2/neu enriched carcinomas (83.3 %) and 6 (85.7) out of 7 triple positive carcinomas were CALLA positive, whereas only one (20%) out of 5 of luminal A and 2 cases (13.3%) out of 15 luminal unclassified carcinomas (16.7 %) were CALLA positive, As in Figure 7.

Table 5: The correlation between molecular subtypes of breast carcinoma with CALLA expression

Molecular subtypes	Total	CALLA expression		P value
		CALLA negative No (%)	CALLA positive No (%)	
Luminal-A	5	4 (80)	1 (20)	0.016
Luminal-B	١٣	6 (46.2)	7 (53.8)	
Luminal unclassified (unknown A or B)	15	13 (86.7)	2 (13.3)	
HER2/neu enriched	10	2 (16.7)	8 (83.3)	
Triple negative	7	1 (14.2)	6 (85.7)	

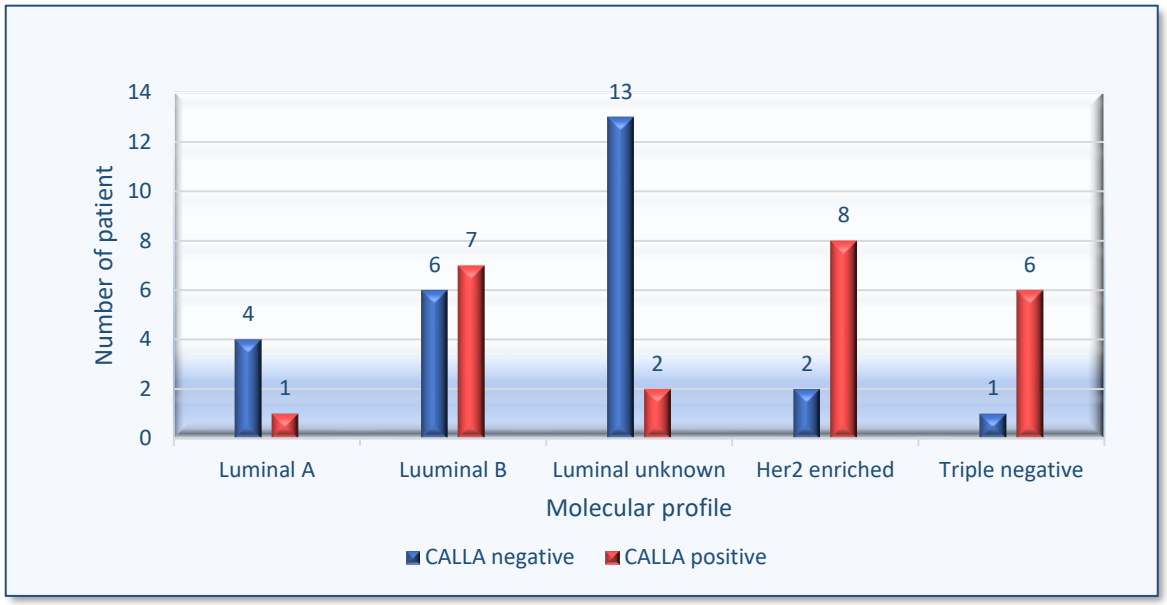


Figure 7: The correlation between molecular subtypes and CALLA expression, *P- value=0.016

3-5- The correlation response to neoadjuvant therapy and CALLA expression in each molecular subtype:

There is complete pathological responses in the patients with CALLA negative tumors in all luminal-A tumors 3 (100%), most Luminal-B cases 5 (55.6%) and luminal unclassified 11 (91.7%) with nonsignificant P value. In her-2/neo all cases 2 (40%) of negative CALLA expression showed complete pathological response while most of positive cases 3 (60%) achieved partial response, in cases with triple negative subtypes, the single case who didn't achieve any response to neoadjuvant therapy was CALLA positive, while all cases with positive CALLA expression showed jut partial pathological responce 4 (100 %) in compassion to 1(50%) achieved complete response. As showed in Figure 8, and Table 6.

Table 6: The correlation between molecular subtypes and responses to neo-adjuvant therapy with CALLA expression

Molecular subtypes	Response to NAT	Total	CALLA expression		P value
			CALLA negative No. (%)	CALLA positive No. (%)	
Luminal A	Partial	2	1 (50)	1 (50)	0.819
	Complete	3	3 (100)	0 (0)	
Luminal B	Partial	4	1 (25)	3 (75)	0.676
	Complete	9	5 (55.6)	4 (44.4)	
Luminal (unknown A or B)	Partial	3	2(66.7)	1 (33.3)	0.849
	Complete	12	11 (91.7)	1(8.3)	
HER2/neu rich	Partial	5	0	5 (100)	0.429
	Complete	5	2 (40)	3 (60)	
Trilep-negative	No response	1	0	1 (100)	0.537
	Partial	4	0	4 (100)	
	Complete	2	1 (50)	1 (50)	

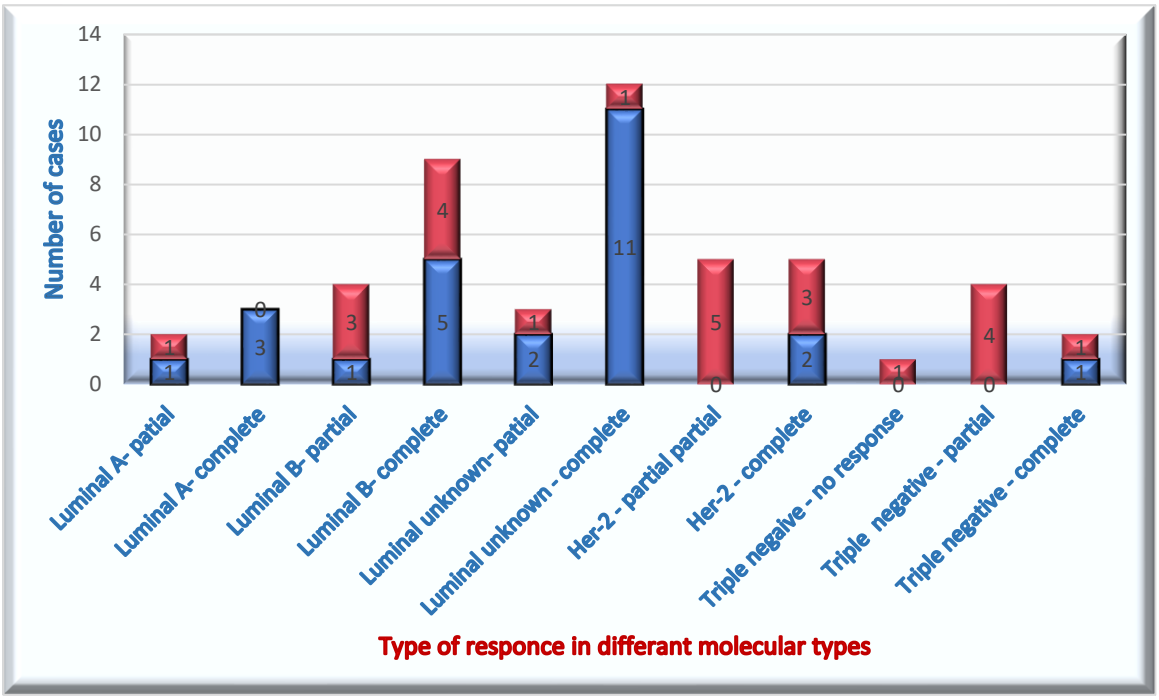
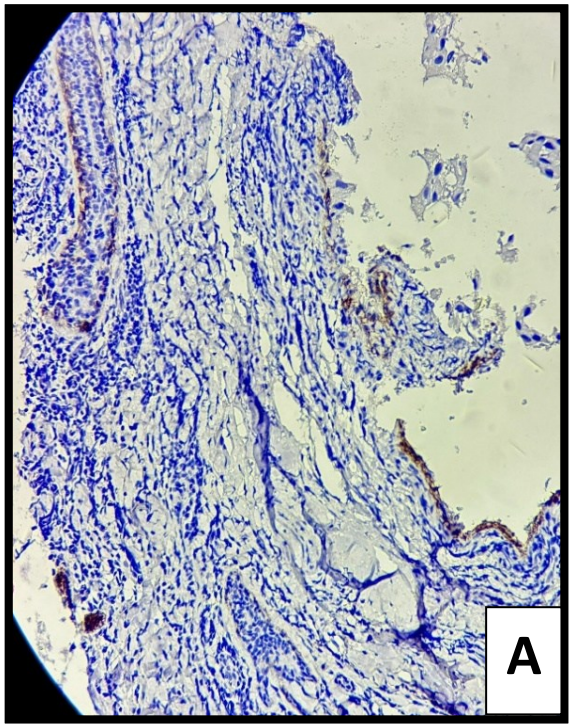
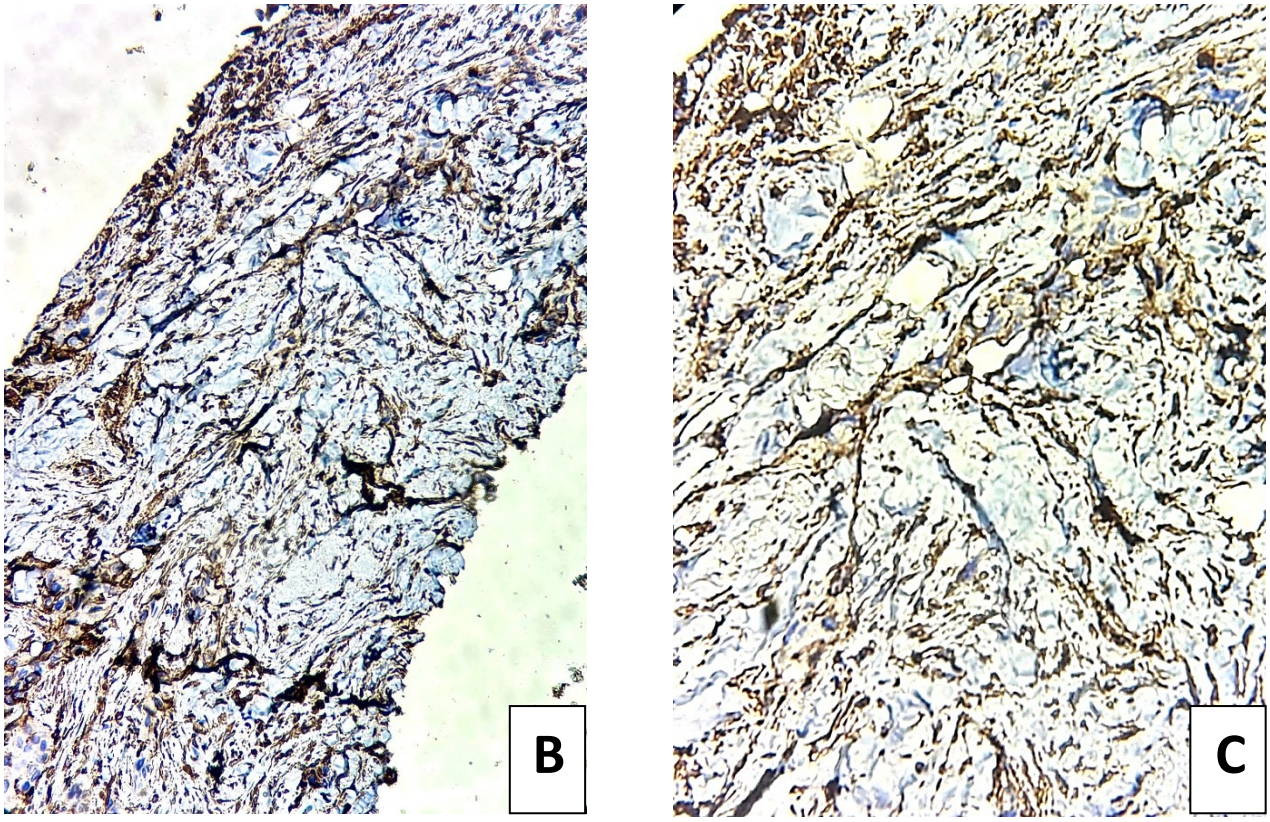


Figure 8: The association between molecular groups and response to neoadjuvant therapy with CALLA expression.



Figures 9: (A- magnification power is 10x) Histological section of CALLA negative immunohistochemistry in the struma (less than 10% of stroma is positive)



Figures 9: (B-magnification power is 20x C-magnification power is 40x) Histological sections of CALLA positive immunohistochemistry in the struma (More than 10% of stroma is positive)

Discussion:

Despite the fact that breast carcinoma is epithelial in origin still the stroma plays a role in carcinogenesis and prognosis, many articles revealed that stromal CALLA expression is associated with more aggressiveness and poor survival in patient with breast carcinomas. But the correlation between CALLA expression and response to neoadjuvant chemotherapy is not well established. So, the optimal integration of many important prognostic factors remains a researcher's field, our data included six parameters (age, grade, hormone receptor status, epidermal growth factor receptors (Her-2/neo), mitotic rate and molecular profiles, which have been accepted worldwide as prognostic parameters [15]. In the current study, we did immunohistochemistry of CALLA marker on 50 FFPE (Formalin Fixed Paraffin Embedded) tissue blocks and detect the staining microscopically for 50 patients with invasive ductal breast carcinomas. The cut-off score in previous studies for CALLA (CD10) was 10%, 20% or 30 % of the struma with or without assessment of intensity [10, 11, 16, 17, 18]. these cut off points were used to categorized the tumors into CALLA (CD10 negative CD10 positive); or categorized the CALLA-positive cases into weak &

strong positive subgroup. In this study we depend on the same basis with the first cut off point which is regarded as negative (CALLA < 10%), and ≥10% regarded as positive regardless the intensity of staining. Despite the CALLA expression had been suggested as important prognostic marker, its expressiion was very variable in previuos studies, it was ranged from 18% (20 /110) [17] to 49.50% (50 /101) [11] to 79.50% (205 /258) [10], while in our study the stromal CALLA expression was 48 % among females with IDCs (24/ 50 patient).

Patient and tumour characteristics:

In our study the average age of the patient was 49.52 ± 11 years 44% of them were < 50 years old. Which agree with the previous Iraqi studies of epidemiology. In a large cross sectional study included 1093 patient from all governorates of Iraq the median age was 46.40 ± 9.5 [19]. Alwaan et al in (2010), reported that out of 721 patients with breast carcinoma, 580 (80.40%) of them were younger than 60. (54.1%) of them were in the pre-menopausal age group and (22.20 %) were younger than 4o years old [20]. A recent Kurdistanian study for 429 patients with breast carcinoma revealed that the average age was 49.6 ±10 years and (53.80) of females were of middle age or younger [21]. According to WHO estimation, about 50% of malignancies in middle east area occur below the age of 55. As the exposure to the risk factors increase, the age- incidence rates of all malignancy in this area are projected to double [22]. This is differ the reports obtained from developed countries and western nations, which estimated that the peak incidences rates tend to occur decades later [23].

In the current study the majority of patients 37 (74%) had Grade-II breast carcinoma and about quarter of patients had higher grade disease. Similarity was illustrated by Abdulkareem et al in Iraqi local study [21], Nevertheless, American cancer statistics reported a higher prevalence of Grade-I breast carcinoma (21%) and Grade-III (29%) [24]. Enviromental and racial factors were established to affect tumour development and behaviour [25].

In this study the examined ER, PR, Her-2/neu and Ki-67 categorized the malignancies as, Luminal-A (10%), Luminal-B (26%), Her-2/neu enriched (20%), and lastly triple negative breast carcinoma (14%). However, there is (30%) of luminal groups categorized as luminal unclassified (Luminal A or B) because absent information about Ki-67. Studies that addressed the molecular classification of breast cancer in Iraqi patients are inconsistent, with variable variation in the Luminal-A and Luminal-B destribution [26,27,28].The range of Luminal-A group was between 29 % [28], 45 % [27], and 56 % [26] While the range of Luminal-B group was betweeen 14 % [27], 17 % [26], and 35 % [28]. All the Luminal cases in our study were 30 case (60 %) which is within the same range of previous publications. But the Her-2/neu rich cases relatively had higher rate when compered with other local and interaetional studies in which the range was 3 % to 10.30 % [24, 26-28].

Relation with CALLA expression:

In our study, <half of the cases (48%) were CALLA positive, a zinc-dependent metalloproteinase that is frequently detected on mature neutrophils, pro-B lymphoblasts, bone marrow lymphoid stem cells, renal cell carcinoma, endometrial stromal sarcoma, and pro-B lymphoblasts [17]. it has a role as a regulator of healthy mammary progenitor cells as well as in the degradation and remodeling of extracellular matrix components [29,30]. It has been established that this molecule is involved in the processes of cell adhesion, migration. It has been shown that tumors with CAFs expressing CALLA (CD10) were more aggressive with rapid progression and inferior prognosis [29]. In breast cancer, high CALLA expression has been associated with hormone receptor negativity and HER-2/neu overexpression. Furthermore, it has been observed that neoadjuvant anthracycline-based chemotherapy induces alterations in the dynamics of stromal CD10 expression [31].

There is discrepancy in CALLA expresion which may be partially due to variation in the charecterstics of each study group specially the compelling evidence that CALLA expression has a relation to age; the significant association with age may be unnoted in the current study due to smaller sample size[32]. CALLA positive cases were significantly correlated with higher tumor grade (grade-III), negative hormonal expression (ER and PR) and Her-2/neu overexpression. Which agree with other study that correlate with tumor grade such as Boler AK et. al. [32]. Regarding tumor phenotype, Puri V et. al (2011) and Diem Thi-Ngoc Vom, et al indicated, in agreement with our findings, a significant association of CALLA expression with low estrogen receptor and progesterone receptor expression, HER2 immunohistochemistry 3+ and also high Ki-67 [33, 34]

In comparison to CALLA negative, the positive cases were significantly less in Luminal-group including luminal unknown (unknown A or B) 23 case vs 10 (46% vs 20%) and more in Her2/neu enriched group (16.70 vs 83.30%). Additionally, 85.7% of triple-negative tumors were CALLA positive. A good deal of studies failed to demonstrate a statistical association between CALLA expression and molecular subtype. But there is scattered studies found a significant correlation between CALLA expression and molecular classification [35]. Tahani Louhichi et-al. (2018) demonstrated that CD10 expression usually associated with her-2 enriched and triple negative breast cancer [35]

CALLA expression and responses to neoadjuvant chemotherapy:

The current study reveals a significant correlation between CALLA expressed carcinoma and limited (partial) responses to NAC (neoadjuvant chemotherapy) in contrast to complete pathological response (P=0.011) in CALLA negative cases. The association between positive cases and partial pathological responce was significant in young patients < 50 years old (P value was 0.003), Her-2 expressed carcinomas (86.7%) (P value 0.042), and molecular subgroup (P = 0.016). this was agreed with A.K. Boler, et al (2021) who observed CALLA positivity was significantly associated with high-tumor grade (*P value < 001*) and odd ratio 26.00 [32], Sayantan H. Jana et-al

found a significant correlation between negative expresion of CALLA prior to NAC and a higher response in 375 Her2/neu negative cases. This significant relationship persist in Estrogen receptor negative subgroup [36] CD10 expression in the stroma was found to be significantly assosiated with higher grade of tumors (P value= 0.04), increased mitotic activity (P value- 0.33), worsened prognosis (Pvalue= 0.01), Estrogen Receptors negativity (P value= 0.0001), Her-2/neu positivity (P value =0.19) and finally with molecular classification (CD10 expressed in HER2/neu enriched subtype, and CD10 was negative in Luminal subtype) [37]

Limitations of the Study:

Small sample size was an issue in our study. Only representative blocks with known molecular profile were subjected to IHC; as a result, there may be some variation in CD10 expression because of the heterogeneity of the tumor. Lack of follow-up at current time which may be done later on to connect with progression-free survival and overall surviva

Conclusions:

1. CALLA expression can be regarded as adverse prognostic marker expressed in higher tumors grade, tumors with Her2/neu overexpression and tumors that don't express Hormones receptors (ER and PR negative breast carcinomas)
2. CALLA positive cases had been associated with partial response to neoadjuvant chemotherapy, while tumors that had negative CALLA expression achieved pathological complete response to NAC
3. Statistically significant association between CALLA expression and partial pathological response in patients who were younger than 50 years old
4. Consequently, we discovered that the molecular subtype is closely related with CALLA expression and concluded that CALLA expression was more frequent in Her2neu positive and triple negative cases compared to luminal A and luminal B subtype.

Recommendations:

Study the association between CALLA expression and progression-free survival and overall survival

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Bacteriophages: Molecular and Virologic Review Study

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Abstract

Earth's most common viruses, bacteriophages, infect bacteria and archaea. Protein capsids and sometimes lipid envelopes encase phage genomes, which vary in kind and structure. Most are double-stranded DNA phages, whereas *Cystoviridae* are RNA. Phages control bacterial populations, transfer horizontal genes, lyse molecules, and alter metabolism in different situations. Based on their structural morphotypes, which are largely determined by tail architecture, phages are classified as *Podoviridae* (short tails), *Siphoviridae* (long, flexible tails), and *Myoviridae* (long, contractile tails). They build genome transport and host recognition virions with portal proteins, tail fibers, and baseplates. DNA is transferred into capsids by ATP-dependent terminases during genome packing. The lysogenic cycle comprises temperate phages integrating into the host genome as prophages and replicating passively until stimulation activates the lytic phase. Phages with non-lytic chronic infections or carriers exist. Phages limit their host range by recognizing lipopolysaccharides, outer membrane proteins, teichoic acids, and flagella. They can make anti-CRISPR proteins to attack bacteria. Phages indirectly affect human health by influencing microbiota and immune systems. Immunoglobulin-like capsid domains connect them to mucosal surfaces for bacterial clearance and barrier protection. They can also enter tissues and circulation, where they are immunologically tolerated. Phages activate innate and adaptive immunity. Innate sensing and cytokine production are enabled by TLR3, TLR7, and TLR9. However, adaptive immunity produces neutralizing IgM, IgG, and IgA antibodies. Recurrent phage therapy can be reduced by phage-neutralizing antibodies. Phage biology is characterized by the discovery of "huge phages" with genomes that rival small bacterial genomes and encode complex functions like tRNAs, translation factors, CRISPR-Cas systems, and nucleus-like compartments that protect phage genomes from host defenses. Phage-encoded CRISPR-Cas systems often lack spacer acquisition or interference genes, resulting in host apparatus repurposing or gene transcriptional silence. Targeting competing phages and host regulatory mechanisms is possible. Phages treat antibiotic-resistant microorganisms. Phage treatment has potential despite bacterial resistance and host immune neutralization. They also carry toxin genes (*cholera, diphtheria*) through lysogenic conversion, boosting bacterial pathogenicity. Dynamic evolutionary arms races affect microbial ecology through phage-host and phage-phage interactions. Recent metagenomics and meta transcriptomics advances have increased the variety of dsRNA and tailless dsDNA phages. This showed unique viral families and the prevalence of phages in human microbiomes, particularly crAss-like phages in the intestine. The molecular details of phage interactions with eukaryotic cells

are still emerging, despite the vast knowledge of bacterial receptors for phage attachment. Mammalian cells can use endocytosis mechanisms to internalize phages for immunological regulation and therapeutic delivery. expanding the taxonomy of dsRNA phages, understanding non-lytic infection mechanisms, characterizing phage-host range and interactions, and using phages for biocontrol in agriculture and medicine. It is required to overcome immune clearance, understand phage immunogenicity, and understand the tri-kingdom interactions between phages, bacteria, and human hosts that maintain microbial and immunological homeostasis to maximize phage therapy. Bacteriophages are complex, diversified, and ecologically important viruses. Bacteria, horizontal gene transfer, immune system contact, and burgeoning biological uses including phage therapy and biotechnology are managed. Phages' complex life cycles, structural biology, and interactions with human and bacterial hosts are being understood beyond their role as bacterial predators. Briefly explain bacteriophages, which infect bacteria and archaea, their diversity, dsDNA and dsRNA phages, structure and replication mechanisms, ecological implications, mammalian immunity, and emerging therapeutic applications, including phage therapy. Phage viruses are assembled by complex structural proteins. Widely studied tailed phages including T7 (*Autographviridae*/*Podoviridae*-like), SPP1 (*Siphoviridae*-like), and T4 (*Myoviridae*-like) have tail and capsid shapes optimized for genome transport and host recognition Biomolecules such lipopolysaccharides and outer membrane proteins bind to baseplate and tail fibers. Genome packing motor ATPases assist phage DNA enter capsids. T4 phages employ the lytic cycle to rapidly multiply and lyse the host cell, while temperate phages use the lysogenic cycle to passively integrate their genome into the host chromosome until induction activates lytic replication. Infection-causing pseudolysogenic or persistent phages do not lyse quickly. In bacterial pathogen phage treatment, these life choices affect phage ecology and application. To identify hosts, phages recognize polysaccharides, proteins, and flagella. Phage host range is limited by specificity. To escape bacterial defenses, phages can encode anti-CRISPR proteins and adapt genomically. Phages transfer toxin genes and other virulence factors to bacteria by lysogenic conversion. Phages influence microbiomes and immunity without infecting humans. Phages can infect tissues and cells via endocytosis, altering immune responses. Phages destroy bacteria to fight antibiotic-resistant illnesses, but immunological responses including neutralizing antibodies can hinder phage treatment. Recent discoveries include "huge phages" with microscopic bacteria genomes. Complex machinery is provided by tRNA, translation factor, and CRISPR-Cas phages. Metagenomic studies have found tailless dsDNA phages from new viral orders and families, enhancing our understanding of phage diversity. CRISPR-mediated phage-host and phage-phage interactions show dynamic evolutionary arms races. Biomedical applications, gene transfer, microbial community dynamics, and bacterial population regulation depend on bacteriophages. Research on their structural biology, life cycles, and host immunological interactions determines their therapeutic and ecological potential. Bacteriophages are everywhere in the biosphere and influence bacterial ecology and evolution. DNA-containing bacteriophages in ecosystems are well-studied, whereas RNA-containing ones are often overlooked. **Keywords**: Bacteriophages, phages, Lytic cycle, Lysogenic cycle, Phage therapy, Phage immunogenicity, Microbiome, Anti-CRISPR proteins

Classification and Diversity:

The ICTV has classified more than 50 virus families of double-stranded DNA bacteriophages. The *Cystoviridae* is the sole family of dsRNA bacteriophages, consisting of seven species. In contrast to dsDNA phages, classified dsRNA phage isolates exhibit a restricted host range. Most phages are double-stranded DNA tailed (order *Caudovirales*), but double-stranded RNA (family *Cystoviridae*) and tailless phages also contribute to viral variety. New metagenomic research have revealed a great diversity of phages, including giant phages with massive genomes that encode complicated functionalities including tRNAs, translation factors, and CRISPR-Cas systems. Phylogenetic investigations of signature genes like RNA-dependent RNA polymerase and capsid protein structures are currently used more than morphology or host specificity to progress taxonomy.

In the present taxonomy, dsRNA viruses are classified as either *Pisuviricota* or *Duplornaviricota*. The majority of animal dsRNA viruses (e.g., rotavirus and bluetongue virus) and several significant microbial and plant viruses are members of the *Cystoviridae* virus family (class: *Vidaverviricetes*, order: *Mindivirales*) of the phylum *Duplornaviricota*. The phylum *Pisuviricota* comprises dsRNA viruses, including amalga-, curvula-, partiti-, and *picobirnaviruses*. This initial taxonomy classification of RNA viruses is based on a comprehensive sequence-based comparison of viral RdRp sequences [1]. Nevertheless, a recent phylogenetic analysis of RdRp sequences based on sequences revealed that cystoviral and dsRNA virus RdRps are classified in *Pisuviricota* [2]. Additionally, higher-order groupings were identified through a structure-based phylogenetic analysis of viral RdRps [3].

The *Cystoviridae* family was recognized by the ICTV in 1978 [4], and the phi6 group and phage phi6 were recognized in 1976 [5]. At present, the Cystoviridae virus family comprises a single genus, Cystovirus (formerly known as the phi6 group), which comprises seven virus species: phi6, phi8, phi12, phi13, phiNN, phi2954, and phiYY. Due to their unique similarities in virion structures and genomes, these phages were classified together, despite their low nucleotide sequence identity (<50%, with the exception of phi6 and phiNN; [6]. The current criteria for identifying *Cystoviridae* species are 95% nucleotide sequence identity. The Cystoviridae family comprises six viruses that remain unidentified: phi7, phi9–phi11, phi14, and phiNY. phiZ98 and CAP3-7 may also be included in the *Cystoviridae* family due to their genetic and structural similarities [7,8]. The ICTV has recently introduced supplementary higher-order ranks for virus taxonomic classification and is in the process of transitioning to a system that more accurately reflects virus phylogenetic relationships than host specificity, morphological features, or disease symptoms [9]. The polymerase gene, which encodes RdRp or reverse transcriptase, is a critical locus for the classification of RNA viruses and phylogenetic analysis. RdRp-encoding RNA viruses are found in the *Orthornavirae* kingdom of the Riboviria domain. *Duplornaviricota*, *Kitrinoviricota*, *Lenarviricota*, *Negarnaviricota*, and *Pisuviricota* are the five official phyla of *Orthornavirae* [10].

In the recently adopted megataxonomy of viruses, double-stranded DNA phages are divided into two huge realms: *Duplodnaviria* and *Varidnaviria* [11]. Viral structures in these realms include MCP and packing ATPases. Archaeal, tailed, and herpesviruses are *duplodnaviria*. The

phylum *Nucleocytoviricota*, which comprises mimiviruses, tailless bacteriophages, and related archaeal viruses, is included in *Varidnaviria*.

In cultivation, tailed *Duplodnaviria* bacteriophages are the most prevalent. The upper oceans are dominated by non-tailed varidnaviruses, which account for 50% to 90% of viral particles, according to metagenomic investigations [121,13]. Varidnaviria's icosahedral capsids are composed of the double jelly-roll (DJR) MCP [14,15]. The DJR MCP core structure is conserved across the entire spectrum of *Varidnaviria* members that infect hosts from all three domains of life, despite the fact that some viruses are difficult to recognize and the sequences are highly varied [16,17]. By employing sensitive profile-based methods to search metagenomic sequence data for DJR MCP contigs, an unexpectedly extensive array of varidnaviruses was identified, which are likely to infect bacterial and archaeal hosts. This diversity surpassed that of prokaryote viruses in the families *Turriviridae*, *Tectiviridae*, *Corticoviridae*, and *Autolykiviridae* in the class *Tectiliviricetes* [18]. In the 'PM2-like' category, which included several prophages, tailless phages were most prevalent. This group includes two *Corticoviridae* phages, PM2 [19] and Cr39582 [20], eleven Vibrio phages of the Autolykiviridae family, and the unassigned f No16 phage [21], which infect *Pseudoalteromonas* species. *Vinavirales* includes *Corticoviridae* and *Autolykiviridae* [22]. Most phages in this group have 9-12 kb genomes that are circular (*Corticoviridae*) or have inverted terminal repeats. Vinavirales viruses encode structural components and genes that govern transcription, genome replication, and cell lysis. Proviruses from Vinavirales members are extensively incorporated into aquatic bacteria genomes. Their occurrence in other habitats is unknown. DJR MCP and packing ATPase are the only Vinavirales-shared proteins [23]. Metagenomics has been used to study the human intestine virome, a major microbiome factor. Most identified viruses are *Duplodnaviria* domain tailed bacteriophages [24,25,26]. The most common human-associated viruses are crAss-like phages, which the ICTV recently categorized into the Crassvirales order. These phages were discovered by metagenomics investigation and have a wide variety of very varied members. Gut metagenomics has found many new abundant-tailed bacteriophage families [27,28] *Varidnaviria* members are likely a minor component of the gastrointestinal virome and are not well-known. Study conducted a search of 23,119 gut metagenomes for DJR MCP and discovered that a significant number of the sequences encoding this hallmark protein of *Varidnaviria* are associated with prophages that are distantly related to the families *Corticoviridae* and *Autolykiviridae*. These prophages suggest a new Vinavirales order with at least three families. It found an extended, conserved gene core of Vinavirales with 12 genes, one of which encodes a hitherto undiscovered lysin protein, using sensitive protein structure prediction and sequence analysis methods. [29,30.31]

Structure and composition:

Unlike cells, phages viruses that infect bacteria cannot perform most biological processes needed for reproduction. They modify ecosystems by preying on certain bacterial populations, mediating lateral gene transfer, altering host metabolism, and redistributing bacterial chemicals by cell lysis [23,33,34,35,26]. They spread antibiotic resistance and pathogenic agents that infect humans

and animals [37,38]. Our knowledge of phages comes from laboratory studies, most of which had genomes a few tens of kb. Many isolation methods remove big phage particles from phage concentrations obtained by 100-nm or 200-nm filters. Only 93 isolated phages with genomes over 200 kb were published in 2017 [32].

Community-wide DNA sequencing is capable of detecting phage-derived fragments; however, fragmentation can obscure large genomes. A novel clade of megaphages associated with humans and animals was identified in the genomes of metagenomic datasets that were manually curated [39]. In order to ascertain the prevalence, diversity, and environmental distribution of large-genome phages, we implemented a more comprehensive investigation of microbial communities. In the past, phages with genomes over 200 kb were called "jumbophages" or megaphages [40]. These phages have evolved a unique "life" strategy that entails substantial host biology interception and enhancement while replicating their massive genomes. [41,42,43].[44] were able to reconstruct 351 phages, 6 plasmid-like, and 4 unclassified sequences. It exclusively retained CRISPR–Cas loci and excluded plasmids. We incorporated three phage sequences that were ≤200 kb in length as a result of the CRISPR-Cas loci. As anticipated, we identified numerous phage-relevant genes, such as those that are involved in the encoding of structural proteins and lysis, as well as other genomic properties of phages. It was anticipated that certain structural proteins would be as long as 7,694 amino acids. 175 phage sequences were circularized, and 35 were manually curated to completion, occasionally by resolving complex repetition sections to disclose encoded proteins. Although the majority of genomes are incomplete, a few may be complete but linear. Bidirectional replication is indicated by the GC asymmetry of 30% of genomes, while unidirectional replication is indicated by 30% of genomes [45]. The largest phage genomes known are our 4 largest complete, carefully curated, and circularized genomes, 634, 636, 642, and 735 kb. The largest circularized phage genome known was 596 kb [46]. The prior work reported a 630-kb circularized genome, but it was an assembly artifact. Concatenation artefacts were so severe in IMG/VR [47] that we excluded these data from future analyses. We revised our phage genome size distribution using full and circularized genomes from our study and published genomes. Without the enormous phages mentioned here, entire phages had median genomic sizes of 52 kb. Thus, these sequences greatly increase the number of phages with extremely large genomes. [44]

Nine of our genomes have coding densities that are less than 78%, which may be attributed to a genetic code that deviates from the standard code. This effect is uncommon in phages; however, it has been observed in Lak phage. The UAG stop codon appears to have been reassigned to encode an amino acid in numerous genomes, primarily human and animal. By transitioning into a flanking bacterial genome sequence, only one region exceeding 200 kb was identified as a prophage. Nevertheless, prophage integration is feasible, as half of the genomes were not circularized. Genomes containing integrases suggest a temperate lifestyle in certain circumstances. [48] There are significant structural differences within these groupings, especially in tail tips and capsid diameters. Since the tail tube is isolated, this knowledge is enough to place the main tail protein in its structural context. Capsid, fiber, and baseplate proteins will also be skipped.

SPP1 is a phage of the tail-morphotype that is similar to *Siphoviridae*. The dodecameric portal protein gp6 is located on one vertex of the icosahedral procapsid of the SPP1 phage. The

maturation of procapsid into capsid is facilitated by the translocation of viral DNA into the head and the release of Gp6 [49,50]. ATP is utilized by the packaging terminase to facilitate the passage of DNA through the portal complex. The hexameric ring–shaped adaptor protein complex gp15 and stopper protein complex gp16 attach to the portal complex after capsid formation to limit DNA leakage, resulting in the formation of the head-to-tail connector [51]. The tail of SPP1 is dependent on the hexameric ring-shaped distal tail protein (Dit) gp19.1. The distal tail adsorption function and the tail tube [52] are connected by this junction. The length of the tail tube [53] is determined by 40 stacked hexameric rings of MTP gp17.1 and its C-terminally extended form gp17.1∗, which are constructed around a tape measure protein gp8, which may be trimeric. A ribosomal frameshift leads to the C-terminal expansion of gp17.1∗, which contains an immunoglobulin (Ig) fold and a fibronectin type III (FN3) domain. The tail tube is composed of gp17.1 and gp17.1∗ in a 3:1 ratio. Nevertheless, virions that contain only gp17.1 are infectious [54]. The FN3 domain may transiently interact with *Bacillus subtilis* cell wall carbohydrates, thereby increasing infection, during two-dimensional diffusion of virions on the outer bacterial surface. The tail tube top is tapered by a hexameric ring of the tail completion protein gp17, which binds to the stopper protein gp16 and connects the tail to the head-to-tail connector. The trimeric tail tip protein gp21, as well as potentially gp22, gp23, gp23.1, and gp24, are involved in the distal tail adsorption function [55]. The positions of these proteins are ambiguous. The YueB receptor ectodomain of Bacillus subtilis is irreversibly bound by gp21 or a protein, resulting in infection. The formation of the tail in λ phages commences with an initiator complex that includes tape measure protein, assembly chaperones, distal tail protein, baseplate proteins, and tail tip proteins. This combination enables MTP to polymerize onto the distal tail protein ring and tape measure protein, resulting in the formation of a helical tube that replaces assembly chaperones. When the tail tube completely engulfs the tape measure protein, MTP oligomerization concludes. The tail completion protein will truncate the tail and connect it to the head-to-tail connector to complete the virion [56].

In contrast, SPP1 phage MTP (gp17.1) can self-polymerize without protein [57]. The tail creation process may differ. Dissociating the tail tip after binding to the YueB receptor primes the release of the metastable tape measure protein from the tail tube. Next, the stopper protein gp16 opens diaphragm-like and ejects DNA into the tail tube to start infection [58]. Tape measure proteins may produce host membrane pores for DNA translocation [59]. T4 phage is a *Myoviridae*-like tail-morphotype phage. This phage has a more complicated head assembly than the others: The membrane-spanning initiation complex of the dodecameric portal protein gp20 and gp40 binds 11 scaffolding proteins to start procapsid assembly [60]. The prohead's fivefold vertices in the capsid shell are produced by the major capsid protein gp23 and the vertex protein gp24. Procapsids are released from the membrane and space is created for DNA by proteolytic cleavage of the scaffolding and capsid proteins [61]. The ATP-dependent terminase translocates DNA through the portal complex and expands the procapsid into the final capsid. This process creates binding sites for the small outer capsid protein (gp soc) and the highly antigenic outer capsid protein (gp hoc) that decorate the capsid [62]. Icosahedral extremities and cylindrical equatorial central regions are features of the T4 phage capsid. The head is completed and the portal complex is sealed by the

binding of the Gp13–gp14 neck complex. The six segments of the baseplate are arranged around a tube [63] to initiate the tail assembly.

The junction for tail tube polymerization [64] at the proximal end of the baseplate is composed of hexameric rings of gp48 and gp54, while the tail tip at the distal end is composed of gp27, gp5, and gp5.4. The trimeric proteins gp5 and gp5.4 puncture host membranes and hydrolyze host peptidoglycan, while gp27 is capable of passing DNA. To create the tail tube, the MTP gp19 polymerizes into 24 hexameric rings around the baseplate-anchored tape measure protein gp29 [65]. The tail tube terminator protein gp3 [66] taper the proximal end of the tail tube. Gp18, the sheath protein, undergoes helical polymerization around the tail tube [67]. Gp15 completes the tail by binding to gp3 and the terminal ring of the tail sheath. The virion is completed by the interaction between gp15 and (gp13–gp14), which connects the tail and head-to-tail connector. T4 is equipped with a head whisker at the head-to-tail connector and short and long tail fibers at the baseplate. The short tail fibers unwind from beneath the baseplate and irreversibly bond to LPS, anchoring the baseplate to the outer membrane, when *E. coli's* long tail fibers bind to LPSs and OmpC of the outer membrane [68]. The tail sheath is constricted by the baseplate's reorganizations, which compel the tail tip to pass through the outer membrane. This process digests the peptidoglycan layer and translocates the tape measure protein gp29 and viral DNA through the tail tube. An inner membrane pore may be generated by the tape measure protein gp29 and/or gp27, which enables DNA to enter the host cytoplasm [69]. Phage virions have complicated designs for host recognition, genome packaging, and genome delivery, with tail morphologies resembling *Podoviridae*, *Siphoviridae*, and *Myoviridae* families. Mechanisms of phage assembly and infection reveal coordinated protein interactions and ATP-driven DNA packaging motors. Unique RNA bacteriophages with lipid envelopes and multilayered capsids, enveloped dsRNA phages (*Cystoviridae*) share structural similarities with eukaryotic dsRNA viruses, suggesting evolutionary linkages.

Currently, phi6 and phi12 are the best-characterized dsRNA phages, their component proteins, and assembly intermediates. However, similar gene sets in known dsRNA phage isolates suggest a similar virion organization. However, new studies show structural variance, especially in host recognition and entrance components. Phi6, phi8, phi12, phi2954, phiNN, phiYY, phiNY, phiZ98, and CAP3 have enveloped spherical virions in negative-stain transmission electron microscopy (TEM) [70,71], cryo-EM, and cryo-ET [72]. Detergent or organic solvent sensitivity studies have shown that dsRNA phage virion lipids exist.

These are the sole membrane-enveloped RNA bacteriophages that are currently known. phi6 and phi8 virions exhibited envelope spikes of 2 and 7 nm following cryo-EM characterization. These structures are host attachment spikes produced by multimeric P3 complexes, as indicated by prior research on P3-deficient phi6 virions [73]. Subsequently, cryo-ET and three-dimensional reconstruction revealed toroidal or elongated structures on phi12 and phi2954 virions. By employing the S and L segments from phi12 and the M segment from phi2954, a recombinant phi12 phage was generated through reverse genetic technique. The recombinant phage exhibited host specificity and envelope surface features similar to those of phi2954 [74].

By dsRNA phage envelopes, icosahedrally symmetric nucleocapsids are enclosed. The nucleocapsid surface shell and polymerase complex were identified in these particles in the initial

EM investigation on phi6 virions [75]. The protein P8 trimer nucleocapsid shell is arranged into an incomplete icosahedral T = 13 lattice, which is interrupted at the five-fold symmetry positions by P4 complexes protruding from the polymerase complex layer, as demonstrated by high-resolution cryo-EM imaging and three-dimensional reconstruction analyses of phages phi6 and phi12 [76]. The cystovirus phi8 deviates from this fundamental structure by lacking a nucleocapsid shell. Phi6 bonds to and penetrates the host plasma membrane through the P8 layer [77]. This suggests that polymerase complex components are responsible for these critical responsibilities, as purified phi8 core particles have the capacity to infect spheroplasts of host cells [78]. Additional research is necessary to ascertain the presence of a nucleocapsid surface shell that enables plasma membrane penetration in other dsRNA phages that are similar. The phi6 polymerase complex, which consists of the proteins P1, P2, P4, and P7, replicates and transcribes the viral dsRNA. Cryo-EM has identified dimers of the primary inner capsid protein (MCP) P1 on an icosahedral T = 1 lattice in the empty and/or genome-containing polymerase complex particles of phi6, phi8, and phi12 [79]. I find it intriguing that the capsid organization of picobirnaviruses, *Reovirales* order animal and plant dsRNA viruses, and members of the families *Partitiviridae*, *Totiviridae*, *Chrysoviridae*, *Quadriviridae*, and *Megabirnaviridae* is similar (referred to as "T = 2") [80,81]. This organization is not present in any other viruses. This suggests that dsRNA viral capsids have a shared ancestry.

The P2 structure of the phi6 polymerase subunit was described by Butcher et al. in 2001. The first high-resolution structure of RNA-dependent RNA polymerase (RdRp) from dsRNA virus. The phi6 P2 structure was found to be remarkably similar to the RdRp of the hepatitis C virus, indicating that the polymerase subunit of a dsRNA phage and a eukaryotic positive-sense single-stranded RNA virus may have shared a common evolutionary origin. Several high-resolution structures for additional viral RdRps have been deposited in the protein data bank over the past two decades. A structure-based computational comparison has demonstrated that the phi6 and phi12 RdRps [82] are structurally similar to all currently structurally characterized viral RdRps [83].

Protein P4 is one of the most extensively studied cystovirus proteins in terms of its physical and functional characteristics. P4, a molecular motor that organizes viral single-stranded genomic precursor molecules into empty polymerase complexes, is powered by NTP hydrolysis. The structures of the P4 proteins of phi6, phi8, phi12, phi13, and phiYY are of high resolution [84]. Despite structural differences, these proteins form hexameric complexes that resemble RecA-type ATPases. A genome with three dsRNA segments: L (large, 6.3–7.1 kb), M (medium, 3.6–4.7 kb), and S is present in each *Cystoviridae* family member and related dsRNA phage isolate. The genome is in the range of 12.7–15.0 kb (phi2954–phi8). Comparative genomic analyses indicate that certain dsRNA phage isolates are closely associated with *Pseudomonas* phage phi6, while others are distant. The L, M, and S segments of *Pseudomonas* phages phiNN and phi6 exhibit 80%, 55%, and 84% nucleotide sequence identity, respectively. However, phiNY does not appear to share any similarities with other cystoviruses or phages.

The genome organizations of the six dsRNA phage isolates CAP3–7 and phiZ98, as well as the currently recognized cystoviruses, are comparable. The L segment encodes proteins for the polymerase complex (MCP P1, RdRp P2, packaging NTPase P4, and assembly factor P7), the M segment encodes proteins for host recognition and outer membrane penetration (P3 and P6), and

the S segment encodes the nucleocapsid shell protein (P8), major membrane protein (P9), putative membrane morphogenetic factor (non-structural protein P12), and the RNA polymerase. In contrast, the majority of the open reading frames that are expected to be generated by phiNY contain proteins of unknown function, and only the RdRp and MCP genes in the L segment and the glycoside hydrolase gene in the S segment could be predicted, necessitating further investigation. Nevertheless, the coding regions are flanked by non-coding sections in all dsRNA phage genome segments. According to phi6 research, these non-coding regions are essential for genome packaging and replication. [85,86]. Despite their high gene synteny, certain cystoviruses possess open reading frames with ambiguous functions. The P3 host recognition spike complex contains a single P3 protein or its multimer in the spike complex of cystoviruses phi6, phi2954, and phiNN [87]. In contrast, the heteromeric spike complex of phi8, phi12, phi13, and phiYY contains two or three viral proteins [88]. Recent metatranscriptomic surveys indicate that dsRNA phages have developed a variety of lytic enzymes. Cystoviridae members encode lytic transglycosylases from the lysozyme superfamily. However, some cystovirus-like contigs encode putative N-acetylmuramoyl-L-alanine amidase, metallopeptidase (families M15 and M23), lipase, and L-alanyl-D-glutamate endopeptidase genes. The variety of lysis genes in these potential dsRNA phages may suggest a broader host range. [89]

Phage Replication Cycles and Infection Strategies:

Bacteriophages undergo lytic or lysogenous cycles. Furthermore, certain phages are pseudolysogenic. Proteins that are anticipated to locate the bacterial membrane or cell surface are encoded by the phage genomes. These factors may influence the susceptibility of the host to phage infection. Almost all of the host metabolism-enhancing genes that were previously identified were discovered. Numerous phages possess genes that synthesize purines and pyrimidines de novo and convert nucleic and ribonucleic acids and nucleotide phosphorylation states. These gene sets are reminiscent of microorganisms that are remarkably minuscule and likely have symbiotic relationships. Numerous phages contain transcription and translation genes. The unique sequences of up to 67 tRNAs are encoded by complete phage genomes. In general, the number of tRNAs increases as the genome length increases (Spearman's $\rho = 0.61$, $P = 4.5 \times 10{-}22$, $n = 201$). Each genome of large phages contains up to 15 tRNA synthetases that are distinct but related to those of their hosts. Via these proteins, phages can charge their tRNA variations with host amino acids. Genes for tRNA modification and ligation of host defense-cleaved tRNAs are present in certain genomes.

Genes that intercept and redirect host translation are present in numerous phages. Among these genes are the ribosomal proteins S4, S1, S21, and L7/L12, as well as the initiation factors IF1 and IF3. Ribosomes were recently discovered in phages. rpS1 and rpS21 are indispensable for the initiation of translation in bacteria, which renders them advantageous for the hijacking of host ribosomes. Additional investigation of rpS21 proteins revealed N-terminal extensions that contain RNA-binding basic and aromatic residues. The anticipation of that these phage ribosomal proteins will supplant host proteins and promote competitive ribosome binding or preferential phage mRNA

initiation. Lytic phages, such as T4, lyse and eradicate bacterial cells following virion multiplication. Phage offspring are capable of infecting other organisms following the cell's death. Lytic phages are more effective for therapeutic purposes. Some lytic phages prevent phage progeny from lysing out of the cell when extracellular phage concentrations are high.

This process differs from temperate phage dormancy and is usually transient. [90] The lysogenic cycle does not immediately lyse the host cell. Lysogeny-capable phages are temperate. Their viral genome integrates with host DNA and replicates harmlessly or becomes a plasmid. Endogenous phages (prophages) activate when host circumstances decrease, such as nutritional deprivation. The host cell lyses when they start reproducing. The lysogenic cycle permits the host cell to live and proliferate, replicating the virus in all offspring. *E. coli's* lambda phage follows the lysogenic and lytic cycles.[91] Prophages can help the host bacterium by adding additional functionalities to the genome during dormancy, called lysogenic conversion. Bacteriophages can turn safe strains of *Corynebacterium diphtheriae* or *Vibrio cholerae* into highly virulent ones that cause diphtheria or cholera. [92] There are ways to fight certain bacterial infections by targeting toxin-encoding prophages. [93] The viruses in this electron micrograph of bacteriophages adhering to a bacterial cell are coliphage-sized. T1

Bacterial cells' polysaccharide cell walls shield them against antibiotics and immunological host defenses. [94] Bacteriophages bind to lipopolysaccharides, teichoic acids, proteins, or flagella on bacteria to enter a host cell. Bacteriophages can only infect bacteria with receptors they can bind to, limiting their host range. Virion-associated polysaccharide-degrading enzymes breakdown their hosts' capsular outer coat during the start of a tightly regulated phage infection. Host growth parameters affect phage attachment and invasion. Phage virions cannot move autonomously, thus they must randomly contact the right receptors in solution, such as blood, lymphatic circulation, irrigation, soil water, etc. Myovirus bacteriophages inject genetic material into cells using a hypodermic syringe. The tail fibers flex to bring the base plate closer to the cell after hitting the receptor. This is reversible binding. Once fully joined, irreversible binding begins and the tail contracts, presumably with ATP, shooting genetic material through the bacterial membrane. [95] The shaft bends to the side, contracts closer to the cell, and pushes up to inject. Unlike myoviruses, podoviruses use their short, tooth-like tail fibers to enzymatically destroy a section of the cell membrane before introducing their genetic material.

In minutes, bacterial ribosomes convert viral mRNA into protein. Early RNA replicase synthesis occurs in RNA-based phages. Proteins make bacterial RNA polymerase prefer viral mRNA. The host must produce viral products instead of proteins and nucleic acids due to disruptions. Ribosomal proteins in some dsDNA bacteriophages may influence protein translation during infection. [96] T4 phage morphogenesis requires catalytic helper proteins to build new virus particles. The foundation plates are produced first, then the tails. Separately built head capsids will spontaneously assemble with tails. Phage genes encode morphogenetic proteins that interact in a specific sequence during phage T4 virion formation. Normal phage T4 morphogenesis requires a balance in these proteins produced during viral infection. DNA is efficiently packed in heads. The entire process takes 15 minutes.

studies of bacteriophage T4 (1962–1964) revealed almost all of the genes required for laboratory development. [97] Two groups of conditional lethal mutations enabled these experiments. Amber mutants were one type. Temperature-sensitive mutants were another type of conditional lethal mutant. These two groups of mutants revealed the activities and connections of proteins involved in DNA replication, repair, and recombination and how viruses are built from protein and nucleic acid components. Sometimes phages are released via cell lysis, extrusion, or budding. Tailed phages lyse by breaking down cell wall peptidoglycan with endolysin. Filamentous phages cause host cells to release new virus particles. Free virions can infect a new bacterium unless faulty. Budding is linked to Mycoplasma phages. Unlike virion release, lysogenic phages become prophages and stay in the host. [98]

The majority of cystovirus isolates are virulent and lyse the bacterial cells of their host during reproduction. Certain dsRNA phages have the ability to produce viral particles within the host cell without causing lysis or integrating into the host chromosome. This single-cell phenomenon be referred to as a non-productive chronic infection or carrier cell in order to differentiate it from the carrier state life cycle, which should be allocated to population-level phage-host interactions. In cell cultures that are continuously evolving, cystovirus carrier cells are identified by intracellular viral genomic dsRNA molecules or viral particles. phi6 and its *P. syringae* host were first reported to infect this way, but the phi13 variant (containing a kanamycin resistance gene) has also been found to infect Salmonella typhimurium and *E. coli*. Recently, dsRNA phage phiNY was shown to infect similarly. The continuous phiNY infection boosted Microvirgula aerodenitrificans host growth, suggesting a mutualistic, parasitic lifestyle. Interestingly, dsRNA phages persist like fungus dsRNA viruses, which do not create external infectious virions and are mostly cryptic.

Bacteriophages are essential to the human microbiome and mediate genetic exchange between pathogenic and nonpathogenic bacteria, even though they cannot infect and multiply in human cells. Transduction occurs when a bacteriophage transfers genes from one bacterial strain to another. Endocytosis pathways are key to eukaryotic cell bacteriophage uptake. Phages cannot multiply in eukaryotic cells but can be absorbed through cellular processes similar to those utilized for other particles or viruses: Phages can be taken up by clathrin-mediated, caveolin-dependent, or macropinocytosis from eukaryotic cells. Phages can enter vesicles and pass epithelial barriers via these internalization mechanisms [99]. Receptor Binding: Polysialic acid-binding E. coli phages can enter neuroblastoma cells by interacting with cell surface chemicals like polysialic acids [100]. After endocytosis, some phages can cross human epithelial cells to reach underlying tissues via intracellular trafficking processes that converge on recycling endosomes [99]. Phage Display and Cell Penetration Peptides: Engineered phages with TAT (transactivator of transcription) peptides increase membrane translocation and phage entry into various eukaryotic cells [101]. Phage uptake by eukaryotic cells is researched, revealing different methods by which phages can interact with and penetrate human cells, modulating immune responses and providing therapeutic benefits.

Recent experiments have illuminated how eukaryotic cells ingest bacteriophages: A 2023 study found that mammalian cells ingest bacteriophage T4 mostly by macropinocytosis, which engulfs extracellular fluid and particles. Intact phages accumulate in macropinosomes and go

through endosomal pathways such lysosomal destruction or exocytosis. Importantly, internalized phages induced protein phosphorylation cascades that promoted cell metabolism and survival without activating inflammatory DNA-sensing immune pathways Phages promote cellular development and metabolism, which has implications for phage therapy and microbiome interactions [102]. Another experiment evaluated neuroblastoma cell internalization of an Escherichia coli bacteriophage (PK1A2) that binds to polysialic acid. Endolysosomal internalization of the phage-receptor complex was shown by fluorescence and electron microscopy. Phages remained intracellular for 24 hours. Phages were redistributed to vesicular structures surrounding the nucleus, confirming receptor-mediated endocytosis as a significant uptake mechanism [103]. Filamentous M13 phages with cell-penetrating peptides like TAT were shown to increase endocytosis and intracellular trafficking in mammalian cells. Phage surface changes promoted cellular penetration and therapeutic administration in this experiment [103]. Active cellular mechanisms such macropinocytosis and receptor-mediated endocytosis are key to human cell phage uptake, according to this research. They show endosomal phage trafficking and putative cellular signaling responses from internalized phages.

Some of the molecular receptors for bacteriophage binding on eukaryotic cells have been experimentally characterized: Polysilic acid: A well-known receptor on human neuroblastoma cells that binds and internalizes Escherichia coli phages. This sialic acid polymer aids receptor-mediated phage endocytosis into eukaryotic cells [105]. Sugars and glycans: Though less well-defined than bacterial phage receptors, eukaryotic cell surface carbohydrate moieties can bind phages [106]. Peptides that penetrate cells: Engineered peptides on phages like TAT interact with negatively charged membrane components to increase mammalian cell uptake, suggesting membrane phospholipid or proteoglycan components may help bind [107]. Many eukaryotic phage receptors' molecular identities are unknown. In contrast, bacterial phage receptors typically contain outer membrane proteins, lipopolysaccharides, teichoic acids, and polysaccharides that have been extensively studied in numerous phage-host systems [108]. Thus, polysialic acid and certain surface glycans are major bacteriophage receptors for eukaryotic cells, while cell-penetrating peptide-targeted membrane components offer tailored uptake options.

Lytic cycles with fast host cell lysis or lysogenic cycles integrating their genome into host chromosomes allow persistence and horizontal gene transfer. Some dsRNA phages cause chronic non-lytic host cell infections. Tail fibers or spikes that identify lipopolysaccharides or proteins bind to bacterial cells specifically. Phage infection involves anti-CRISPR proteins and nucleus-like compartments that protect phage DNA to avoid host resistance.

Horizontal Gene Transfer, Phages:

Phages transfer horizontal genes by universal and specialized transduction, affecting bacterial virulence, antibiotic resistance, and evolution. Lysogenic conversion via prophages encodes toxins and other virulence factors, causing bacterial disease.

Transduction:

As the host cell disintegrates from lytic replication, generalized transduction bundles random bacterial genomic DNA in phage capsids instead of phage genomic DNA. The bacterium's genome and that of its progeny cells may be altered if this phage injects this bacterial DNA into a healthy host cell and integrates into the bacterium's chromosome. When initiating a lytic replication cycle in specialized transduction, lysogenic phages that have been amplified in a population of bacteria may eliminate a portion of the bacterial DNA with their genome. Lysogens share an integration site, which is why all progeny phages transfer the same bacterial gene to their hosts.

In addition to genetic exchange, bacteriophages can affect microbial populations by preying on select bacteria species while ignoring others. This characteristic has been studied to treat pathogenic bacterial infections in humans and animals for over 100 years. Wild phages may temporarily affect wild bacterial populations, [109], but lytic bacteriophages as antimicrobial therapy (phage therapy) in humans face significant challenges. Various wild bacterial strains resist phages. Famous resistance mechanisms include the CRISPR-Cas9 system, which was created for genetic manipulation in the lab and started as a bacterial defensive mechanism against bacteriophage invasion. [110] Phages are more immunogenic than antimicrobials and removed from the blood by the reticular endothelial system quickly. If effective phage mixtures are developed, their size may limit their topical use compared to antimicrobials. Some researchers recommend using phage enzymes, which can permeate bacterial cell walls, for simplicity. [111] No randomized, controlled, double-blind trials have proved either technique works in humans.

Host Specificity and Phage-Host Interactions

Bacteriophage-human cell interactions are a new field of study that combines microbiology and immunology and reveals exciting intricacies beyond basic bacteria-phage dynamics. The gut is full of phages, viruses that infect bacteria, which regulate bacterial ecosystems and immunological activities. Phages can directly interact with human cells, affecting immunological responses, inflammation, and tissue homeostasis, according to recent studies. Phages seldom infect humans, although they can adhere to eukaryotic cells and be internalized, influencing cell signaling pathways and immunological activation.

Besides altering microbiota, phages interact with human cells in therapeutic ways. Phages can infiltrate human macrophages and epithelial cells and give antibiotic effects to intracellular microorganisms. This intracellular trip of phages implies a sophisticated way they can manage bacterial illnesses inaccessible to some drugs. Phages also interact with receptors on human immune cells to control innate immune responses and shape inflammatory and antiviral defenses. Understanding that phages' interactions with human cells rely on the type of phage, the bacterial environment, and the host's immunological condition is crucial. Some phages have developed unique tactics to avoid human immune detection or alter host cells to help their bacterial hosts survive. Phages are not human pathogens, but their close interactions with human cells can affect gut homeostasis, immunological tolerance, and dysbiotic inflammation. Phages are being studied for their antibacterial and cell-interacting medicinal potential. Phage-human cell interactions reveal

a dynamic dialogue where phages are more than bacterial predators. Phages modulate the immune system and control intracellular pathogens in human cells, offering a viable therapeutic avenue. Phages are natural partners in our microbiota, promoting health through diverse cellular interactions. [12-18]. Phages, or bacteriophages, infect bacteria, but recent study has shown their complicated connections with human cells. Phages are plentiful in the gut, skin, and respiratory system, where they maintain microbial balance and prevent bacterial infections. Phages can attach directly to human cell surfaces, entering cells and affecting biological activities, offering promising therapeutic and immune system regulation potential. Phages are internalized by mammalian cells, which is critical for phage-human cell contact. Several investigations have shown that certain phages can endocytose human epithelial and immunological cells. Phages can interact with intracellular components, regulate signaling pathways, and deliver genetic material. Phages' intracellular presence challenges the idea that they work only extracellularly and shows they may regulate host immunity and inflammation.

Phages directly stimulate immune cells or interact with bacterial populations. They activate immune cell pattern recognition receptors, producing cytokines and modulating inflammation. Phages are interesting candidates for innovative therapies for bacterial infections and inflammatory illnesses due to their immunomodulatory and antibacterial properties. Phages are typically safe for humans, with little negative effects in clinical trials. Phages' capacity to interact with human cells is being used to treat antibiotic-resistant intracellular bacterial infections. Phages can enter host cells to reach buried germs, improving therapy success in persistent infections. Phage-host cell interactions are being studied to increase delivery, reduce resistance, and boost immunomodulation while maintaining safety. Besides bacterial targeting, bacteriophages and human cells interact directly, modulate the immune system, and have therapeutic potential. This new knowledge calls into question phages' role in human physiology and immunological homeostasis as well as antibacterial agents. [19-126]. Phages change the bacterial microbiome and affect human physiology and immunology without infecting cells. Endocytosis or receptors allow phages to enter tissues and cells, altering mucosal immunity and cell activity. Microbial ecology and immunological homeostasis are regulated by a complex tri-kingdom interaction network of bacteriophages, bacteria, and human cells.

Interactions with Mammalian Immunity:

Clinical aspects:

Phages are clinically significant for many reasons. First, bacteriophage genomes encode several highly pathogenic bacterial toxins, making the host bacterium only pathogenic when lysogenized. *Vibrio cholerae*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium difficile*, and *Shigella species* produce cholera, diphtheria, botulinum neurotoxin, binary, and Shiga toxins, respectively. [127]. These bacteria are either harmless or nonpathogenic without phage-encoded toxins. Phages encode these poisons for unknown reasons. Botulinum toxin paralysis seems to have the opposite effect of cholera toxin, which causes watery diarrhea and helps the

phage and host find their next victim. Second, bacteriophages enable horizontal gene transfer, including antibiotic resistance. They have been developed to incorporate genes into specific strains for clinical use, but this is still in testing. [128] A third therapeutically relevant component of bacteriophages is their usage as a biomarker for their host in complicated environmental samples. This usually indicates water feces pollution. The host is likely present if the phage is. To identify bacteria in mixed environmental samples, phages have been designed to produce luciferase when they infect their host. Bacteriophages may identify strains of the same bacterial species, making them clinically valuable even though they are generally replaced by newer technology. As humans are susceptible to many viruses, most bacteria have multiple bacteriophage pathogens. Not all strains of a species are phage-resistant. Infecting each strain routinely with a standardized panel of phages for that species reveals its sensitivity and resistance to each phage type. To distinguish S. aureus strains, phage typing used a standardized panel of bacteriophages common internationally. Phage typing was the standard epidemiological strain monitoring approach before molecular methods like multilocus sequence typing and pulsed-field gel electrophoresis. Finally, bacteriophages were the first virus discovered and contributed to several molecular biology breakthroughs. Bacteriophages proved that DNA transferred genetic information, set up gene control, and revealed the genetic code. [129]

Immune Responses to Phages in Humans

Phages greatly influence the immune system: Innate immunity: Phages activate pattern recognition receptors, producing cytokines and modulating immunity. Adaptive immunity: Strong humoral responses generate IgM, IgG, and mucosal IgA antibodies that neutralize phages and alter clearance and therapy. Cellular adaptive responses include T cell activation. Repetition of phage therapy reduces bioavailability due to host immunological neutralization by antibodies and complement. Phages can cause pro- or anti-inflammatory immune responses depending on context and purity. Phages can influence immune cells to help eliminate microorganisms synergistically or inhibit immunological processes, demonstrating diverse immunomodulatory actions.

Phages' indirect impact on mammalian immunity:

Bacterial disease, ecology, and genetic evolution are all significantly influenced by phages. Consequently, phages indirectly influence host defense and immunological function. Bacterial virulence and human immunity are influenced by the proteins encoded by phages. Lysogenic phages encode proteins that enable their bacterial hosts to penetrate tissue barriers, which serve as the initial line of defense against infections. The temperate phage Φctx is renowned for its ability to parasitize *Vibrio cholerae* hrough the production of cholera toxin. Bacterial adhesion, colonization, tissue invasion, and biofilm formation are all facilitated by phage-derived virulence factors [130,131].

The immune-clearing phagocytes are either destroyed or discouraged by phage-encoded proteins. The phage-encoded chemotaxis inhibitory protein of Staphylococcus aureus binds to and inhibits neutrophil receptors for complement and formylated proteins, thereby safeguarding it from neutrophil-mediated mortality. Exotoxin [132], cytotoxicity, intracellular infection [133], and superantigen are all produced or delivered by other phage-encoded proteins. Chromosome transcription factors primarily regulate phage-encoded virulence genes, which are synthesized during lysogeny, when other genes are not actively transcribed. For example, the lytic phage genes are located on the coding strand, while the λ-encoded bor of *E. coli* and the phage-encoded vir of M. arthritidis are located on the noncoding strand. Lastly, phages horizontally transfer antibiotic resistance genes within and between bacteria [134]. Prophages improve their bacterial hosts' fitness and propagation. Prophages can shed genes, including virion-producing genes, and domesticate [135]. When they still benefit, prophage-derived genomic elements can be selectively retained [136]. Thus, many phage-bacteria interactions are complex coevolutionary interactions.

Direct Interactions with Phages and Innate Immunity:

Innate immunity controls the microbial-human contact through structural and germline-encoded characteristics. Phages may be essential to our relationship with bacterial flora, as they contribute to and cross these barriers. In the mucosa, circulation, and cells, phages are identified on the cell surface and in endocytic vesicles. Phages are abundant at bacterial colonization sites and may directly contribute to mucosal barrier defenses. Barr et al. [137] discovered that mucosal surfaces can retain as many as 109 adhering phages per biopsy. Phages can be modified to facilitate these interactions. Immunoglobulin (Ig)-like domains are present in *E. coli* T4 phage capsid proteins, which interact with epithelial cell mucins and surface glycoproteins. Many phage families include Ig superfamily-like protein domains, suggesting mucosal layer enrichment of other phages [138]. Mucosal binding increased the susceptibility of some bacteria to phage-mediated lysis [139] and phage diffusion in the mucus layer [140]. Thus, mucosal phages may prevent bacterial invasion in a ubiquitous, strain-specific, and non-host-derived manner. These findings suggest a complicated interaction between phages, bacteria, and mucosal surfaces that needs additional study. Antiphage antibodies may weaken or change barrier immunity, among other questions.

In mice models of colitis, the intestinal virome affects innate immunity, which may affect outcomes. Yang et al. [141] found that antiviral medicines worsened DSS-induced colitis in mice, but gut-resident viruses detected by TLR 3 and TLR7 protected via IFN-β production. Phages make up most of the intestinal virome, however this study did not focus on them. In contrast, Gogokhia et al. [142] found that oral phage cocktail exacerbated DSS colitis via TLR9. Phages' significance in intestinal homeostasis needs further study. Mucosal-associated phages may affect tissues beyond the epithelial barrier. Transcytosis across cells transports many phages across the gut and into systemic circulation, possibly for paracytosis at inflammatory sites. Based on in vitro studies of cell monolayers [142], this transit is expected to occur at 3.1 × 107 particles/day. Transcytosis across the Golgi apparatus allows phage T4 to absorb apically to basolaterally. Certain phages and peptide

sequences may be more easily taken up [144]. T1 phage straight entering the small intestine entered gut-draining lymph and blood. Bioactivity against bacteria in circulating phages may help hosts defend against bloodstream bacteria. These investigations demonstrate the potential importance of phage interactions with mucosal immunity and how little we know about them. It is unclear how many phages transverse mucosal tissues, what processes are involved, if M cells, which sense the intestinal environment, aid transmission, or whether inflammation influences this transit.

Peripheral circulation and tissues contain several phages. The pharmacokinetics of some of these phages have been widely studied for lytic phage treatment. Regardless of method, circulating phages clear temporally and spatially. Phages last several days, with the biggest decline (>99%) in the first hour. After infusion, phages are discovered in most major organs, with the liver and spleen having the greatest and most lasting titers, indicating that these organs remove circulating phage particles. Splenic and hepatic macrophages phagocytose phage quickly and efficiently, according to research. Active phage is less persistent in the liver than in the spleen. Kupffer cells endocytose better, retain greater basal ROS levels, and produce fewer proinflammatory cytokines in response to TLR ligation than splenic macrophages [145]. These cells' phagolysosomes digested and inactivated internalized phage. Phage clearance involves two steps: quick elimination of most particles in 24 hours, followed by slower clearance of the remaining ~1% of circulating phages over many days. This slower tail may be phages trapped by antibodies or other immune response components. Inflammation and bacterial contamination may influence phage pharmacokinetics [146]. Immunologically, circulating and peripheral phages are well tolerated. Phages at immunological privilege areas including the placenta and CSF are also well tolerated. The recently obtained CSF virome shows that phage are abundant (104 pfu/mL) without inflammation [147]. This is noteworthy since phages are related with LPS, bacterial DNA, and other powerful immune stimulants. Even with remaining bacterial contaminants, lytic phage treatment infusion is well tolerated. It is probable that mechanisms exist to facilitate the immunological reception of numerous phages in peripheral tissues and circulation. Phage capsid proteins may be engineered to mitigate immune detection. Phages that are designed to exhibit peptides for epitope identification or phage vaccines are immunogenic; however, lytic phage therapy is well-tolerated. The clearance of phages in the liver and spleen may also be influenced by tolerogenic pathways. In the liver and spleen, there is an abundance of tolerogenic macrophages and DCs. Finally, endogenous phages may possess the unique ability to stimulate tolerogenic immunological responses, such as the differentiation of M2 macrophages. Phages are absorbed by a diverse array of eukaryotic cells through nonspecific uptake, receptor-mediated endocytosis, and the uptake of bacteria-harboring prophages [148,149].

Most phages are cleared by phagocytes. This literature focuses on tumor cell lines and biotechnology-engineered phages, however wild (unmodified) phage uptake is widespread and may follow comparable routes. The capsid protein gp24 of the E. coli phage T4 is characterized by a Lys-Gly-Asp motif that interacts with β3-integrin receptors on target cells. Chondroitin sulfate proteoglycans are associated with phage absorption in other studies. The filamentous E. coli phage M13 [150] is subjected to a variety of receptor-mediated endocytosis mechanisms by various cell

lines. Most phages' cellular uptake receptors and processes are unclear. Phages are degraded in endosomal vesicles, cytoplasm, nucleus, Golgi, and lysosomes after internalization. Recently, intracellular phages were demonstrated to be bioactive against intracellular bacterial infections, including *Mycobacteria abscessus* [151]. Phagocytosis can also expel Listeria from phagosomes by removing prophages. Phages can reach the nucleus and create RNA and protein, as shown by phage DNA vaccines [152]. A new review [153] details intracellular phage. Multiple cell-surface and intracellular pattern-recognition receptors (PRRs) identify phages during cellular uptake and transit [153]. Most pathways include detecting ssDNA and dsDNA and inducing IFN responses.

Several studies have linked endosomal PRR TLR9 to phage recognition. TLR9 detects unmethylated CpG patterns found in phage and bacteria DNA [154]. TLR9 activates proinflammatory and antiviral cytokines via MyD88. Recent gut phage sensing research has also linked TLR9 to phage-induced inflammation. The use of oral E. coli tailed phages enhanced IFN-γ-producing CD4+ T cells, mediated by DC sensing of phage DNA via TLR9. The role of TLR9 in phage responses may be complicated. Hashiguchi et al. [155] found that MyD88−/− mice's weak antibody responses to M13 phage immunization indicate the importance of TLR signaling in antiphage adaptive immunity. However, TLR9−/− animals had much higher IgG levels. The authors suggested that TLR9 regulates M13 ssDNA genome sensing. The primary integrating axis for cytosolic dsDNA sensing is the STING route. STING has not been implicated in phage sensing, despite the fact that modified phages enter the cytosol. Phage sensing is also facilitated by TLR3, an endosomal RNA sensor [156]. Only TLR3 strongly induces antiviral cytokines by signaling through the adaptor Toll/interleukin-1 receptor (IL-1R) resistance (TIR) domain-containing adapter-inducing IFN-β (TRIF). Sweere et al. [156] discovered that Pf, a filamentous phage that infects P. aeruginosa, activates IFN-β in DCs through TLR3 and TRIF. In eukaryotic cells, this RNA-sensing receptor is activated by phage-derived RNA synthesis; however, the mechanism by which Pf initiates transcription in mammalian cells remains enigmatic. This was not the initial report of phage genome transcription in a eukaryotic environment, as phage DNA vaccines require RNA to generate protein. However, mammalian cell bacterial absorption is still poorly understood. It is uncertain if phages are cell type-tropic [157].

The immune system's innate and adaptive systems respond to phages in human tissues. Pattern recognition receptors on immune cells allow the innate immune system to recognize phages in tissues, triggering cytokine release and inflammation. Dendritic cells and macrophages can take up phages and transmit phage-derived antigens on MHC molecules to T cells, starting adaptive immunity. In the adaptive immune system, phages induce particular antibodies. IgM antibodies are the first reaction to phage exposure, formed within days. After recurrent phage exposure, IgG antibodies are produced to neutralize phages by attaching to them and clearing them from circulation and tissues. Antibody-mediated neutralization combined with complement system enhances phage inactivation. This antibody/complement collaboration mirrors immune response mechanisms for eukaryotic viruses, suggesting that the immune system handles phages similarly despite their bacterial host specialization. T helper cells triggered by phage antigens stimulate B cell development into plasmablasts and memory B cells, resulting in stronger antibody responses

to phages after repeated exposure. If immunity develops against administered phages, these immunological memory responses enable quick neutralization in subsequent encounters, which can reduce therapeutic phage efficacy. Phages may modulate immune cell activation and inflammatory equilibrium in human tissues, which could be used therapeutically. Thus, human tissues respond to phages with immediate innate recognition, cellular antigen presentation, and a powerful, adaptive antibody response that neutralizes phages and regulates the immune system. Learning these pathways is crucial to improving phage therapy and predicting immune responses to phages delivered into the body. [159-163]

Human tissues respond to phages with innate and adaptive immunity. Innate immune cells produce cytokines when they recognize phages via pattern recognition receptors. Antigen-presenting cells absorb phages and present MHC molecules with phage-derived antigens to T cells, initiating adaptive immunity. Adaptive immunity develops phage-specific IgM antibodies days after exposure and stronger IgG antibodies following re-exposure. By binding and clearing phages, these antibodies work with the complement system to neutralize them. Phages, bacterial viruses, have similar immunological treatment to eukaryotic viruses, as seen by this antibody/complement action. Phage antigen-activated T helper cells differentiate B cells into antibody-producing plasma blasts and memory B cells for long-term immunity. Therapy with phages can be neutralized by immunological memory, but they can also control immune activation and inflammation. Complex immunological responses to phages in human tissues include quick innate recognition, antigen presentation, and robust adaptive antibody responses that neutralize and modulate immunity. Optimizing phage therapy and anticipating immunological reactions requires understanding these interactions.

Phage-specific antibodies created by the host immune system during phage therapy can greatly impact repeated treatments. The immune system develops IgM, then IgG, then IgA antibodies against therapeutic phages after exposure. These antibodies destroy phages by attaching to them, inhibiting their interaction with bacterial targets, and helping them leave the body, reducing the potency of following phage dosages. Animal models suggest that repeated phage delivery, especially intravenous or intraperitoneal injection, increases antibody titers. After subsequent administrations, this antibody response increases phage clearance from the bloodstream, reducing their circulation time and ability to reach infection sites. After treatment stoppage, IgG antibodies persist, and IgA titers can rapidly rise when the same phage is given again, improving neutralization. Route of delivery affects antibody induction. Phage-specific antibody responses are milder in oral delivery than parenteral approaches, which may allow extended phage activity throughout treatment. Inhalation (nebulization) and systemic injections may produce higher immune reactions that can hinder repeated phage therapy unless formulations or treatment regimens are tailored to avoid or regulate the immune reaction. Due to phage-neutralizing antibodies, repeated phage therapy may involve rotating phage types (cocktails), altering phage surface proteins to minimize immunogenicity, or immunosuppressive methods to maintain therapeutic benefits. Designing efficient repeated phage therapy regimens without losing efficacy requires understanding antibody induction kinetics and phage pharmacokinetics. Repeated exposure to phage-specific antibodies neutralizes therapeutic phages and accelerates their

clearance, reducing phage therapy efficacy. To overcome this immunological barrier and sustain clinical effectiveness, tailored treatment techniques are needed. [146-168]

IgG and IgA antibodies affect phage biodistribution and efficacy differentially due to their immune system roles and sites. In the blood and extracellular fluids, IgG antibodies destroy phages by attaching to them, clearing them from the bloodstream and lowering their bioavailability for bacterial targeting. Phage therapy can be less effective systemically due to increased IgG levels, especially after parenteral delivery, which expedite phage neutralization and clearance. However, IgA antibodies are mostly found on mucosal surfaces including the gastrointestinal, respiratory, and secretions. Secretory IgA neutralizes phages locally at mucosal barriers, preventing them from entering or surviving. Phage activity in the gut is limited by IgA, therefore if phage-specific IgA levels rise sufficiently, active phages are no longer detectable in feces, indicating diminished phage passage and efficacy in the gut. Primary IgA response is slower but grows faster after repeated phage exposure, limiting mucosal tissue phage bioavailability. IgG mostly affects systemic phage biodistribution and clearance from circulation, while IgA mostly controls phage activity in secretory mucosal tissues. Both antibody types neutralize phages, although their spatial effects differ: IgG influences systemic exposure and IgA mucosal activity. This difference emphasizes the necessity of addressing phage administration method, therapeutic location, and antibody kinetics when designing phage therapy regimens. [169-171]

IgG and IgA antibodies alter phage biodistribution and therapeutic efficacy differently according to their localizations and immunological activities. IgG antibodies bind and remove phages in the circulation and extracellular fluids, lowering systemic phage availability. Systemic phage injection accelerates phage clearance from circulation and limits their therapeutic reach to tissue microorganisms. By contrast, IgA antibodies work on mucosal surfaces like the gastrointestinal tract, respiratory tract, and secretions. Secretory IgA binds phages in the mucosa, inhibiting their survival and transit. In oral phage therapy, the formation of phage-specific IgA in the gut limits phage survival and efficacy. When IgA levels grow, active phages fail to show in feces, indicating restricted mucosal phage activity. IgA response is sluggish at first but accelerates with repeated exposure, reducing mucosal phage bioavailability. Thus, IgG controls systemic phage clearance and effectiveness, while IgA controls mucosal phage neutralization. The administration route and target infection location determine how phage therapies balance phage activity with immune neutralization. [172-174]

Mucosal IgA neutralizes gut phages by adhering to phage particles in the intestinal mucus and lumen, preventing them from targeting bacteria. IgA neutralizes phages by crosslinking them into mucus-trapped clumps. Phages are clumped and can be flushed out by intestinal peristalsis because they cannot move or access bacterial hosts. IgA-phage binding covers phages and limits their penetration beyond the mucus barrier, maintaining the mucosal barrier and homeostasis. This activity prevents phage invasion and immunological activation in epithelial cells. IgA-bound phages can also be picked up by gut dendritic cells and other antigen-presenting cells to orchestrate specific immune responses to balance gut elimination and tolerance. Capsid-displayed Ig-like protein domains allow phages to bind to mucin glycoproteins in mucus, which may increase their persistence at mucosal surfaces and provide a symbiotic antimicrobial defense. Specific IgA

antibodies against phages dominate phage clearance by immunologically targeting these particles, decreasing their persistence and therapeutic potential in the gut. Thus, mucosal IgA neutralizes gut phages by trapping and aggregating them in mucus, avoiding bacterial infection and promoting phage clearance while preserving immunological homeostasis. [175-177]. Mucosal IgA neutralizes gut phages by adhering to them in the mucus layer, blocking phage access to bacteria. This binding clumps phages, which get immobilized in mucus and less infectious. Intestinal peristalsis flushes phage particles from the gut. Immune exclusion protects the mucosal barrier against microbial invasion and maintains homeostasis. IgA coats phages and blocks their host cell connections, preventing epithelial penetration. Dendritic cells can deliver antigens from IgA-bound phages, modulating immune responses between tolerance and clearance. Phages that bind non-specifically to mucin glycoproteins via Ig-like domains can survive in mucus, but IgA responses neutralize and remove them. Thus, mucosal IgA traps and neutralizes gut phages in mucus to prevent bacterial infection and regulate immunological homeostasis through coordinated clearance and immune signaling. [175-177]

Phages and Bacterial Clearance

Contradictory data exist on the effects of phages on inflammation, bacterial clearance, and PRRs in phage identification. Proinflammatory phage vaccinations use modified T4 or other phages [178]. In response to such phages [179], mixed T helper (Th) 1 and Th2 responses and significant proinflammatory cytokine production indicate a strong antibacterial response. Endotoxin levels in phage vaccines are rarely studied, and some investigations employ bacterial lysates as immunogen [180]. Bacterial contamination may make these preparations immunogenic. Phages may work with the innate immune response to remove germs, consistent with their proinflammatory effects. Bodner et al. [181] used a unique fluorescent lysis reporter system to detect lambda prophage lysis of E. coli in response to macrophage phagosome ROS. It appears that phages offer macrophages an alternate bacterial killing route. Tiwari et al. [182] tested phage treatment on wild-type and neutropenic mice for P. aeruginosa. Wild-type mice survived phage injection 100%, however neutropenic mice did not. Phage cocultured with isolated neutrophils killed bacteria better than phage alone in vitro, suggesting phage-neutrophil synergy is necessary for bacterial clearance. Treatment with the lytic phage PAK_P1 was equally effective in wild-type and Rag2−/−IL2rg−/− mice, demonstrating that lymphocytes are not crucial for bacterial clearance in this time frame [183] in a Pseudomonas lung infection model. The study found that phage therapy was unsuccessful in mice missing neutrophils and MyD88−/− mice. These findings indicate that phage control of bacterial infections requires cooperation with the host immune system, particularly neutrophils and myeloid cell TLR-sensing pathways.

Other investigations found limited phage-induced inflammation [183] (sd104–106) and no phagocytosis effect [184]. Proinflammatory cytokines were not altered by T4 phages in naive and LPS-activated monocytes [185]. Similar to pure T4 and A3/R phages, monocyte and neutrophil ROS generation was low. Endogenous phages in circulation and lytic phage treatment infusions are well tolerated in people with low inflammation [185]. Phages decrease bacterial phagocytosis and reduce proinflammatory cytokine production in response to endotoxin, according to another research. In

response to LPS, P. aeruginosa Pf4 phages suppressed phagocytosis and tumor necrosis factor production, contributing to chronicity of mouse wound and lung infection models and to chronic wound and lung infections in humans [187]. These effects were caused by type 1 IFN-dominated antiviral responses that inhibited bacterial clearance. T4 and F8 phages from E. coli suppressed phagocytosis and ROS generation but not phorbol myristate acetate. Jahn et al. [188] found that phage-expressed protein ANKp inhibited proinflammatory cytokine production and macrophage phagocytosis of E. coli producing ANKp under an inducible promoter. Van Belleghem et al. [189] observed that S. aureus and P. aeruginosa phage stimulation affected endotoxin-induced transcription in human peripheral blood mononuclear cells. These investigations show that some phages directly affect local immune responses, changing bacterial infection susceptibility. They also suggest that phages control local immunity to protect their bacterial hosts. How can we interpret these conflicting phage pro- and anti-inflammatory data? It is interesting that T4 phages are pro-inflammatory in some circumstances [190] and anti-inflammatory in others. The immunological response may depend on the purity of the phage preparation [191]. Phages appear to decrease the immune response to bacteria but no other inflammatory conditions, suggesting that their immunomodulatory effects may be context- and possibly phage-dependent. This could happen if numerous phages trigger IFN responses that counteract bacterial immunity but boost other inflammatory responses.

Innate phage immune responses are poorly understood. Most of our data comes from a few phages; it would be important to determine if common responses exist. It is also unknown how different phages (lytic versus filamentous) affect immune responses. Adaptive immunity targets specific infections. Phages can induce antibody (humoral immunity) and T cell responses (cellular immunity), which affects phage display vaccines, phage therapy, and microbiome interactions. Phages induce neutralizing antibodies that aid in their absorption and elimination. Most synthetic and environmental phages generate neutralizing antibodies without adjuvant [192]. Animal experiments have shown how some phages induce antibody production. These results show that the spleen is needed for a humoral response to circulating phages and that phagocytes are crucial to APC absorption, processing, and presentation of phage antigens. After presenting the target peptides on MHC-I and MHC-II pathways, B and T cells respond to the viral or tumor antigen in vitro and in vivo [193]. Antiphage antibodies are mostly IgM, but IgG and IgA are also generated [194]. In a heterogenous phage therapy investigation, local phage delivery at the infection site induced stronger antibody responses than oral phages in people, but controlled studies have not been conducted.

The long history of study on the coliphage phiX174 as a diagnostic for adaptive immune responses gives crucial data on human antiphage antibody responses. PHX174 circulates for 3–4 days after first IV immunization in healthy controls. This time window produces phage-specific IgM antibodies that peak at 2 weeks. Secondary immunization 6 weeks later produces a higher IgG/IgM peak after 1 week. X-linked agammaglobulinemia patients, who lack functioning B cells, have extended active phage circulation and no antiphage antibody response. Because these patients cannot manufacture natural antibodies or mount an adaptive response, phage clearance is difficult. B cell depletion with anti-CD19 antibody (Rituximab) normalizes phiX174 clearance despite

reduced primary and secondary antibody responses. This study emphasizes the role of humoral proteins in phage clearance as anti-CD19 therapy will not influence patients' natural IgM/IgA antibodies and complement. Antiphage antibodies may regulate phage bioactivity against our microbiome. Repeated T4 phage administration caused antiphage IgAs, which reduced bioactivity. Intravenous phage therapy may also be affected by antiphage adaptive immunity. Human antiphage antibodies are rarely studied, although one study reveals that many people have neutralizing antibodies against phages used in phage therapy. Opportunistic pathogens that are part of our commensal flora may cause several chronic infections, exposing us to their phages. Patients acquire neutralizing antibodies during phage treatment. Antiphage antibodies may affect phage therapy success, however this is uncertain. In one study of 122 individuals receiving various phages for various illnesses, robust humoral immunity did not affect treatment outcomes. The lack of controlled trials employing standardized phage product, administration routes, and treatment time makes it difficult to monitor patient antiphage antibody formation and treatment outcome.

Peptide display techniques may make phages with changed capsid shapes more immunogenic and clearer from circulation faster [195], making the data harder to interpret. Phages given intravenously or at the infection site may be more immunogenic than gastrointestinal phages. Both complement and antiphage antibodies remove phages from peripheral circulation. Sokoloff et al. injected rats with a random-peptide T7 phage library. IgM and IgG reduction in rat serum increased phage survival by 62% and 16%, respectively. Dabrowska et al. found that complement is essential for serum antibodies to inactivate phages. These results indicate that antibody binding and complement fixation opsonize and eliminate phages. Much regarding phage antibody response is unknown. It is unclear how much humoral immunity exists against the body's larger pool of phages and how these antibodies modulate phage activity, transcytosis, or opsonization of commensal or pathogenic bacteria targeted by phages. Phage treatment and phage display vaccine development require a better grasp of these and related concepts. [196]

Phages activate cellular adaptive immunity. APCs endocytose and process phages, presenting phage-derived peptides on MHC surface molecules to induce B and T cell responses against the viral antigen in vitro and in vivo. E. coli fd phage modified to express a peptide antigen is taken up by phagocytes and displayed on cell-surface MHC-I and MHC-II molecules, resulting in CD8+ and CD4+ T cell responses. Phages can also activate T cells by secreting costimulatory molecules. Phage display vaccines induce cytotoxic CD8+ T cell responses against eukaryotic virus-infected cells and malignancies. Phages prime CD4+ Th cell immunity. Phages can induce Th1 and Th2 responses in T cell line stimulation experiments and phage vaccines. PhiX174 research shows that T cells contribute generate the initial B cell IgM phage-specific response and class-switch to IgG [197]. There are signs that endogenous phages may help train T cells and fight cancer. Fluckiger et al. [196] (sd146) found MHC-I-restricted epitopes in an Enterococcus hirae prophage that drive CD8+ T lymphocytes to attack tape measure protein, a cross-reactive tumor-associated antigen. Mice with E. hirae harboring this prophage responded to cyclophosphamide or anti–PD-1 antibodies with a TMP-specific cytotoxic T-lymphocyte response, clearing the tumor. In human renal and lung cancer patients, enterococcal prophage and tumor TMP expression linked with long-term PD-1 blocking benefit. These intriguing findings show that the phageome and

immunomodulatory medications that modify antigen responses may affect anticancer immunity. To completely understand this topic, further research is needed on patient tumor phageomes and how composition and abundance affect cancer outcomes. These findings may apply to other microbiome and cellular immunity issues. Autoimmunity coupled with PD-1 blockage may also be impacted by similar dynamics. [179]

Interactions Between phages Human Hosts

Recently, many study has shown how commensal bacteria affect host health and development. Microbiota greatly impact host immunity, metabolism, and commensal makeup and structure, according to research. Only a few studies have considered bacteria's role in this dynamic. These findings show that bacteriophages are essential to human biology. Phages directly affect bacteria immune responses and indirectly damage human cells and tissues through their hosts. Thus, phages influence immunologic-bacterial interactions. Bacteria, bacteriophages, and human cells form a microbiome network. These components' tri-kingdom interactions may determine network stability. Phages reduce colonization site microorganisms and inflammation. Phages modulate host immunity directly and indirectly to increase commensal colonization immunologic tolerance. Exogenous phages, microbial dysbiosis, and immunological dysregulation can upset this balance, impacting metabolism and immunity. Unfortunately, these encounters have limited data and options. Focus is on a few phages. Many phages have been altered for biotechnology or lytic phage therapy. Expand this research to encompass steady-state and inflammatory commensal (unmodified) phages. Current approaches are often unsuitable for examining phages' physiological impacts in vivo. These projects need prophages, free phage particles, and cell and tissue phage data. Despite computational biology advances, phage biology is still difficult to explain from sequencing data. We need well-controlled, extensive human studies to understand how phages alter immunity. Most of our evidence comes from mice. Optimizing these efforts yields huge returns. Better understanding our endogenous phages may help us understand human health and disease and these tri-kingdom links.

The evolution of phages with enormous genomes—whether they are the product of recent genome expansion within clades of normal-sized phages or a persistent strategy—is intriguing. Phylogenetic trees for big terminase subunit and main capsid proteins using public database sequences to examine. Many of our phage genome sequences form clades with significant bootstrap support. The public sequences in these clades are from phages with genomes at least 120 kb, according to database sequence genome size analysis. The Mahaphage clade, named after Sanskrit for enormous, encompasses all of our biggest genomes plus the 540–552 kb Lak genomes from human and animal microbiomes. A Nine more clusters of enormous phages and called them "huge" in certain authors' languages. Despite modest differences in gene and dataset tree topologies, protein family and capsid studies support grouping. Large phages are reliably sorted into clades, indicating that large genomes are stable. Phages were sampled from many environments within each clade, suggesting the diversity of these massive phages and their hosts throughout ecosystems. In 20 cases, phages that are so closely related that their genomes may be matched occur in at least

two cohorts or habitat types. Most main CRISPR–Cas systems in phages, including Cas9-based type II, the recently discovered type V-I [198], new variants of type V-U systems, and new subtypes of type V-F systems [199]. Phages have not been reported to have class II systems (types II and V). Most phage effector nucleases (for interference) have conserved catalytic residues, indicating function.

Unlike the well-known phage with a CRISPR system, most phage CRISPR systems lack spacer acquisition machinery (Cas1, Cas2, and Cas4) and interference genes. Two similar phages have a type I-C variant system without Cas1 and Cas2 and with a helicase protein instead of Cas3. Phages have a second system with a 750-amino-acid CasΦ (Cas12j) effector protein, located near CRISPR arrays. Phages without genes for interference and spacer integration may use host Cas proteins since they have homologous CRISPR repeats. Alternatively, systems without an effector nuclease can inhibit target sequence transcription without cleavage [200]. Spacer-repeat guide RNAs may also silence host CRISPR systems or nucleic acids they hybridize with using an RNA-interference-like mechanism. Phages encode compact CRISPR arrays (median, six repetitions). This range is much less than bacterial genomes (mean 41 repetitions for class I systems) [201]. Several phage spacers target other phages' structural and regulatory genes. To defend against competing phages, phages appear to boost their hosts' immune systems.

Some spacers in phage-encoded CRISPR loci target bacteria from the same sample or study. Other host prediction analyses corroborate our assumption that these phages host focused bacteria. Not all loci with bacterial chromosome-targeting spacers encode Cas proteins that can break the host chromosome. The phage infection cycle may benefit from targeting host genes to inhibit or change their control. Phage CRISPR spacers may block promoters or silence non-coding RNAs in bacterial intergenic regions, affecting genome control. Transcription and translation genes are known CRISPR targets for bacterial chromosomes. For example, one phage constructs its own σ70 by targeting a transcription factor gene in its host's genome. Several large phage genomes contain anti-sigma factor-like proteins (AsiA), supporting prior reports of σ70 hijacking by phages with AsiA. In another example, a phage spacer targets the host glycyl tRNA synthetase, but the Cas14 effector lacks a catalytic residue, suggesting suppression (as a ‘dCas14’). No host-encoded spacers targeting CRISPR-bearing phages were detected. However, phage CRISPR targeting of other phages that are similarly targeted by bacterial CRISPR revealed phage–host connections, which the phage taxonomic profile corroborated.

Some big Pseudomonas-infecting phages encode anti-CRISPRs (Acrs) and proteins that form a nucleus-like compartment to separate their replicating genomes from host-defense and other bacterial processes. We found proteins in large phage genomes that may be Acrs and cluster with AcrVA5, AcrVA2, AcrIIA7, and AcrIIA11. We also found tubulin homologues (PhuZ) and proteins that form a proteinaceous phage "nucleus". Recently, the phage nucleus physically blocked CRISPR–Cas degradation to protect the genome from host defense.

Clinical and Therapeutic Implications

Phage treatment may replace antibiotics for antibiotic-resistant microorganisms. However, phage mixtures and surface changes are needed to reduce immunological neutralization by phage-specific antibodies. Optimizing phage therapy, vaccine development, immunomodulation, and intracellular pathogen targeting requires understanding the immune-phage interaction. Phages' ability to cling to mucosal surfaces and modify local immunity can be used to prevent and treat disease.

Host resistance and anti-phage defense

Because bacteriophages are dangerous to bacteria, prokaryotes have developed several host resistance and anti-phage defensive mechanisms. Examples include CRISPR. Retron anti-toxin system. Thoeris' NAD+ degradation method for bacterial antiphage resistance is unique. Hailong's anti-phage defense system has two genes: HalA, a transmembrane ion channel effector, and HalB, a nucleotidyltransferase. Cell death and membrane depolarization result from phage HalA activation. This kills the sick cell but stops bacterial spread. Although phages do not infect humans, the microbiome contains many phage particles. The human phageome includes the "healthy gut phageome" (HGP) and "diseased human phageome" (DHP). Some dozens to thousands of viruses replicate in a healthy person's active phageome. There is evidence that gut microbiome bacteriophages and bacteria interact negatively and positively. According to preliminary research, 62% of healthy people and 42% and 54% of UC and Crohn's disease patients had common bacteriophages. Older persons may have fewer phages. CrAssphages rule the gut worldwide. CrAssphages are transferred from mother to child shortly after delivery, with some local transmission. People create distinct crAssphage clusters. Non-human apes may have CrAss-like phages. Most bacteriophages infect human bacterial populations. Recent investigations have shown that bacteriophages can infiltrate eukaryotic tissues and impact the immunological, respiratory, gastrointestinal, and central neurological systems. They directly affect immune responses and indirectly affect human health and disease-causing microbes [202,203]. Phages, bacteria, and human cells form a complex tri-kingdom network, revealing their interactions with people. Phages maintain immunological homeostasis and commensal microbiota tolerance by regulating bacterial populations and minimizing overgrowth and inflammation. Phage-bacteria-human ecosystem disruptions such microbial dysbiosis or immune dysfunction can cause sickness. These interactions affect phage therapy for bacterial infections and immune control in health and sickness [204,205].

Phages control innate and adaptive immunity. These engage immunological sensors, alter cytokine production, and are eliminated by the immune system. Phage immunomodulation can treat intracellular infections and inflammation [202,204]. In conclusion, bacteriophages effect the microbiota, immunity, and health directly and indirectly. Phages should be regarded human microbiological and immunological milieu members, not just bacterial predators.

Conclusions:

The following are the detailed points that can be used to summarize the conclusions of the appended research on bacteriophages:

1. Bacteriophages are the most prevalent viruses on Earth, infecting bacteria and archaea with a variety of genome types, including dsDNA and dsRNA.
2. In recent metagenomic investigations, the vast diversity of phages was revealed, including "huge phages" with genomes that rival those of small bacteria. These phages encode complex functions such as tRNAs and CRISPR-Cas systems.
3. The taxonomy of phages is undergoing a transformation, with phylum and realm classifications being determined by phylogenetic analyses of critical genes such as polymerases, rather than morphology or host specificity.
4. Bacteriophages are viruses that infect bacteria and archaea, and they are extremely diverse. They are essential for the control of bacterial populations, the modulation of host metabolism, and the transfer of horizontal genes. They possess intricate structures and a variety of replication cycles, which include lytic, lysogenic, and non-lytic chronic infections.
5. Phages indirectly affect human health by interacting with the microbiota and immune systems. They are present in mucosal surfaces, tissues, and circulation, where they can be immunologically tolerated and influence innate and adaptive immunity. They can restrict the efficacy of repeated phage therapy by activating immune receptors (e.g., TLR3, TLR7, TLR9) and inducing neutralizing antibodies (IgM, IgG, IgA).
6. The discovery of large "huge phages" with genomes that are comparable to those of small bacteria has significantly enhanced our comprehension of phage biology. These phages evade host defenses and compete with other phages by encoding complex functions, including tRNAs, translation factors, CRISPR-Cas systems, and protective nucleus-like compartments.
7. Phage therapy has the potential to combat antibiotic-resistant bacteria; however, there are still obstacles to overcome, such as immune neutralization, bacterial resistance, and the necessity of phage cocktails or engineered phages to enhance their efficacy. It is imperative to comprehend immune responses and phage-host interactions in order to advance therapeutic applications.
8. The taxonomy and evolutionary comprehension of phages have been improved by the discovery of extensive phage diversity, including tailless dsDNA phages and diverse RNA phages, as a result of advancements in metagenomics and metatranscriptomics.
9. Specific receptors on bacterial surfaces, such as lipopolysaccharides and outer membrane proteins, determine the host range.
10. The molecular interactions of phages with eukaryotic cells are a developing field that demonstrates phage internalization through mechanisms such as endocytosis, as well as potential immunomodulatory and therapeutic functions that extend beyond their bacterial

hosts. Anti-CRISPR proteins are present in phages to thwart the defense mechanisms of bacteria.

11. Without replicating in human cells, they can adhere to mucosal surfaces, interact with immune cells, and penetrate tissues through endocytosis mechanisms.
12. Bacterial virulence and evolution are influenced by horizontal gene transfer by phages. The function of prophages in shaping bacterial pathogenicity is underscored by the clinical significance of phage-encoded toxins and antibiotic resistance genes.
13. With both innate and adaptive mechanisms, the immune system has a robust response to phages. Inflammation, bacterial clearance, and immunological homeostasis can be influenced by phages, which can modulate immune responses in a positive or negative manner, depending on the context.
14. Mucosal IgA antibodies are instrumental in the neutralization of phages at mucosal surfaces by encasing them in mucus, thereby reducing their capacity to infect bacteria and regulating local immunity.
15. Improvements in the characterization of dsRNA and other phage types, their non-lytic infection mechanisms, the tri-kingdom interactions between phages, bacteria, and human hosts, and the surmounting of immune clearance to optimize phage therapy and biocontrol applications should be the primary focus of future research.
16. Chronic non-lytic infections (carrier states) can be caused by certain dsRNA phages.
17. Phages indirectly influence human health by influencing the composition of the microbiota and immune responses. Neutralizing antibodies frequently impede the efficacy of repeated phage therapy, necessitating strategies to circumvent immune clearance.
18. The utilization of phages as biomarkers and instruments for clinical and environmental surveillance is advantageous. It is imperative to comprehend the immunogenicity of phages and the immune responses of the organism in clinical environments in order to optimize therapeutic strategies.
19. In general, bacteriophages are essential elements of microbial ecology and human microbiomes, and they possess significant potential in the fields of biotechnology, medicine, and immunology. Their implementations in the management of health and disease will be furthered by ongoing interdisciplinary research.
20. Phages are promising agents against antibiotic-resistant bacteria; however, they encounter obstacles such as host immune neutralization and bacterial resistance. In order to enhance efficacy, it is necessary to implement phage cocktails, engineered phages, and delivery optimization.

Future Research Directions

Further isolation and whole-genome sequencing of dsRNA and other phages from varied habitats are needed to improve taxonomy and genetic diversity. Non-lytic infection strategies, host interaction molecular processes, and ecological distribution of large-genome phages need further investigation. The tri-kingdom interactions between phages, bacteria, and the immune system can reveal health and disease and inspire new treatments. Translational breakthroughs require better

methods to analyze endogenous phages and their physiological consequences in vivo, especially in humans. Bacteriophages are diverse biological agents important to microbial ecology, bacterial evolution, human health, immunity, and therapeutic innovation, according to current knowledge.

Recommendations on Bacteriophage Research and Applications

Taxonomic and Genomic Characterization

Continue and expand the isolation and whole-genome sequencing of double-stranded RNA (dsRNA) phages to better understand their genetic diversity and taxonomy. Update and refine the taxonomy of dsRNA bacteriophages using both sequence-based phylogenetic methods (especially RNA-dependent RNA polymerase genes) and structural approaches. Employ metagenomic and metatranscriptomic analyses with advanced computational and structure-based methods to uncover novel phage diversity, especially for RNA phages that are genetically diverse and under-studied.

Study of dsRNA Phage Cycles

Investigate the molecular mechanisms underlying alternate non-lytic infection cycles of dsRNA phages, such as the carrier cell chronic infection mode. Apply reverse genetics and related molecular tools, particularly using well-studied model phages like phi6, to dissect host and viral factors regulating these infection states. Explore environmental and host cues influencing lytic versus non-lytic phage replication strategies.

Phage Therapy and Biocontrol Development

Assess the host range, host shift potential, and alternative infection modes of dsRNA phages before applying them as biocontrol agents in agriculture and as therapeutics for human bacterial infections. Conduct stability and efficacy studies of phages (like phi6 and phiYY) under diverse environmental and clinical conditions. Monitor the development and impact of phage-specific neutralizing antibodies in therapeutic contexts to optimize dosing schedules and phage formulations. Consider usage of phage cocktails or engineered phages to overcome bacterial resistance and host immune neutralization.

Phage-Host Interaction and Immunomodulation

Expand studies of tri-kingdom interactions involving phages, bacteria, and human hosts to understand their dynamic roles in microbiome stability, immune tolerance, and inflammation. Investigate molecular mechanisms by which phages modulate innate and adaptive immunity, including their influence on antigen-presenting cells, cytokine profiles, and immune cell activation. Examine how mucosal immunity, especially secretory IgA, modulates phage populations and impacts phage therapy at mucosal sites. Characterize molecular receptors and entry mechanisms mediating phage internalization into eukaryotic cells, including endocytosis and transcytosis pathways.

Phage Defense and Anti-CRISPR Systems

Study phage-encoded CRISPR-Cas systems and anti-CRISPR proteins that influence phage-bacteria immune arms races and the regulation of phage infection outcomes. Investigate the genomic and functional diversity of phage defense systems, including novel mechanisms such as Thoeris and Hailong systems that bacteria use against phages. Explore phage strategies that protect their genomes during infection, including nucleus-like compartments that evade bacterial immune defenses.

Clinical and Environmental Surveillance

Use phage presence as biomarkers for detecting bacterial contamination in environmental and clinical samples. Develop and refine phage typing methods for epidemiological tracking and strain differentiation of bacterial pathogens. Systematically evaluate immune responses to therapeutic phages in clinical trials, aiming to understand the impact of humoral and cellular immunity on phage therapy efficacy.

Future Research Directions

Improve physiologically appropriate and high-throughput approaches to study phage biology and host interactions in vivo, including microbiomes and immune system modulation. To completely understand phage diversity and function, use structural biology, genome sequencing, bioinformatics, immunology, and microbiology. Help researchers overcome immune neutralization and bacterial resistance to implement phage-based therapeutics in clinical practice. Increasing molecular and ecological understanding of bacteriophages, maximizing their therapeutic usage, and studying their functions in the human microbiome and immunity are crucial. These fields will improve phage therapy, biocontrol, and understanding of phage biology.

Directions for Future Research:

1. To enhance our understanding of genetic diversity and taxonomy, it is imperative to conduct additional genome sequencing and isolation of phages, particularly those that are dsRNA-based.
2. More research is necessary to investigate the molecular mechanisms of non-lytic infection cycles and host specificity.
3. It is imperative to have a more comprehensive understanding of the interactions between phages and mammalian immunity and microbiomes in order to optimize phage therapy.
4. The comprehension of phage-bacteria evolutionary arms races will be facilitated by the expansion of knowledge on phage-encoded CRISPR-Cas and anti-phage defense systems.
5. The development of biomarkers and epidemiological instruments that are based on phages will facilitate clinical and environmental surveillance.

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A Exploratory Study to Investigate the Prevalence of Vitamin D Deficiency Among Pregnant Women

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Abstract

This study aims to identify the prevalence of vitamin D deficiency among pregnant women attending Al-Jalaa Maternity Hospital in Tripoli, and those attending the Obstetrics and Gynecology Department at Al-Hadaba Al-Khadra Hospital. The study used a descriptive and analytical approach. The study also used a questionnaire to collect data and information, with a sample of (250) A pregnant woman attending Al-Jalaa Maternity Hospital in Tripoli, and attending the Obstetrics and Gynecology Department at Al-Hadaba Al-Khadra Hospital. The study also used a number of statistical methods to achieve its goals, including the arithmetic mean, the standard deviation, the percentage, the relative weight, the Pearson correlation coefficient, and the reliability coefficient. Cronbach's alpha, the reliability coefficient using the split-half method, and the t-test. The results of this study resulted in a low percentage of vitamin D deficiency according to the responses of the study sample members, and there was also a moderate awareness of the symptoms of vitamin D deficiency and its importance to the body. There are some symptoms of vitamin D deficiency in some pregnant women, although this does not confirm the existence of a deficiency of this vitamin in these women. The responses of the study sample members also confirmed that a large number of women do not follow a healthy diet because of its negative impact on the mother's health. And the fetus, and on the levels of vitamin D in the body, and after subjecting the study sample members to a test to determine the rate of vitamin D deficiency, the results indicated that there is a small percentage of the study sample members who suffer from vitamin D deficiency, and this deficiency is not to a large degree. There are statistically significant differences in the level of responses of the study sample members, which are attributed to the health institutions variable, in favor of Al-Jalaa Hospital for Women and Maternity in Tripoli.

Keywords: Vitamin D - women - pregnant women – hospitals.

Introduction:

Vitamin D deficiency represents a significant global health concern that has attracted considerable scientific attention due to its widespread prevalence across diverse populations. Although the existence of Vitamin D was suspected as early as the 17th century, it was not officially discovered until 1920 (Giovannucci et al., 2008). Classified among the fat-soluble vitamins (A, D, E, K) (Anaizi, 2010), Vitamin D can be obtained through natural dietary sources, fortified foods,

and nutritional supplements. However, the majority of the body's requirement-approximately 90%-is synthesized endogenously through skin exposure to ultraviolet B (UVB) sunlight Vitamin D functions as a hormone when synthesized in the body and as a vitamin when obtained through external sources (Tasset, 2014). Its deficiency has been directly linked to several musculoskeletal disorders, such as rickets in children and osteoporosis in adults, as well as to a range of chronic conditions, including cardiovascular diseases, infections, and diabetes (Basil et al., 2013) Beyond its physiological functions, Vitamin D has garnered research interest for its role in brain health and cognitive function Studies have highlighted its involvement in neurochemical balance and neurological processes, with implications for academic performance. Adequate Vitamin D levels are associated with enhanced learning, memory, and concentration, while deficiency may impair information retention, focus, and cognitive flexibility (Tolppanen, 2012). Furthermore, Vitamin D status has been linked to emotional and psychological well-being, with lower levels correlating with fatigue, depressive symptoms, and diminished academic motivation Although Vitamin D-rich foods such as fortified milk and fatty fish are commonly recommended (Muthanna et al., 2023), recent research has raised questions regarding the assumption that higher serum levels confer additional health benefits. Several studies have indicated that excessive levels of Vitamin D do not necessarily offer greater protection against disease, emphasizing the importance of maintaining optimal-not merely elevated-levels Recent investigations have also focused on the role of Vitamin D in brain development, as numerous receptors for the vitamin have been identified in brain regions critical to learning and memory processes (Abdulrahman et al., 2018). In addition to its role in cognitive health, Vitamin D is essential for maternal and fetal well-being during pregnancy. As a result, growing attention has been directed toward assessing pregnant women's awareness of Vitamin D, particularly in relation to its deficiency and health implications This study aims to explore the level of awareness regarding Vitamin D among a sample of pregnant women attending Al-Jalaa Maternity Hospital in Tripoli, as well as to examine the prevalence of deficiency in this population.

Research Problem:

Given the significant role that Vitamin D plays in overall human health-and particularly in the health of pregnant women -this study emerges within the framework of improving maternal healthcare services. Through the researcher's observation of the widespread prevalence of Vitamin D deficiency among pregnant women, and based on various collected observations and supporting information, the necessity of this study became evident Accordingly, the researcher conducted this study to explore the level of awareness among a sample of pregnant women attending Al-Jalaa Maternity Hospital in Tripoli, as well as those attending the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital, regarding the importance of Vitamin D. The study also aims to identify the symptoms associated with its deficiency and determine the prevalence rate of Vitamin D deficiency among these women.

Research Questions:

The study seeks to address the following research questions:

1- What is the prevalence rate of Vitamin deficiency among pregnant women attending a number of medical hospitals?

2- Are there statistically significant differences in the responses of the study sample that can be attributed to the variable of healthcare institutions?

Research Objectives:

This study aims to identify the prevalence rate of Vitamin D deficiency among pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and those visiting the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital.

Significance of the Study:

The significance of the current study can be highlighted through the following points:

1-The study provides valuable insight into the current field reality regarding the prevalence of Vitamin D deficiency among pregnant women attending Al-Jalaa Maternity Hospital in Tripoli.

2- It serves as a scientific contribution toward the development of medical and healthcare services offered to women, particularly in the context of pregnancy and childbirth.

3- The findings of the study may serve as a foundation for proposing recommendations and suggestions that could assist specialists in reducing cases of Vitamin D deficiency, thereby contributing to the protection of both mothers and their unborn children from future health complications.

Scope and Delimitations of the Study:

Topical Scope**:**

1- The study focuses: on identifying the prevalence rate of Vitamin D deficiency among pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and those visiting the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital**.**

2- Geographical Scope: The study was conducted at Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital, involving pregnant women receiving care at these institutions.

Time Scope: The study was carried out during the year 2024.

4- Human Scope: The study sample consisted of 250 pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital**.**

Study Procedures:

Research Methodology: To address the research questions, the researcher employed the descriptive-analytical method, as it is considered the most appropriate approach for studies aiming to investigate phenomena as they exist in reality.

Study Sample: The sample was selected through direct communication with a number of pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and those visiting the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital. The sample was randomly selected from the study population.

Pilot Sample: The pilot sample consisted of 30 pregnant women, including participants from Al-Jalaa Maternity Hospital in Tripoli and others from the Obstetrics

Number	Health institutions
15	**Al -Galaa Hospital for birth, Tripoli**
15	**Department of Women and Gynecology at Al Hadaba Green Hospital**
30	**The total number**

The table number (1) shows the distribution of the exploratory study sample

Actual Study Sample: The actual study sample consisted of 250 pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital.

The following table presents the distribution of the actual study sample according to the healthcare institutions**.**

Table No. (2) shows the distribution of the actual study sample according to the variable of medical institutions

The percentage	Number	Health institutions
60%	150 pregnant women	**Al -Galaa Hospital for birth, Tripoli**
40%	100 pregnant women	**Department of Women and Gynecology at Al Hadaba Green Hospital**
100%	250 pregnant women	**The total number**

Study Instrument:

The study instrument consisted of:

Questionnaire (prepared by the researcher):

The questionnaire included 14 items aimed at assessing the level of awareness among pregnant women regarding the symptoms of Vitamin D deficiency, its importance to human health, and its prevalence among women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital.

Medical Tests for Vitamin D Levels:

The study participants underwent medical testing to determine their serum Vitamin D levels. This was conducted to identify the prevalence of Vitamin D deficiency among the pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital.

Validity and Reliability of the Study Instrument:

1-Content Validity: The questionnaire was presented to a panel of five experts specialized in the field to review its items and provide feedback. Their opinions were carefully considered and incorporated to ensure the content validity of the instrument.

2- Internal Consistency Validity: The questionnaire was administered to a pilot sample of 30 pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital. The Pearson correlation coefficient was used to measure the correlation of each item with the overall instrument. The results were as follows:

Table No. (3) shows the correlation of each statement with the questionnaire using the Pearson correlation coefficient

Statistical significance	Person correlation coefficient	The number of arrest paragraphs
Statistically signified	0.788	**14 paragraphs**

Based on the results presented in the previous table, it was found that the correlation coefficients of all questionnaire items with the overall instrument were statistically significant at the 0.01 level, indicating that all items of the questionnaire exhibit a high degree of internal validity.

Reliability of the Study Instrument:

To assess the reliability of the questionnaire, the following method was used:

Cronbach's Alpha Coefficient: The Cronbach's alpha reliability coefficient was calculated to measure the internal consistency of the questionnaire. This analysis was conducted using the SPSS

statistical software, based on the data obtained from the pilot sample. The results are presented in the following table:

Table No. (4) presents the reliability analysis of the questionnaire, assessed using Cronbach's Alpha coefficient

Statistical significance	Alfirronbach stability coefficient	number of arrest paragraphs
Statistically signified	0.847	14 paragraphs

Based on the results presented in the previous table, it can be concluded that the questionnaire demonstrates a high level of reliability.

Split-Half Method: The questionnaire items were divided into two halves-odd-numbered items versus even-numbered items. The Pearson correlation coefficient was used to determine the correlation between the two halves. The reliability was then adjusted using the Spearman-Brown prophecy formula and the Guttman split-half coefficient, as shown in the following table:

Table (5) displays the reliability of the questionnaire as determined by the split-half method

Spearman and Bruun laboratory	Jetman laboratory	Person correlation coefficient	number of arrest paragraphs
0.882	0.862	0.877	14 paragraphs

Based on the results obtained from the previous table, it can be concluded that the questionnaire exhibits a high degree of reliability.

Data Collection: Data were collected through a paper-based questionnaire, which was distributed to a number of pregnant women attending Al-Jalaa Maternity Hospital in Tripoli and the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital. Out of 300 distributed questionnaires, a total of 250 completed questionnaires were returned. Additionally, medical tests for detecting Vitamin D deficiency were used to support the study findings.

Data Analysis: In order to achieve the objectives of the study and answer its research questions, the researcher utilized the Statistical Package for the Social Sciences (SPSS) to analyze the collected data. A set of appropriate statistical methods was employed, including the arithmetic mean, standard deviation, relative weight, percentage, frequency distribution, and the t-test to examine the significance of differences between means.

Study Results:

Results for Research Question:

What is the prevalence rate of Vitamin D deficiency among pregnant women attending various hospitals? To answer this question, the relative weights for each item in the questionnaire were calculated. The results were as follows:

Table (6) shows the relative weights of the responses of the study sample individuals to the questionnaire items

Relative weight	Phrases
30%	**I suffer from bone pain**
10%	**I suffer from muscle pain**
50%	**I feel fatigued even with minimal physical effort**
60%	**I experience mood swings**
72%	**I suffer from depression**
5%	**I suffer from hair loss**
48%	**I experience back pain**
15%	**I frequently contract infections**
26%	**My wounds heal slowly**
46%	**I am well aware of the importance of vitamin D for the body**
30%	**I am well aware of the symptoms of vitamin D deficiency**
75%	**I am in good health**
12%	**I am exposed to sunlight for sufficient periods**
18%	**I follow a healthy diet**
20%	**I have a vitamin D deficiency**

Based on the results obtained from the previous table, it is observed that there is a low reported prevalence of Vitamin D deficiency according to the responses of the study participants. Additionally, there appears to be a moderate level of awareness regarding the symptoms of Vitamin D deficiency and its importance to the body Some pregnant women reported symptoms that may be associated with Vitamin D deficiency; however, these symptoms alone do not confirm an actual deficiency. The participants' responses also indicated that a significant number of women do not

follow a healthy dietary regimen, which negatively affects both maternal and fetal health, as well as the body's Vitamin D levels.

After the study participants underwent clinical testing for Vitamin D deficiency, the results were as follows:

Table (7) shows the average vitamin D level among the study sample participants who visit Al-Jalaa Maternity Hospital in Tripoli

The percentage	The level of vitamin D in the blood	Study sample / Al -Galaa Hospital for birth, Tripol
86.66%	Normal	**130 women**
13.33%	Low rate	**20 women**

Based on the results obtained from the previous table, it was observed that a normal level of Vitamin D was found in a large portion of the study sample, with 130 participants (representing 86.66%) exhibiting adequate levels. In contrast, 20 participants (representing 13.33%) were found to have low Vitamin D levels.

As for the pregnant women in the study sample who were attending the Obstetrics and Gynecology Department at Al-Hadhba Al-Khadra Hospital, the results were as follows:

Table No. (8) shows the average level of Vitamin D among the study sample of women attending the Obstetrics and Gynecology Department at Al-Hadbah Al-Khadra Hospital

The percentage	The level of vitamin D in the blood	Study sample / Obstetrics and Gynecology Department at Al Hadaba Green Hospital
70 %	Normal	**70 women**
30 %	Low rate	**30 women**

Based on the results of the previous tables, we conclude the following:

Table No. (9) shows the overall results of the average Vitamin D levels among the study sample

The percentage	The level of vitamin D in the blood	Study sample
80 %	Normal	**200 woman**
20 %	Low rate	**50 woman**

Based on the results obtained from the previous table, it was observed that a normal level of Vitamin D was present in a large portion of the study sample, with 200 participants representing 80% of the total. In contrast, 50 participants (representing 20%) were found to have low Vitamin D levels.

Results for Research Question:

" There statistically significant differences in the responses of the study sample that can be attributed to the variable of healthcare institutions? To answer this question, the means, standard deviations, T-values, degrees of freedom, and significance levels for the responses of the study participants were calculated. The results were as follows:

Table No. (10) shows the use of several statistical methods to determine the level of differences in the responses of the study sample individuals attributed to the variable of healthcare institutions

Level of Significance	DV	T	Standard deviation	avg	Number	Health institutions
0.02	34	0.51	0.29	0.92	150	**Al -Galaa Hospital for birth, Tripoli**
			0.49	0.57	100	**Green Plateau Hospital**

The results obtained from the previous table indicate the presence of statistically significant differences at the 0.01 significance level, attributed to the variable of healthcare institutions, favoring Al-Jalaa Maternity Hospital for Women and Childbirth in Tripoli.

Conclusions:

After a thorough examination of the chapters of this study, the following conclusions can be drawn:

1-There is a low prevalence of Vitamin D deficiency based on the responses of the study participants.

2-There is a moderate level of awareness regarding the symptoms of Vitamin D deficiency and its importance to the body among the study participants.

3-The responses indicated that a significant number of women do not follow a healthy diet, which negatively impacts both maternal and fetal health, as well as Vitamin D levels in the body.

4-After subjecting the study sample to a test for Vitamin D deficiency, the results indicated that a small proportion of participants were found to have a deficiency. However, the deficiency was not of a severe degree.

5-There were statistically significant differences in the responses of the study sample, attributed to the variable of healthcare institutions, in favor of Al-Jala Women's and Maternity Hospital in Tripoli.

Recommendations:

Following the comprehensive journey through this study, the researcher recommends the following:

1-Emphasize the importance of a healthy diet for women, especially during pregnancy stages.

2- Raise community awareness about the importance of Vitamin D, its symptoms, and preventive measures through educational lectures, seminars, and various media programs (visual, audio, and stages.

3- Encourage individuals to visit healthcare facilities for medical check-ups upon experiencing any symptoms of Vitamin D deficiency.

4- Advise against the unsupervised use of medications and dietary supplements, as this may pose serious health risks.

5- Drioritize the healthcare and monitoring.

Suggestions:
The researcher suggests the following:
Conducting further studies that examine the subject of this research with greater precision and depth. Healthcare authorities should consider the results of this study, along with previous and future related studies, and incorporate them into research planning and policy-making.

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- All studies and research sent are subject to dual objective scientific arbitration by the Reading Committee, with complete confidentiality, scientific honesty, transparency and complete impartiality, so that the journal has the right to make some necessary formal amendments to the research sent for publication without prejudice to its content, and by specialized arbitrators with experience, knowledge and a distinguished scientific reputation

The Original Article formats

Papers reporting original research findings should follow this format; Title page, Abstract, Background, Objectives; Methods; Results; Discussion, Conclusion, acknowledgement and references. If the research is a review article, the main and sub-headings are classified according to the topic and scientific content. **Title page;** manuscript's title, all authors full names, highest degrees and affiliations. The corresponding author both e-mail address and fax number should be provided.

Abstract; should be structured in this format: Background; Aims; Methods; Results; and Conclusion.
An Arabic summary with the title, authors' names, highest degrees and affiliations must be provided.

Keywords 4 –8 keywords should be provided.

Background: State the purpose of the article and its rationale. Give only pertinent references and do not include data from the work being reported.

Methods

A clear description of the methodology used in the study (patients, laboratory materials and other methods used) as well as the subjects' selection. Inclusion and exclusion criteria should be mentioned. Identify apparatus (give the manufacturer name and address in parenthesis) and procedures in sufficient details. Identify precisely all chemicals and drugs used. State clearly the nature of the study, the tools used in data collection and statistical methods used for each analysis.

Results

Present results in logical sequence. Emphasize and summarize only important findings. Results may be presented in form of text, tables and figures. Avoid repetition of data in the three forms. Tables and figures should be accompanied with clear descriptive legends.

Discussion

This part should focus on discussing the results obtained. Avoid repetition of the results. Relate the observations and findings to other relevant studies. This part ends with "In conclusion" which should summarize the final outcome of the study, linked with the objectives of the study and should be supported by study data.

Acknowledgement

This also includes Authors contributions, Conflict of Interest, Ethical Clearance

References

To follow the Vancouver style of referencing. They should be numbered consecutively in the order they are first mentioned – Arabic numbers. All references must be cited in the text or tables. All six authors must be provided. If there are more than six authors, then write only the first 3 authors and et al. They should contain the following elements as appropriate: name(s) and initial(s) of author(s); title of paper or book in its original language plus translation; for research articles, abbreviated name of journal plus volume number ,Issueand page range; for books and other texts, place of publication (city and country) and name of publisher (commercial or institutional); and date of publication; for texts published exclusively on the Internet, exact URL of the page cited and date when last accessed.

Tables & Figures including the legends must be placed at the end of the manuscript. Number tables and figures consecutively in order of their first citation in the text and supply a brief title for each table or figure. Give each column a short or abbreviated heading. Explain in footnotes all nonstandard abbreviations that are used in each table.

Review articles:

(i.e. critical assessments of research on topics of relevance to health problems and health sciences). These should contain sections dealing with objectives, sources, methods of selection, compilation and interpretation of data and conclusions. The text should not exceed 25 page (excluding the accompanying abstract, references, tables and figures), and should be accompanied by an abstract of not more than 250 words. The number of tables and figures should not exceed 5

Case reports:

Only reports of cases of an unusual nature are considered for publication. Text should include an Introduction, the Report of the case(s) and a Discussion. The text should not exceed 1500 words and the number of references kept to a minimum. The abstract should not exceed 150 words.

Reports

Manuscript specifications (length, references, tables/ figures) are the same as a research article, but abstract length should not exceed 150 words.

Short research communications

Articles which do not constitute a complete research study but are of particular relevance or importance to health issues in the region may be considered for publication. The text should not exceed 1500 words (excluding references), and should be accompanied by a structured abstract of not more than 150 words. The number of tables and figures should not exceed 3.

Commentaries

Manuscript specifications (references, tables/figures) are the same as a short research communication, but maximum length is 1000 words. The abstract (unstructured) for submissions purposes should not exceed 150 words.

Letter to Editor

Should be no more than 400 words, no more than 4 references, no more than one table or figure and no more than 4 authors.

Academic activities

Journal of contemporary medical sciences to health Sciences publishes short reports of academic activities (seminars, conferences and workshops), the report should include; a brief description of the activity, it's main objectives, summary of the important papers presented and the main recommendations of that activity.

Units and measurements

Give measurement of length, height and weight in metric units. Give all hematological and clinical chemicals in SI units; equivalent values in traditional units could be used with or without the SI units.

Abbreviations and Symbols

Use only standard abbreviations. Avoid abbreviations in title and abstract. The full term for which abbreviation stands should precedes its first use in the text.

Ethics

In experiment on human subjects should follow the guidelines of Ethics Committee (country or institution). The manuscript should include a statement confirming that an informed consent was obtained from each subject.

Research Paper should be sent in the form of a Microsoft word to the following email

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