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Front Pages

NAJWA ALHARBİ & REHAM YAHYA
Characterization of a Multidrug-resistant Escherichia Coli Lytic Bacteriophage Isolate as a Safe Alternative to Synthetic Antibiotics

SIDDHARTH GUPTA & PRATIK MAN SHRESTHA
A Research Study on Tobacco Associated Oral Potentially Malignant Disorders (OPMDs) Prevalent in Oral Mucosa of Lumbini Province/District Rupandehi Population of Nepal

KOKO WANGJAM & NAOREM SHARAT SINGH
Effects of Socio-Economic and Health Variables on Survival Time of HIV/AIDS Patients in Manipuir

SHANMUGA NATHAN SELVARAJ, ARULCHELVAN SRIRAM & SUGANYA S
Impact of Obesity-Related Social Media Contents on Urban Men: Application of the Health Belief Model

Athens Journal of Health and Medical Sciences

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Download the entire issue (PDF)

Front Pages i-x

Characterization of a Multidrug-resistant Escherichia Coli Lytic Bacteriophage Isolate as a Safe Alternative to Synthetic Antibiotics 179
Najwa Alharbi & Reham Yahya

A Research Study on Tobacco Associated Oral Potentially Malignant Disorders (OPMDs) Prevalent in Oral Mucosa of Lumbini Province/District Rupandehi Population of Nepal 197
Siddharth Gupta & Pratik Man Shrestha

Effects of Socio-Economic and Health Variables on Survival Time of HIV/AIDS Patients in Manipuir 205
Koko Wangjam & Naorem Sharat Singh

Impact of Obesity-Related Social Media Contents on Urban Men: Application of the Health Belief Model 215
Shanmuga Nathan Selvaraj, Arulchelvan Sriram & Suganya S

Athens Journal of Health and Medical Sciences
Editorial and Reviewers' Board

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The current issue is the fourth of the eleventh volume of the *Athens Journal of Health and Medical Sciences* (AJHMS), published by the **Health & Medical Sciences Division of ATINER.**

Gregory T. Papanikos
President
ATINER

Athens Institute for Education and Research

A World Association of Academics and Researchers

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The Health Economics & Management Unit of ATINER will hold its **24th Annual International Conference on Health Economics, Management & Policy, 23-26 June 2025, Athens, Greece s**ponsored by the Athens Journal of Health and Medical Sciences. The aim of the conference is to bring together academics, researchers and professionals in health economics, management and policy. You may participate as stream leader, presenter of one paper, chair of a session or observer. Please submit a proposal using the form available (https://www.atiner.gr/2025/FORM-HEA.doc).

Academic Members Responsible for the Conference

- **Dr. Paul Contoyannis**, Head, Health Economics & Management Unit, ATINER & Associate Professor, McMaster University, Canada.
- **Dr. Vickie Hughes**, Director, Health & Medical Sciences Division, ATINER & Assistant Professor, School of Nursing, Johns Hopkins University, USA.

Important Dates

- Abstract Submission: **3 March 2025**
- Acceptance of Abstract: 4 Weeks after Submission
- Submission of Paper: **26 May 2025**

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5-8 May 2025, Athens, Greece

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Important Dates

- Abstract Submission: **21 January 2025**
- Acceptance of Abstract: 4 Weeks after Submission
- Submission of Paper: **7 April 2025**

Academic Member Responsible for the Conference

- **Dr. Vickie Hughes**, Director, Health & Medical Sciences Research Division, ATINER & Assistant Professor, School of Nursing, Johns Hopkins University, USA.
- **Dr. Carol Anne Chamley**, Head, Nursing Research Unit & Associate Professor, School of Health and Social Care, London South Bank University UK.
- **Dr. Andriana Margariti**, Head, Medicine Research Unit, ATINER & Lecturer, Centre for Experimental Medicine, Queen's University Belfast, U.K.

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Athens Journal of Health & Medical Sciences – Volume 11, Issue 4, December 2024 – Pages 179-196

Characterization of a Multidrug-resistant *Escherichia Coli* Lytic Bacteriophage Isolate as a Safe Alternative to Synthetic Antibiotics

By Najwa Alharbi[*] & Reham Yahya[±]

The control of pathogenic bacteria depends mainly on the use of antibiotics; however, several problems can arise due to the overuse of broad-spectrum antibiotics that destroy not only pathogens, but also natural beneficial microbes in the gut micro-flora. Furthermore, the development of antibiotic resistance poses a major challenge in clinical medicine. Therefore, there is an urgent therapeutic need to develop safe alternative bactericidal or bacteriostatic approaches that will selectively kill only pathogens without disrupting the micro-flora. One such approach is the use of bacteriophages to suppress the growth of bacterial pathogens in humans and other animals. Sewage water can be a rich source of pathogenic bacteria and their lytic phages; thus, we sampled sewage water in this study and successfully isolated an Escherichia coli strain and its lytic phage. We demonstrated that the phage could repress bacterial growth of Salmonella enterica (ATCC 14028), Klebsiella oxytoca (ATCC49131), Shigella sonnei (ATCC 25931), and E. coli (ATCC25922). The isolated phage was stable at room temperature, but survival declined with an increase in temperature to 70 °C, and complete inhibition was observed at temperatures ≥80 °C. In addition, the phage was stable over a pH range of 6–8, and was completely inactivated at pH 14.

Keywords: *bacteriophage, Escherichia coli strain M00057, EC phage, multidrug- resistance*

Abbreviations: *EC phage, Escherichia coli lytic bacteriophage; EHEC, enteric hemorrhagic E.coli; PFU, plaque forming units; STEC, Shiga toxin-producing E. coli; Stxs, Shiga toxins; TEM, transmission electron microscopy*

Introduction

Antibiotics are widely used to control pathogenic bacteria; however, excessive use poses several problems. In particular, broad-spectrum antibiotics may destroy the pathogen of concern, but they may also destroy beneficial microbes in the gut micro-flora. Furthermore, excessive antibiotic use can lead to the development of antibiotic resistance, which is a major challenge facing clinical medicine today (Cieplak et al. 2018). Safe alternative bactericidal or bacteriostatic approaches need to be developed that will selectively kill only the pathogen, and

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not lead to resistance. One of these approaches is the use of bacteriophages to repress the growth of bacterial pathogens in humans and other animals. One of the unique features of bacteriophages is their high host specificity; bacteriophages usually attack only specific bacterial strains and they do not infect the cells of any human organs, unlike broad-spectrum antibiotics (Woolston et al. 2013). Bacteriophages also play an important role in Figurehting some pathogenic viruses; therefore, they may contribute to the formulation of vaccines as well as antiviral compounds (Wdowiak et al. 2022).

Several bacterial species commonly cause diseases in humans and other animals; however, some of these bacteria are also commensal within the gut. For example, *Escherichia coli* lives within the digestive tract of humans and warm-blooded animals, but it also causes the highest frequency of infections in healthcare settings (Kaper et al. 2004, Nguyen et al. 2012). Furthermore, *E. coli* is involved in the progress of diseases caused by other types of bacteria; for instance in the urinary tract, bloodstream, and intra-abdominal infections, in addition to enteritis and neonatal meningitis. In contrast, several research studies have documented that the majority of *E. coli* strains are harmless to humans.

Severe foodborne diseases are associated with particular types of *E. coli*, e.g., Shiga toxin-producing *E. coli* (STEC). STEC is passed to humans mainly through eating contaminated foods, such as undercooked ground meat, raw milk or milk products, and uncooked vegetables or their products (Kaper et al. 2004, Nguyen et al. 2012). *E. coli* was first described by Theodor Escherich in 1885 as an anaerobic, rod-shaped bacterium. *E. coli* pathogenicity comes from the acquisition of virulence factors from phages, such as plasmids and transposons.

Based on clinical symptoms, serogroups, virulence factors, and mechanisms of pathogenicity, *E. coli* can be classified as enteric hemorrhagic or invasive *E. coli* (EHEC) (Kaper et al. 2004, Nguyen et al. 2012). EHEC as a group was recognized previously to include pathogenic strains of *E. coli* that secrete Shiga toxins (Stxs). These toxins lead to certain illnesses, such as hemorrhagic colitis. Moreover, infection by hemolytic *E. coli* can be life-threatening disease.

Phages, also known as bacteriophages, were first discovered by Frederick Twort and Félix d'Hérelle in 1915 and 1917 respectively. These virus particles consist of genetic material enclosed in a protein capsid or head, often accompanied by a protein tail. The term "bacteriophage" literally translates to "something that eats bacteria." Biologists consider phages as natural parasites or enemies of bacteria, playing a crucial role in maintaining microbial balance on Earth (Wittebole et al. 2014). Tailed phages, belonging to the Cudoviricetes class, encompass various types such as podoviruses, myoviruses, and siphoviruses (Liu et al. 2021), along with the polyhedral Microviridae family. These phages are commonly associated with applications in phage therapy (Harper and Enright 2011, Lin et al. 2017). Scientists attribute the antibacterial effectiveness of phages to a well-established life cycle, which can be either lytic or lysogenic. In certain situations, phages have the ability to adapt and employ both lysogenic and lytic strategies, known as pseudolysogenicity, as alternative infection pathways in response to different host strains, host physiology, and changes in the environment (Mäntynen et al. 2021). Given their widespread presence, it is not surprising that

phages can be found and isolated from various sampling sites, including feces, seawater, sewage, soil, sludge, and any environment where bacteria can thrive (Khawaja et al. 2016, Bhetwal et al. 2017). Bacteriophages (phages) were discovered by Frederick Twort and Félix d'Hérelle in 1915 and 1917, respectively. Phages are virus particles consisting of genetic material wrapped in a capsid or head, most often with a protein tail. The term "bacteriophage" literally means "something that eats bacteria." Biologists describe them as natural parasites (or enemies) of bacteria and associate them with maintaining microbial balance on Earth (Wittebole et al. 2014). Tailed phages of the class Cudoviricetes, which includes podoviruses, myoviruses, siphoviruses (Liu et al. 2021), and the polyhedral Microviridae family are commonly associated with applications in phage therapy (Harper and Enright 2011, Lin et al. 2017).

Scientists attribute the antibacterial activity of phages to an established life cycle (lytic or lysogenic). In some cases, phages adapt both lysogenic and lytic strategies (i.e., pseudolysogenicity) as alternative infection steps in response to different host strains, host physiology, and environmental changes (Mäntynen et al. 2021). Because phages are ubiquitous, it is not surprising that phages can be isolated from a variety of sampling sites such as feces, seawater, sewage, soil, sludge, and anywhere bacteria can grow (Khawaja et al. 2016, Bhetwal et al. 2017). A number of studies have focused on phages that control *E. coli*; these phages usually present in sewage, polluted river water, hospital waste water, and the feces of humans and other animals (Dalmasso et al. 2016). Our aim in this study was to isolate a safe alternative agent, such as a bacteriophage, in sewage water that could be exploited to specifically repress the development of pathogenic bacteria.

Materials and Methods

Phage Host Bacteria Isolation and Identification

Sample Collection

This study was carried out between October 2020 and December 2020. A grand total of 30 samples of sewage were gathered from the waste disposal site in Jeddah, with the gracious authorization of the sewage plant management. These samples were meticulously collected in hermetically sealed zip-lock plastic bags, ensuring the preservation of aseptic conditions. Each sample contained a volume of 50 ml and was promptly stored at a temperature of 4 °C, diligently labeled to denote their origin and specific location. To ensure their integrity, the collected samples were then transported to the esteemed King Fahd Medical Research Center (KFMRC) microbiology laboratory in an ice box, where they were carefully maintained at refrigerator temperature until they could be processed.

Isolation of Bacteria from Sewage Sample

The sample underwent thorough agitation to achieve a uniform mixture, and it was subsequently filtered using a syringe filter with a pore size of 0.22 μm

before a portion was extracted for culturing. All bacteriological analyses followed standard protocols. Wastewater samples were subjected to serial 10-fold dilutions in sterile distilled water. This dilution process was based on the principle that obtaining a reduced number of bacterial colonies in the presence of water samples would yield purer colonies. Each sample, measuring one milliliter, was mixed with nine milliliters of sterile distilled water and subsequently subjected to serial dilution, starting from one tube containing nine milliliters of sterile distilled water and progressing to the next tube in a 10-fold dilution until an 8-fold dilution was achieved. Subsequently, 0.5 milliliters of the desired serial dilutions of bacterial suspensions were plated for bacterial cultivation. Duplicate samples were plated on various differential media, including MacConkey (MAC) agar, Eosin methylene blue agar (EMB), Blood agar, S.S agar, and nutrient agar. The plates were then incubated at a temperature of 37 ºC for a period of 24-48 hours. Identification of the most significant pathogenic bacteria present in the sewage water was carried out based on colony appearance, gram staining, growth on selective media, and biochemical testing, as per the standard methods outlined in the examination of water and wastewater by the American Public Health Association (1999).

Pure colonies were obtained by subculturing on MacConkey (MAC) agar, Eosin methylene blue agar (EMB), Blood agar, S.S agar, and nutrient agar. Once pure colonies were obtained and their important characteristics were recorded, the isolated organisms were further identified to the species level using biochemical methods following standard procedures outlined by Vandepitte (1996). Upon successful growth of the microorganisms, the pure cultures of bacteria were sub-cultured in slants, nutrient broth, and glycerol stock. These cultures were then incubated at a temperature of 37 °C to promote vigorous growth and subsequently preserved in 20% glycerol vials at -80 °C, as described by Williams (1971). The sample was thoroughly shaken to get a homogenous mixture, and filtrated by syringe filter (0.22 µm) before a portion was taken for culture. Standard procedures were followed in all bacteriological analyses. Serial 10-fold dilutions of wastewater samples were prepared in sterile distilled water. Serial dilution was done based on the principle that when water samples along with bacterial colonies are taken, the result obtained in the form of reduced bacterial colonies would be more appropriate to get pure colonies. One ml of each sample was poured into nine ml of sterile distilled water and serially diluted from one tube (containing 9 ml sterile distilled water) to the next in a 10-fold dilution until an 8-fold dilution was reached. The bacteria were cultivated by plating 0.5 ml each of the desired serial dilutions of the bacterial suspensions. Duplicate samples were plated onto differential media, such as MacConkey (MAC) agar, Eosin methylene blue agar (EMB), Blood agar, S.S agar and nutrient agar, then incubated at 37 ºC for 24-48 hours. Identification of the most important pathogenic bacteria found in sewage water was done based on their colony appearance, gram staining, growth on selective media and biochemical test according to standard methods for examination of water and wastewater (American Public Health Association 1999).

Pure colonies were prepared by subculturing on MacConkey (MAC) agar, Eosin methylene blue agar (EMB), Blood agar, S.S agar and nutrient agar. After

obtaining pure colonies and recording important features, isolated organisms were further identified to the species level biochemically, following standard methods (Vandepitte 1996). After successful growth of microorganisms, the pure cultures of bacteria were sub-cultured in slants, nutrient broth and glycerol stock; incubated at 37 °C to achieve vigorous growth and then preserved in 20% glycerol vials at -80 °C (Williams 1971).

Molecular Identification of the Bacterial Isolate Through 16S rRNA Gene Sequencing

The isolated bacteria were cultivated in nutrient broth medium for 24 h. The cells were then harvested using centrifugation and subjected to genomic DNA extraction using a QlAamp® DNA Mini Kit (Venlo, Limburg, Netherlands) according to the manufacturer's guidelines, with minor changes as previously recommended (Atashpaz et al., 2010). The extracted genomic DNA was used to amplify the 16S rRNA gene, employing an MJ Research Peltier Thermal Cycler (Watertown, MA. USA). The following primers were used: 5′-AGAGTTTGATCMTGGCTCAG-3′ (forward) and 1492R 5′-TACGGYTACCTTGTTACGACTT-3′ (reverse). The amplification products of the PCR were cleaned up utilizing a Millipore kit (Sigma-Aldrich, Burlington, MA, USA) according to the manufacture instruction, and then sent to Macrogen (Seoul, South Korea) for sequencing.

Isolation of the Bacteriophage

Collection and Transport of Samples

Phages were isolated from the thirty previously-described samples. The selection of wastewater sample collection sites was based on the amount of pollution in the water sample, in addition to the distance between the collection site and the laboratory analysis site. In each case, the samples were collected under aseptic conditions and placed in screw cap 50 ml bottles. All samples were then transported in an icebox to the KFMRC and stored until further processing within 2 h.

Spot Assay of Bacteriophage Lytic Activity

A spot assay was used to assess bacteriophage lytic activity against host strains according to Pereira et al. (2011). Following incubation of the plates at suitable conditions (37 °C for 24 h), they were scanned for the existence of any plaques associated with the added lysate. If a clear plaque was detected from the tested phages, they were identified as virulent phages.

Bacteriophage Purification and Titration Assay

Identified phage were purified using a top agar (soft agar) overlay protocol according to Gencay et al. (2017). To confirm the purification of a phage, the protocol was repeated three times, until the appearance of single plaques characterized by the same morphological features. The filtrate containing the purified phage was stored at 4 °C. A double-layered protocol was applied to assay

the phage titers, as well as to determine the concentration of each bacteriophage according the following formula (https://barricklab.org/twiki/bin/view/Lab/ ProtocolsPhageTiters (https://barricklab. org)):

$$\text{Titration of phage} = \frac{\text{PFU}}{mL} = \frac{\text{Plaque number}}{\text{D} \times \text{V}}$$

where PFU stands for plaque forming units, D is the quantity of phage (10^8), and V is the purified phage volume.

Effect of Physical Conditions on Phage Stability

Assay of Phage Thermal Stability

The isolated phage were subjected to thermal stability testing using the procedures of Jurczak-Kurek et al. (2016). Briefly, 100 µL (108 PFU/mL) of phage lysate were collected in an Eppendorf tube under sterile conditions and then incubated for 120 min at temperatures ranging from 30–90 °C in a water bath**.** At each temperature, the bacteriophage titer was determined using the double-layer agar protocol. The stability percentage was calculated based on the ratio of titers of live phage remaining after treatment, to that prior to treatment (×100). The thermal stability data were plotted using Excel 2010 software (mean of triplicate experiments vs. time).

Assay of Phage pH Stability

The pH stability of the phage was assessed by making a slight modification to the procedure outlined by Verma et al. (2009). To create a pH range of 2–14, 1 M HCl or 1 N NaOH, prepared in nutrient broth, was added drop by drop. The pH values were measured using a pH meter (JENWAY, Bibby Scientific Ltd, Stone, UK). Following this, 1 mL of phage suspension was added to 9 mL of growth medium that had been adjusted to a specific pH and then incubated at 37 ºC for 3 hours. The phage titer at each pH was subsequently determined using the double-layer agar technique, and the percentage of surviving phage was calculated. To ensure accuracy, triplicate experiments were conducted to calculate the mean survival percentages. These data were then plotted as a pH stability curve using Excel 2010 software.

Determination of Phage Host Range

The range of hosts that the phage isolated in this study can infect was evaluated using different strains of bacteria, specifically six bacterial species. The evaluation method used was based on the approach described by Jurczak-Kurek et al. in 2016. To carry out the evaluation, a lawn of each bacterial species was prepared by mixing 1 mL of a bacterial culture suspension that had been incubated for 12 hours with liquefied soft agar containing 7% agar. The molten agar mixture was then poured onto the surface of nutrient agar plates. Next, 5 mL of a purified lytic phage lysate, with a concentration of 108 plaque-forming units (PFU) per milliliter, was spotted onto the bacterial lawn. The plates were then incubated for

12 hours at a temperature of 37 ºC. The lytic potential of the phage lysate was assessed by examining the appearance of plaques that formed on the bacterial lawn. The results were recorded as follows: no plaques (-), turbid plaques (+), or clear plaques (++).

The host range of the isolated phage was tested against different strains of bacteria (six bacterial species) based on the method of Jurczak-Kurek et al. (2016). Briefly, each bacterial species was prepared as a lawn by mixing 1 mL of 12 h-incubated bacterial culture suspension with liquefied soft agar (7% agar). The prepared molten agar was then poured over the surface of the nutrient agar in the plates. Subsequently, 5 µL of purified lytic phage lysate (10^8 PFU/mL) was spotted onto the lawn of bacteria and the plates were incubated for 12 h at 37 ºC. The lytic potential of the phage lysate was determined based on the clearness of the produced plaques and recorded as no plaques (-), turbid plaques (+), or clear plaques (++).

Examination of Phage Using Transmission Electron Microscopy (TEM)

The phage isolates were analyzed using transmission electron microscopy (TEM), specifically the Hitachi H-7000 instrument in Tokyo, Japan. The technique employed was based on the method described by van Regenmortel et al. (2000), with some minor modifications. In brief, a volume of 20–30 µL of the purified phage lysate (at a concentration of 108 plaque-forming units per milliliter) was applied onto a 400-mesh carbon-coated copper grid and allowed to incubate for 2 minutes. Subsequently, the samples were treated with a solution of phosphotungstic acid (2% weight/volume, pH 7.0) for staining purposes. Excess stain was eliminated by gently blotting the side of the grid with soft paper, followed by air-drying of the samples. The examination of the prepared samples was conducted at magnifications ranging from 15,000 to 25,000 times, using an accelerating voltage of 80 kilovolts. Based on the TEM images acquired, the classification of the phage isolate was determined by referencing the available data on the International Committee on Taxonomy of Viruses website (https://talk.ictvonline.org/).

Results

Isolation of Bacteria from Urban Sewage

This research was conducted using three samples of urban wastewater collected in Jeddah, Saudi Arabia. A total of 30 bacteria found in the wastewater were isolated. These bacteria were divided into three groups: ten isolates from sample I (coded as MB1, MB2, MB3, MB4, MB5, MB6, MB7, MB8, MB9, and MB10), ten isolates from sample II (coded as MN1, MN2, MN3, MN4, MN5, MN6, MN7, MN8, MN9, and MN10), and ten isolates from sample III (coded as MB11, MB12, MB13, MB14, MB15, MB16, MB17, MB18, MB19, and MB20). Additionally, two pathogenic isolates were obtained from King Abdelaziz hospital.

After analysing the bacteria on the plates, we selected six strains of E.coli for

further identification, which accounted for 31.57% of the total isolates. Table 3.1 provides a summary of the micromorphology characteristics of the bacterial strain MB1. As the colonies of the isolated bacteria developed on nutrient agar plates, they displayed various morphological features (Figures 1-2).

Bacterial Identification

The tables labelled 1 and 2 showcase the biochemical and sugar fermentation test outcomes for the bacterial strain MB1 that has been isolated. In Table 3.1, the identified bacteria are presented after undergoing phenotypic characterization.

The biochemical and sugar fermentation test results of the isolated bacterial strain MB1 are represented in Tables 1 and 2. The bacteria as identified, following phenotypic characterization, are depicted in Table 1.

Table 1. *Colony and Cell Characteristics of the isolated Bacterial Strains from Urban Sewage*

	Bacterial Isolation	**Colony features**		**Cell feature - Name of Bacteria**		
		Color of colony	**Type of Media**	**Gram stain**	**Cell morphology**	**Bacterial identity by 16s RNA**
1	MB1	Metallic green	EMB	Negative	Rod	*Escherichia coli* strain M00057

Morphology and Biochemical Tests of Bacterial Isolate

Several biochemical tests were used to document the properties of the bacterial isolates found. The results of most of the biochemical tests were negative; however, tests for D-mannitol, D-trehalose, lysine decarboxylase, D-glucose, D-mannose, coumarate, D-sorbitol, β-galactosidase, D-sorbitol, and β-glucoronidase were positive (Table 2).

Figure 1. *Results for Gram Stain of Bacteria Isolated from Wastewater Samples*

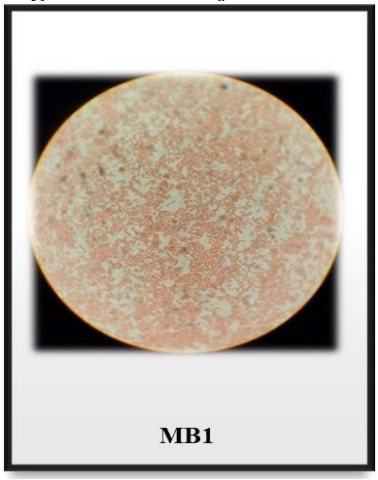


Figure 2. *Colony Characteristics of Bacteria Isolated from Wastewater Samples*

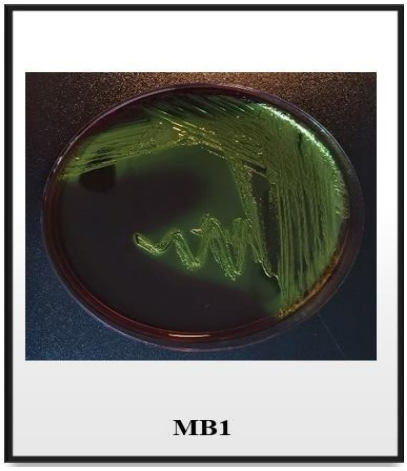


Table 2. *Biochemical Test Results for Bacteria Isolated from Wastewater Samples and a Hospital Clinic*

Biochemical test	**Results**
β-Glucoronidase	+ve
D-Mannitol	
D-Trehalose	
Coumarate	
β-Galactosidase	
D-Sorbitol	
Lysine decarboxylase	
D-Glucose	
D-Mannose	
Production of H_2S	-ve
β-Glucosidase	
L-Proline arylamidase	
Saccharose/Sucralose	
L-Lactate alkalinization	
Glycine arylamidase	
O/129 Resistance	
Adonitol	
β-N-acetyl-glucosaminidase	
D-Maltose	
Lipase	
D-Tagatose	
α-Glucosidase	
Ornithine decarboxylase	
Glu-Gly-Arg-arylamidase	
L-Pyrrolydonyl arilamidase	
Glutamyl arylamidase pNA	
Palatinose	
Succinate alkalinization	
L-Malate assimilation	
L-Arabitol	
Tyrosine arylamidase	
Citrate (sodium)	
β-N-acetyl-galactosaminidase	

L-Histidine assimilation	
Ellman	
D-Cellobiose	
γ-Glutamyl-transferase	
β-Xylosidase	
Urease	
Malonate	
Alpha-galactosidase	
L-Lactate assimilation	
Fermentation-glucose	
β-Alaninearylamidase pNA	
5-Keto-D-gluconate	
Phosphatase	
α-Phe-Pro-arylamidase	

Sensitivity of *E. coli* Strain to Different Antibiotics

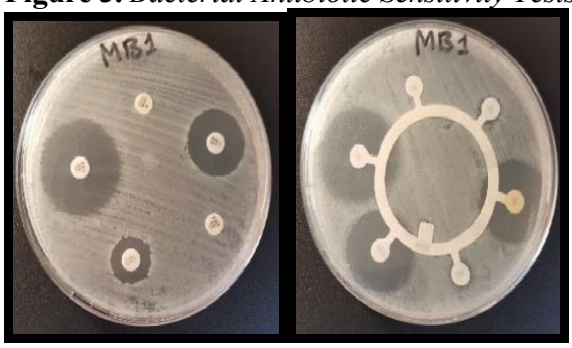
The isolated *E. coli* strain MB1 was susceptible to a number of antibiotics, including gentamicin (aminoglycoside), norfloxacin (fluoroquinolone), and colistin sulfate (polymycin), with inhibition zones of 20 mm, 24 mm, and 15 mm, respectively (Table 3, Figure 3). In contrast, MB1 was resistant to ampicillin, cefoxitin, cephalothin, nalidixic acid, and amoxycillin.

Table 3. *Sensitivity of Isolated E. coli Strain to Different Antibiotics*

		Antibiotic	Result	MIC
ViTEK2 2 technique		Ampicillin	R	-
		Tazobactam/Piperacillin	S	≤ 4
		Imipenem	-	-
		Amikacin	-	-
		Cefepime	-	-
		Ceftazidime	-	-
		Meropenem	-	-
		Gentamicin	-	-
		Tigecycline	-	-
		Ciprofloxacin	-	-

	Antibiotic	Result	MIC
Agar disc diffusion technique	Nitrofurantoin	S	22 mm
	Norfloxacin	S	24 mm
	Cotrimoxazole	S	27 mm
	Nalidixic acid	R	-
	Cephalothin	R	-
	Sulphamethoxazole	S	29 mm
	Gentamicin	S	20 mm
	Colistin sulphate	S	15 mm
	Amoxycillin	R	-
	Cefoxitin	R	-

Figure 3. *Bacterial Antibiotic Sensitivity Tests by the Disc Diffusion Method*



Phylogenetic Analyses

Sequences were analyzed for similarity against existing databases using the BLAST (Basic Local Alignment Search Tool) network service provided by the National Center for Biotechnology Information (NCBI). The BLAST tool, developed by Altschul et al. in 1997, allows for the comparison of sequences and identification of potential matches. Additionally, partial sequences of the 16S rRNA gene were compiled using the AlignIR 2.0 Fragment Assembly and Contig Editor software.

Figure 4. *16S rRNA Gene of Bacteria (1100bp)*

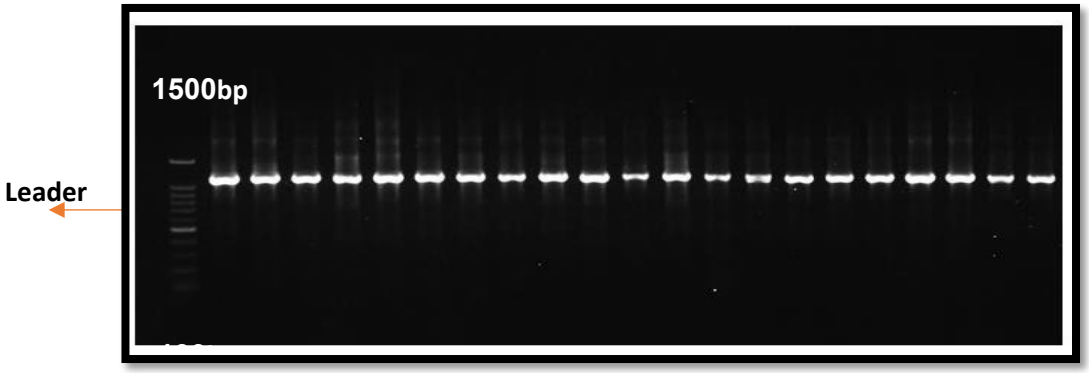
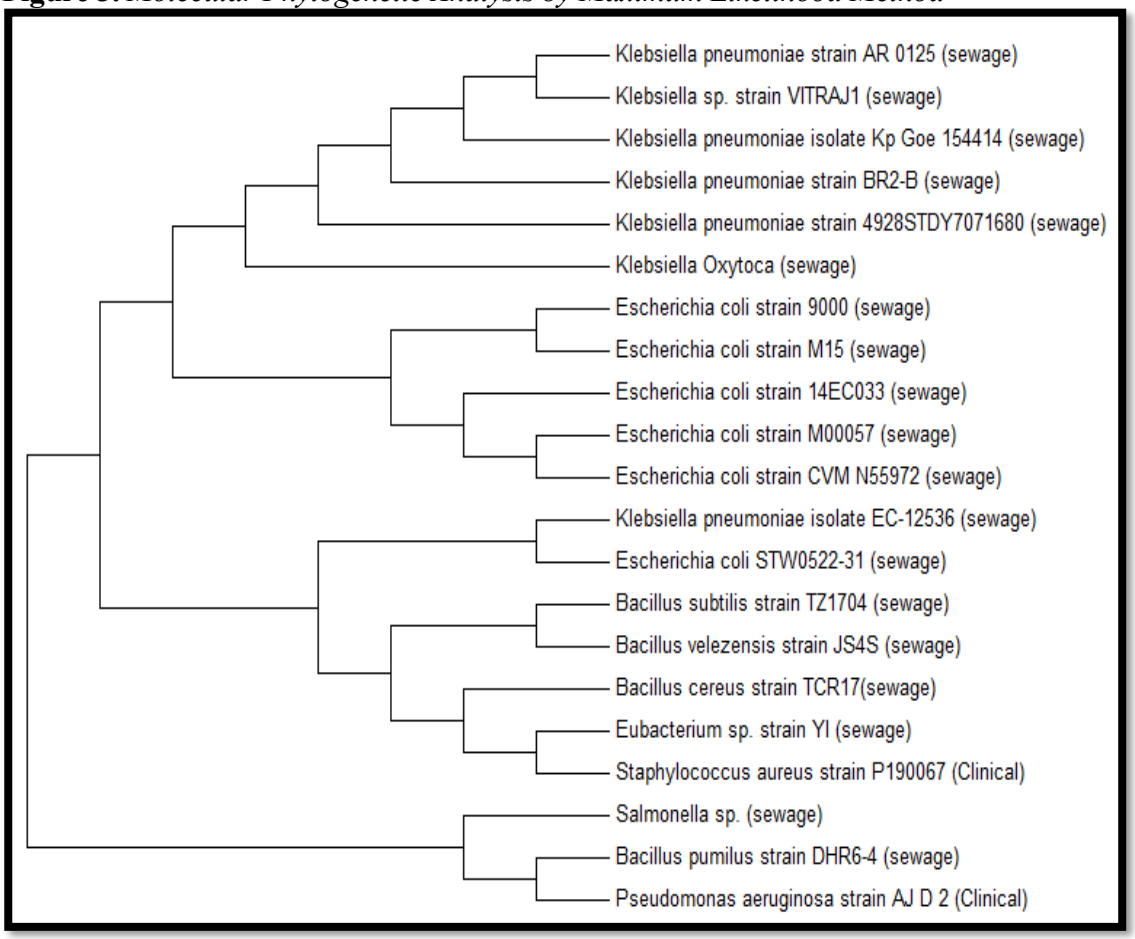


Figure 3. *Molecular Phylogenetic Analysis by Maximum Likelihood Method*



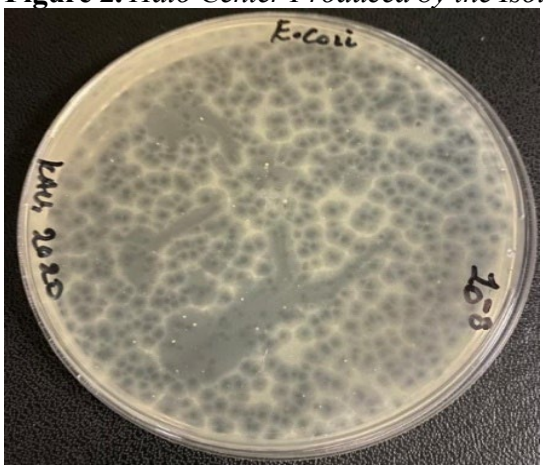
Isolation and Morphological Characteristics of the Bacteriophage

From these results, and using *E. coli* as a host, one lytic bacteriophage was detected in the sewage water ("EC phage"). After incubation of the EC phage with *E. coli* at 37 ºC for 48 h, the bacteriophage titration was calculated to be 10^9 PFU/mL. According to this assay, 131 PFU/mL was effective for *E. coli* inhibition (Table 4). In this context, the formation of clear plaques by the bacteriophage was observed with a 1 mm halo center, and well-defined boundaries (Figure 2).

Table 4. *Bacteriophage Titration at 10^{-9}*

Phage type	Equation used	Total PFUs
EC titer	$\frac{131\ \text{PFU/mL}}{0.1 \times 10^{-8}}$	$1.31 \times 10^9\ \text{PFU/mL}$

Figure 2. *Halo Center Produced by the Isolated Bacteriophage*



Bacteriophage–Host Range Interaction

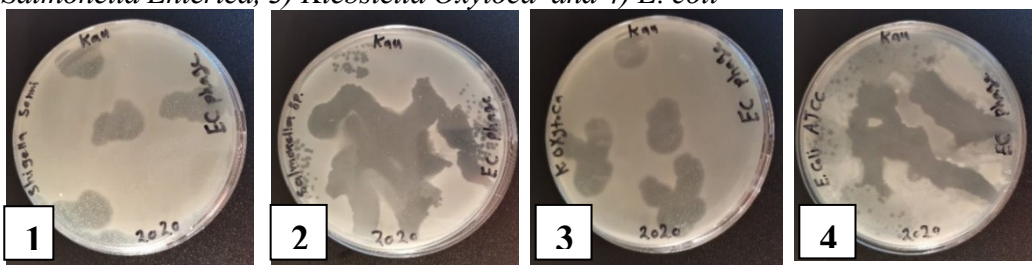
Analysis of the host range of the EC phage showed that a number of bacterial hosts were sensitive to its lytic potential, including *Salmonella enterica* (ATCC 14028), *Klebsiella oxytoca* (ATCC49131), *Shigella sonnei* (ATCC 25931) (Table 5). On the other hand, no lytic activity was observed against *Enterococcus faecalis* (ATCC 29212) and *Proteus vulgaris* (ATTC49132). The lytic activity of PaMAsh phage was then tested using a spot assay against four out of the six bacteria, *Shigella sonnei* (ATCC 25931), *Salmonella enterica* (ATCC 14028), *Klebsiella oxytoca* (ATCC49131), and *E. coli* (ATCC25922) (Figure 3).

Table 5. *Host Range of Isolated Phage*

	Enterococcus faecalis	***Shigella sonnei***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Escherichia coli***	***Salmonella enterica***
	-	+	+	-	+	+

(+) lysed, (-) not lysed.

Figure 3. *Host Range of EC Phage Using Spot Test Against (1) Shigella Sonni, 2) Salmonella Enterica, 3) Klebsiella Oxytoca and 4) E. coli*



Bacteriophage Stability Testing

The isolated bacteriophage was 100% stable at room temperature (Figure 4). EC phage incubated for 1 h exhibited excellent activity, as reflected in their

survival percentages, at temperatures of 37 and 65 °C; however, activity declined with incremental increases in incubation temperature. At 70 °C, the phage showed minimal activity, and at ≥80 °C, complete inhibition was observed. The influence of pH on survival of the EC phage showed that it was more susceptible to acidic pH than alkaline pH (Figure 5). The graph of phage survival over a pH range of 2–14 indicated an increase in survival with an increase in pH from 2 to 7, where survival peaked. There was no survival at pH values ≥11.

Figure 4. *EC Phage Thermal Stability*

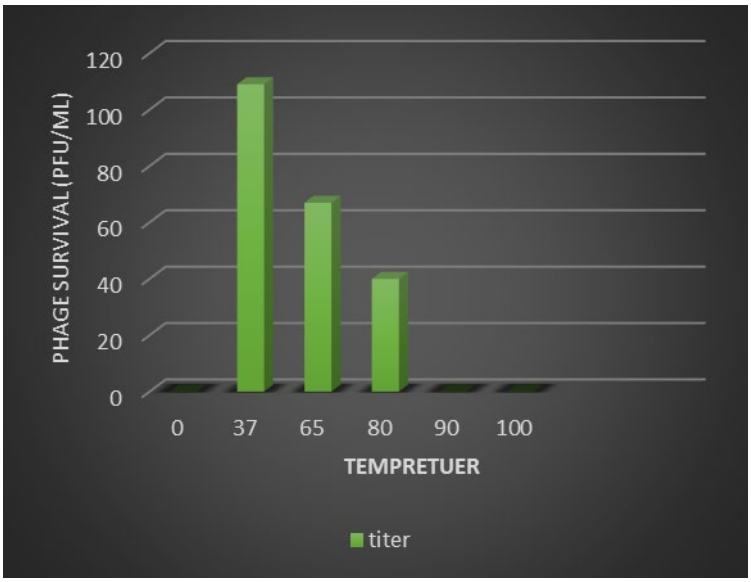
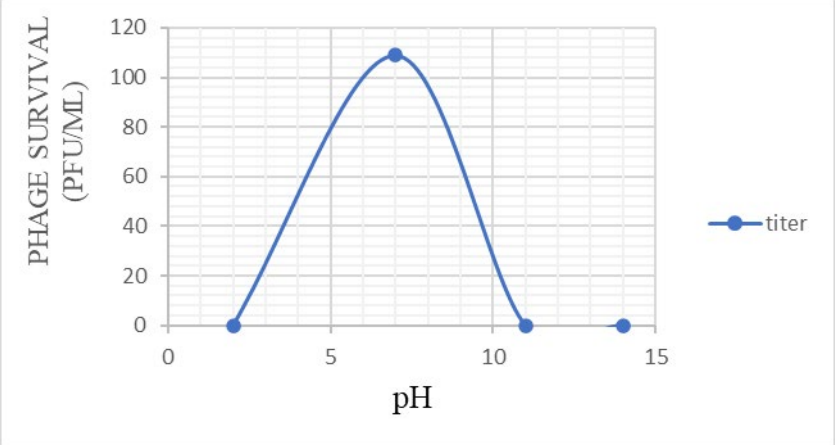


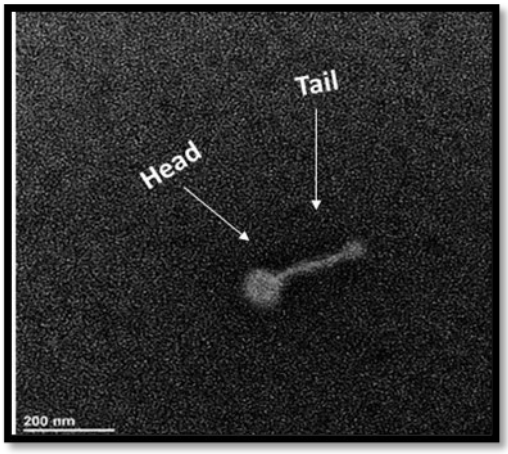
Figure 5. *EC Phage Stability at Different pH*



Morphological Characteristics of the Isolated Phage

The morphological features of the EC phage were examined using TEM, revealing the presence of a non-enveloped, head-tail structure. The head is about 60 nm in diameter (Figure 6), indicating its relationship to members of the family Siphoviridea.

Figure 6. *EC Phage Image via Transmission Electron Microscopy*



Discussion

The current study focused on wastewater as a source of bacteria and bacteriophages, owing to contamination of the water with fecal material exhibiting a great diversity of coliform species that can cause several diseases. The *E. coli* isolated from the wastewater coliform bacteria population (MB1) showed resistance to five antibiotics, classifying it as one of the MDR bacteria. Multi-drug resistant *E.coli* strains have been isolated previously from various sources, including drinking water and waste water, by Odonkor and his colleagues in 2018. Wastewater, in general, contains a large diversity of coliforms due to fecal contamination. Therefore, wastewater is a tank of intestinal pathogens, as well as their lytic phages, proposed in this study as a possible substitute for antibiotics.

The greatest recognizable plaque characteristic of the EC phage recovered from the wastewater sample in the present study was a distinctive halo zone, whose width incrementally increased over time, and which was encircled by a plaque that remained the same diameter. This is explained by Cornelissen et al. (2011), who demonstrated that bacteriophages produce a diverse array of hydrolytic enzymes that are responsible for the degradation of polysaccharides in the bacterial cell wall, as well as other constituents. Thus, the halo zones observed with our EC phage are presumed to be associated with these enzymes. The phage titer of 1.31×10^9 PFU/mL in our investigation is similar to the 7×10^8 PFU/mL titer reported by Swati et al. (2019) for an EC phage also isolated from wastewater.

The morphology of phages is one of the most important criteria for their characterization (Shukla & Hirpurkar 2011). In our study, the variation in the morphology of plaques may correspond to the difference in gel strength, phage strain and addition of cations (calcium chloride) (Ghasemian et al. 2017). However, Jothikumar et al. (2000) reported that the morphology of phage plaques was not affected by cationic supplements. Pedrosa & Martins (Pedroso & Martins 1995) also could not find any association of specific plaque morphology among the coliphage family.

Host range is an essential factor to consider when choosing phages for phage therapy (Duc et al. 2018). One of the fundamental biological properties of a bacteriophage is its host range (Kutter 2009). The EC phage isolated in this study exhibited successful and efficient hydrolytic activity against *K. oxytoca, Shigella sonnei, E. coli*, and *Salmonella enterica* (Figure 3, Table 4); however, the current results are not in agreement with the findings of (Kafshgari et al. 2019). The phage they isolated was effective, but its host range was limited to five strains of *E. coli*; other bacterial species, such as *Staphylococcus aureus* and *Salmonella enterica,* were not affected. A similar result was found by Dalmasso et al. (2015) who isolated three narrow host range phages from human feces in wastewater against nine strains of *E.coli*, so he proposed that using a cocktail of mixed phages would be more successful as a biocontrol than using a single phage.

Temperature is known to be a significant factor in the stability of phages under various conditions, but in particular during storage and/or transportation (Kering et al. 2020). The EC phage isolated in this study exhibited a high thermal stability over a wide range of temperature (37–65 ºC), but it was unstable when exposed for 2 h to high temperatures ≥80 ºC. In addition, pH influences phage stability; the isolated phage was not viable at a very low (pH 2) or very high (pH 14) pH, but remained viable over a pH range of 7–10 Earlier studies by Tiwari et al. (2010) indicated that *E. coli* phage remained viable at 70 °C for 2 min, but increasing the exposure time to 3 min resulted in death of the phage. They also found that phage propagation at a low (pH 3) or high (pH 11) pH was completely inactivated, whereas it remained active within the pH range of 5–9.

Conclusions & Limitations

In this study we successfully isolated a lytic phage active against *E. coli* strain M00057*,* which can cause stomach cramps, fever, and occasionally diarrhea. The ability of the isolated phage to completely lyse the cell makes it a good candidate to treat disease caused by this bacterium. However, more *in vivo* studies are needed to expand this research, and more phages against *E.coli* need to be isolated to cover related bacterial strains.

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A Research Study on Tobacco Associated Oral Potentially Malignant Disorders (OPMDs) Prevalent in Oral Mucosa of Lumbini Province/District Rupandehi Population of Nepal

By Siddharth Gupta[*] & Pratik Man Shrestha[±]

***Background**: Tobacco associated Oral mucosal lesions (OML) oral potentially malignant disorders (OPMDs) that comprise of oral sub-mucous fibrosis, oral leukoplakia, tobacco pouch keratosis, chewer's mucositis, pan encrustation are the most prevalent diseases in Rupandehi District, Lumbini province (Province Five) of Nepal. These OPMDs are usually caused by the consumption of both smokeless and smoked forms of tobacco. Smoking, chewing of the tobacco products including alcohol consumption are the common prevalent habits in Nepal and have been positively associated with such lesions. **Aim of the Research Study**: With this background, a research study has been conducted to assess the prevalence of OPMDs and their association among the patients visiting the outpatient department in Rupandehi district of Nepal. **Research Methods and Material**: This descriptive, cross-sectional prevalence study included five hundred participants who visited the Outpatient Department and fulfilled the inclusion criteria. The study data was carried out from August 2003 till April 2024. Institutional Review Committee (IRC) provided the ethical clearance to the study, which was duly obtained along with patient consent that were part of the study. Standardised proforma was used for recording demographic details, and WHO Assessment form for oral mucosal lesions were used to record the tobacco use status and findings of the clinical and laboratory investigations. Statistical analysis was performed using IBM SPSS version 23, and the results were calculated along with the level of significance. **Research Study Results**: The final sample was comprised of 500 cases of which 392 were males and 108 were females. The mean age of the study population was 58.19 ± 12.33 years ($p<0.001$). Leukoplakia in its varied forms (OPMD) accounted for almost 82% of the sample cases. The lesions from patients aged from 41 to 80 years presented moderate and severe dysplasia more often than lesions from patients in other age groups. The prevalence of tobacco use among study participants was 41.25% and that of the OML (oral mucosal lesions) positively attributed with use of tobacco was 39.28%. Tobacco chewing (areca nut) were significant predictors of oral sub mucous fibrosis in this population. **Conclusion:** The prevalence study emphasizes the deleterious effects of tobacco use on oral mucosa and also serves as a path for future tobacco cessation programs that would be helpful to prevent OPMDs in the Nepalese population especially in Province V (Rupandehi District) of Nepal.*

Keywords: *OPMDs, oral diagnosis, oral leukoplakia, oral sub mucous fibrosis, chewer's mucositis, tobacco cessation*

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Introduction

Oral potentially malignant disorders (OPMDs) are tissue changes that may precede squamous cell carcinoma (SCC) (Villa and Gohel 2014, Johnson 2011, El-Naggar 2017). SCC is a malignant neoplasm that accounts for 80-90% of all cancers in the oral cavity. The most common disorders in this group are leukoplakia, speckled leukoplakia, erythroplakia, and oral submucous fibrosis (Warnakulasuriya 2016, Scully 2017, Johnson 2011, Sarmento 2014).

Considering the various characteristics of OPMD in different populations, knowledge of their clinical-pathological profile is an important diagnostic tool. Therefore, it may play a pivotal role in preventing malignant transformation of these lesions in oral mucosa.

Given the low number of epidemiological studies about this topic in the Nepalese population especially in Rupandehi District region, this study aimed to analyze the clinical-pathological features of OPMD diagnosed in Province Five of Nepalese population.

Material and Methods

A descriptive, cross-sectional prevalence study has been done among five hundred patients with histopathlgically diagnosed oral potentially malignant disorders, from the period of August 2023 to December 2023 duration, attending the outdoor clinic of Department of Oral Medicine, Diagnosis and Radiology, Universal College of Medical Sciences (UCMS), Bhairahawa, Lumbini Province of Nepal.

Exclusion Criteria

A consecutive sampling technique was used and patients who were already operated on for oral cancer, underwent radio/chemo therapy for oral cancer, with recurrent cancer and patients who failed to submit sufficient information were excluded from the study.

Specific Objectives of the study were to identify current demographics of oral cancer with risk factors, types, histopathological types and presenting varied and as well as specific grades and stages.

Ethical clearance was duly obtained from the Institutional Review Board of Universal College of Medical Sciences and Departmental permission for the study was granted. The informed written consent was taken from each patient included in the study. A standardized data collection sheet was used to collect the data. Collected data was summarized and analyzed by Statistical Package for the Social Sciences (SPSS) Version 24.0 and results were presented and tabulated in table form and the level of significance Chi square test was used to test the statistically significant differences with level of p-value<0.05 considered to be statistically significant.

Results

The study was carried out in 500 patients of which 78.26% were male patients and 21.74% were female patients. The most common site was buccal mucosa 97(59.9%). Other characteristics are duly depicted in Table 1.

Table 1. *Gender Characteristics of Patient and Prevalent Site of OPMD*

Gender Characteristics & Site Prediction		**n (%)**
Gender	Male	78.26%
	Female	21.74%
Intraoral site and location of OPMDs Oral Potentially Malignant Disorders	Mandibular Lower alveolus	10.5%
	Buccal mucosa	19.0%
	Gingivobuccal sulcus	15.4%
	Lower lip	20%
	Lip and palate	23%
	Lateral border of Tongue	13%
	Retromolar area	16%
	Maxillary alveolar mucosa	10%

The lateral border of the tongue was the most frequently affected anatomical site 13%, followed by the lower lip 20%, buccal mucosa/vestibule 19.0%, mandibular alveolar mucosa 10.5%, and maxillary alveolar mucosa 10% respectively.

Leukoplakia predominantly represented 82% of the sample, followed by actinic chelitis 12%, and speckled leukoplakia 6%. Pure erythroplakias were not found in the sample. Ulceration was described in 10%.

The mean size of the leukoplakias was approximately 13 mm (ranging from 1 to 100 mm) and the mean size of speckled leukoplakias was 15 mm (ranging from 5 to 30 mm) (p=0.460). With regard to smoking and drinking, 53% of the patients were smokers/ex-smokers and 30% were drinkers.

Approximately 63% of the males and 46% of the females reported smoking (p=0.001), and approximately 48% of the males and 15% of the females reported drinking (p<0.0001).

Histological analysis showed that 49% showed no dysplasia, and 28%, 12%, and 11% showed mild, moderate, and severe epithelial dysplasia, respectively. The anatomical distribution of lesions showed statistically significant differences according to the sex of the patient (p<0.0001).

The mean age of the patients did not show statistically significant difference with regard to the anatomical location of the lesions (p=0.207). The anatomical distribution of the lesions according to the final diagnosis showed statistically significant difference between leukoplakia and speckled leukoplakia (p<0.001).

Among the OPMDs diagnosed oral leukoplakia, speckled leukoplakia, and actinic cheilitis presented ulceration in 9%, 10%, and 18% of the cases, respectively (p=0.03). The distribution of the different degrees of dysplasia showed statistically significant difference between the various anatomical sites were the lesions were found (p=0.002). The floor of the mouth and ventral tongue were the anatomical sites with the greatest percentage of cases with moderate and severe dysplasia.

Oral leukoplakias and speckled leukoplakia's presented moderate or severe dysplasia in 15% and 41% of the cases, respectively ($p<0.0001$).

The distribution of the different degrees of dysplasia among the various age groups showed that patients aged between 41-60 years, 61-80 years, and older than 80 years presented moderate and severe dysplasia in 24%, 27%, and 30% of the lesions, respectively, contrasting with the patients younger than 40 years old ($p<0.001$).

Discussion

OPMDs are relatively common, with a worldwide prevalence of 4.4%, while leukoplakia alone has a prevalence of 4.1%.

Numerous studies on OPMDs have been conducted worldwide in the past few years (Kumar 2015, Kavarodi 2014, Hassona 2014, Foy and Bertulos 2018, Dionne 2015, Muller 2018), though not much of these studies focused on Nepalese populations in Province V region.

Knowledge of the social-demographic profile of patients with OPMDs in a given population is important for understanding the most prevalent risk factors and for outlining prevention and early diagnosis strategies (Mello et al. 2018a, Queiroz 2014, Liu et al. 2011, Porter 2018, Pires 2013).

In the present study, male patients made up 78.26% of the sample the predominance of male patients has also been observed in previous studies on Indian and South East Asian subcontinent populations conducted by Adhikari et al. (2015), Rahman et al. (2018), Habib et al. (2017), in other worldwide studies conducted by Liu et al. (2011), Mello et al. (2018b), and Hussein (2017). However, this predominance was not observed in several studies involving different populations (Gheno 2015).

Over 80% of the patients were aged between 41 and 80 years, which highlights the connection between age and increased risk for developing OPMDs, as previously reported in the scientific literature (Queiroz 2014, Pires 2013).

It is important to note that the age distribution observed in the present study was similar to a descriptive study that evaluated 346 cases of SCC between 2005 and 2012 in accordance with Avon and Klieb (2012). In our study, the age of the patients did not show a statistically significant difference with regard to the anatomical site where the lesions were found.

Leukoplakias are the most common oral OPMDs, with a worldwide incidence between 2 and 4% (Martin 2012, Nun 2009, Saba et al. 2011). In the present study, 82% of the patients had a diagnosis of leukoplakia, similarly to a previous study on a South East Asian Bangladeshi population in accordance with findings from Kumar et al. (2016), and Sultana and Malik (2014).

None of the cases in our sample were diagnosed as pure erythroplakia, possibly due to the lack of precise clinical correlation required for a lesion to be classified as pure erythroplakia. Cases of oral lichen planus were excluded from our sample, due to the difficulties in distinguishing its clinical features from other OPMDs. Some earlier studies have included cases diagnosed as dysplastic oral lichen planus

(Shah 2009, Anis 2013, Dhanuthai 2017), but lacked detailed individual histological information to document the malignant transformation process in those lesions.

In the present sample, leukoplakia and speckled leukoplakia were more frequent in females in all anatomical sites, except for the lips. Furthermore, the frequency of moderate/severe dysplasia in lesions of the lower lip was greater than the frequency of moderate/severe dysplasia in most of the other anatomical sites, which corroborates the findings from previously done studies (Adhikari 2015, Rahman 2018, Rai et al. 2016).

A comparison between the mean size of leukoplakias and speckled leukoplakias did not show a statistically significant difference. This finding suggests that the presence of erythroplakia does not appear to be part of the natural evolution of OPMD. Therefore, the size of the lesion alone is not an indicator of its potential for malignant transformation. Nonetheless, Speight et al. (2018) listed lesion size greater than 200 mm^2 as a clinical parameter associated with increased risk of malignant transformation in OPMDs.

The tongue – especially the lateral border – was the most frequently affected anatomical site, a finding that is in agreement with other studies. This site was also most frequently affected by SCC, according to an earlier study carried out with the same population. Other studies including from Anis (2013). However, reported greater frequencies of buccal mucosa or alveolar mucosa involvement.

This may be due to population variations, difficulty in distinguishing leukoplakia from other lesions (for example, reactional hyperkeratosis) clinically or pathologically, or even inclusion of oral lichen planus lesions in the sample.

The degree of dysplasia is based on structural and cytological characteristics of the epithelium and is one of the findings commonly used to evaluate the risk of malignant transformation in OPMDs.

Half the lesions included in this study showed no epithelial dysplasia on histological analysis but were still considered OPMDs as the clinical aspect was compatible with leukoplakias or leuko-erythroplakias and they could not be diagnosed as any other oral lesions. In the present study, comparison of the anatomical location and degree of dysplasia showed that most of the lesions found on the lateral border of the tongue either did not present dysplasia or only presented mild dysplasia 52% and 28% of the cases, respectively.

The floor of the mouth and ventral tongue were the anatomical sites with the greatest percentage of moderate 23% and severe dysplasia 16%. These results are in agreement with previous studies on South East Asian population and reinforce the notion that OPMDs located on the tongue and on the floor of the mouth have higher risk of malignant transformation (Kumar 2016, Sharma 2018).

Accordingly, these anatomical sites deserve special attention because they are the most commonly affected by squamous cell carcinoma (SCC) in this population.

The frequency of severe epithelial dysplasia increased as patient age increased; no correlation with age was observed for lesions with mild or moderate dysplasia. This finding supports the theory that the malignant transformation process is slow and gradual over the years, and that older patients are at greater risk for malignant transformation than their younger counterparts.

In addition, analysis of the degree of dysplasia based on the clinical aspect of the lesions revealed that leukoplakias and speckled leukoplakias presented moderate or severe dysplasia in 15% and 41% of the cases, respectively ($p<0.0001$), similarly to the findings from previous studies (Pires 2013, Saba et al. 2011, Dhanuthai et al. 2017, Sharma et al. 2018).

Conclusion

In conclusion, in Nepalese population oral leukoplakias were the most commonly observed OPMDs, followed by oral submucous fibrosis. The lateral border of the tongue, the lower lip, and the buccal mucosa/vestibule were the most frequently affected anatomical sites. Lesions found on the floor of the mouth/ ventral tongue presented the highest frequency of severe epithelial dysplasia, and the highest frequency of severe epithelial dysplasia was observed in older patients.

The prevalence study emphasizes the deleterious effects of tobacco use on oral mucosa and also serves as a path for future tobacco cessation programs that would be helpful to prevent OPMDs in the Nepalese population, especially in Province V (Rupandehi District) of Nepal.

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Effects of Socio-Economic and Health Variables on Survival Time of HIV/AIDS Patients in Manipuir

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Background: The survival time of patients with human immunodeficiency virus (HIV)/ acquired immune deficiency syndrome (AIDS) is associated with many socio-economic, education, employment and socio-cultural factors. High prevalence rates of HIV/AIDS are observed in some Indian States including Manipur. Objectives: It is to examine the effects of socio-economic and health related variables on the survival time of HIV and Hepatitis-C virus (HCV) patients. Materials and Methods: Under simple random sampling without replacement (SRSWOR), it analysed a sample of 200 HIV+ patients attending the ART Centre of Jawaharlal Institute of Medical Science (JNIMS), Imphal during March – July 2016. Using multiple regression models, the significant covariates of the survival time of patients are explored. Findings: The five factors – sex of patient ($P<0.05$), marital status ($P<0.05$), family income ($P<0.05$), patient type ($P<0.01$) and CD4 count ($P<0.05$) are found to be significantly influencing the dynamics of survival duration of the patients. Conclusion: In Manipur, the average survival time of the HIV/ AIDS patients is observed to be about six years varying with their sex, marital status, size of family, employment and CD4 count.

Keywords: *Manipur, regression model, sex, marital status, CD4*

Introduction

The economic life of HIV/AIDS patients is influenced by various socio-economic, socio-cultural, behavioural and health care factors. In Europe and the USA, low income and low education have been associated with poorer outcomes for several diseases (Woods et al. 2006, Saydah and Lochner 2010, Hawkins et al. 2012). In USA, in people with HIV receiving antiretroviral therapy (ART), lower education, unemployment and household poverty are associated with having poorer virological and immunological outcomes (Shacham et al. 2010, Simoni et al. 2013, Beer et al. 2014, Burch et al. 2016). HIV-positive populations in UK and Europe also comprise distinct demographic groups, with substantial variation in social circumstances. The association between socio-economic factors and HIV outcomes in the USA might not be generalisable to settings with free universal health care (Burch et al. 2016). In the Italian ICoNA cohort study (Saracino et al. 2016) in individuals who had been taking ART for at least 6 months, unemployment was associated with double the risk of virological failure compared with working full-time. The findings of some European studies (Burch et al. 2016,

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Moralejo et al. 2006, Glass et al. 2006, Collazos et al. 2009), have also shown that lower socio-economic status measured by education, employment, and social support is associated with ART non adherence, but a minority of studies found no evidence (Sherr et al. 2010).

In this new millennium, HIV/AIDS has become a serious socio-economic and health problem with 33 million people living with HIV virus in glove and 2.4 million people in India in the year 2007 (UNAIDS 2008). In 2009, the National AIDS Control Organization (NACO) reports that there are 4987 integrated counseling and testing centers (ICTC) and 211 ART centers where ART treatment is given free of cost to over 2 lakh people living with HIV (NACO 2009). The quality of life (QOL) of people living with HIV has become a salient issue after increase in availability of anti-retroviral treatment and increase in average life span. The QOL is defined as individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns (Anand et al. 2012). Patients who are diagnosed late until their CD4 count fell to below 200 and also are estimated at age 20 to have a life expectancy that was at least four years less than those who had a CD4 count of 200-499 (Mohammadi-Moein et al. 2013) When comparisons between the genders were made, it indicated that females scored lower on all the domain of QOL compared with males (WHO 2002). In India, the first HIV infection case was detected in 1986 among female sex workers in Chennai. A finding also observed four southern states – Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, and two North East States – Manipur and Nagaland were categorized as high-prevalence states (Paranjape and Challacombe 2016). In case of co-infection, IDUs residing in two districts of Manipur that among the 31% of HIV-positive IDUs, 95% were co-infected (Kermode et al. 2016). Thus, the present study aims to explore the effects of socio-economic and health related factors on the variation in the duration of survival time of patients with HIV and HCV in Manipur.

Objectives

The study is to investigate the economic life of the patients with HIV and HIV+HCV, to examine the effects of covariates influencing the survival duration of the patients after detection of the diseases and also to explore the dependence of patient's survival time on socio-economic and health related variables which are hypothesized to have significant impacts on the survival time.

Materials and Methods

A cross-sectional study was carried out through simple random sampling without replacement (SRSWOR) on 200 HIV+ patients who attended the ART Centre of Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal during March - July 2016. Out of these study subjects, 100 patients were HIV+ only and

the other 100 patients were HIV+ with Hepatitis-C virus (HCV) co-infection. The eligibility criteria for the subjects include firstly, the HIV+ patients enrolled in ART centre; secondly, regular or 100% adherence to the program and thirdly, aged between 21-70 years. The exclusion criteria include 1) children with HIV; 2) HIV+ patients with chronic co-morbidity like cancer, diabetes etc.; 3) patients whose adherence to the program is less than 50%; and 4) HIV-patients who are on ART-II program also known as second-line treatment. After completion of each interview the fill-in questionnaire was co-signed by an attending medical officer. As an econometric tool, regression analysis infers the functional relations between patient's survival time (dependent variable) and explanatory variables say socio-economic and health indicators. It is to estimate and/or predict the average values of the dependent variable in terms of known or fixed values of explanatory variables under analysis. Though regression analysis does not necessarily mean causation, it reveals the precise causation on the basis of relation supported by the theory. The binary dummy variables were also used to represent categorical variables such as sex of patient (male/female), employment status (employed/ unemployed) etc. The dummy variables take on only two values, *0* and *1* for two categories. The empirical data was so analysed using SPSS.

Variables Specification

The response variable is considered to be survival time duration of patient (in year). The longevity of survival time after detection of the disease (HIV/ both HIV and HCV) is assumed here to be functionally related with only eleven explanatory variables of interest. They are sex of patient (male = 1 and 0, otherwise that is female), age of patient (count in year), marital status of patient (currently married = 1 and 0, otherwise say single, widow, separated etc.), family size (count discrete number of family members), number of children (count discrete number), status of respondents employment (employed = 1 and 0 otherwise), monthly family income of the patient (in '000Rs., ordinal: 1 for <1; 2 for 1-3; 3 for 3-5 and 4 for 5+), type of patient (patient with HIV = 1 and 0 otherwise that is with both HIV and HCV), mode of transmission of the disease (sexual = 1 and 0 otherwise – IUD, blood, vertical, unknown etc.), CD4 count (ordinal: below 200 = 1, 200-500 = 2 and above 500 = 3) and application of supplementary medicine (yes = 1 and 0 otherwise – no).

Functional Relationship and Hypothesis

In case of functional relationship, the patient's survival time after detection of HIV/HCV (Y) is assumed to be a function of eleven variables namely sex of patient, age, marital status, family size, number of children in the family, respondents employment, family income, type of patient, mode of transmission of the disease, CD4 count and application of supplementary medicine. The null hypothesis (H_0) of the present investigation may be spelt out as $H_0: \beta_i = 0$, each regression coefficient is zero indicating that the survival time period of the patients is not influenced by

their socio-economic and health related factors as against the alternative hypothesis (H_1), pronounced by $H_1: \beta_i \neq 0$, that is the survival time is significantly influenced by the socio-economic and health related factors under study.

Analysis and Results

To quantify some qualitative variables, binary dummy variable (0, 1) and ordinal scale techniques are used and 0.40 was also taken as a cut off zero-order correlation value for scanning the multi-collinearity problems among the explanatory variables. While interpreting the findings measuring the effects of the independent variables on duration of survival time of the patients, the regression coefficient (β) with its 95% confidence interval (CI) and P-values of the t-test for β are used. The probability levels of significance of the effects of independent variables have been advocated by 5% (P<0.05) as statistical significance and 1% (P<0.01) as highly significance. It is evident that the null hypothesis is rejected in the sense that all regression coefficients (β_i) cannot be zero indicating that some of the explanatory variables had significant impacts on the survival time of the patients. It is evidenced by F-value of the regression model, say 3.02 (P<0.01) shown in Table 1. Here, the total variation in the survival time is explained about 25% (R^2=0.251) by the independent variables in the model. Out of eleven variables only two were observed to have their statistically significant contributions on the variation of patient's survival time in the population. They were patient type that is patients with HIV and that of both HIV and HCV (P<0.01) and CD4 count (P<0.05). Each statistically significant variable is observed only when adjusted the joint effects of ten remaining variables under investigation. Despite, five independent variables could be detected to have their significant effects on the survival time of the patients in the step-wise regression models.

Table 1. *Regression Coefficients on Patient's Survival Time After Detection of HIV/ HCV*

Factors	β	t	P-value	95%CI for b	
				Lower	Upper
(Constant)	6.912	2.18	0.031	0.652	13.172
Sex of patient	1.545	1.46	0.147	-0.546	3.636
Age of Patient	-0.001	-0.02	0.982	-0.106	0.103
Marital status of patient	-1.167	-1.55	0.123	-2.652	0.319
Family size	-0.553	-1.74	0.083	-1.179	0.074
Number of children	-0.107	-0.34	0.736	-0.735	0.521
Respondents employment	-0.363	-0.39	0.696	-2.189	1.464
Monthly income	-0.129	-0.45	0.657	-0.702	0.443
Type of patient	3.439	4.28	0.000	1.854	5.023
Mode of transmission	-0.713	-0.69	0.489	-2.743	1.316
CD4 count	2.531	1.99	0.040	-0.004	5.066
Supplementary medicine	0.425	0.54	0.587	-1.116	1.966

Model diagnostics: Model F=3.02, P<0.001; Durbin-Watson=1.95; R^2=0.251.

It is observed that the mean patient's survival time is found to be nearly 7 years irrespective of effects of the independent variables. The survival time of patient with HIV can extend at least 3 year 5 months (β = 3.44) from that of the patients with HIV+ and HCV while controlled the joint effect of other ten variables say sex of patient, age of patient, marital status of patient, family size, number of children, status of respondents employment, monthly family income of the patient, mode of transmission of the disease, CD4 count and application of supplementary medicine. This positive effect of type of patient is highly significant as evidenced by the value of t-statistics, 4.28 (P<0.01). In the similar manner, the survival time may enhance 2.5 years corresponding to each advancement of one level in CD4 count say from below 200 to 200-500 and again to above 500 when adjusted the joint effects of other ten variables. This increment in the patient's survival time is statistically significant as witnessed by t-statistics (1.99, P<0.05). Apart from the statistical significance, the survival time may also visibly enhance 1.5 years in male patient than those in female (β =1.55). While controlled the joint effects of ten variables, the survival time may be reduced by eight and half months (0.7 year) in sexual transmission of the disease than those of IUD, blood, vertical, unknown etc. (β = -0.71). The fitted regression model so obtained is given by the Model - 1 with model diagnostics.

To identify more influencing factors on the variation in patient's survival time, backward stepwise regression analysis is again applied. Screening of significant covariates or explanatory variables to the response variable (patient's survival time) has been performed through seven steps (Table 2). The 1st model is same as above regression model in which the effects of independent variables are explained. The last, 7th model is achieved with five covariates indicating that the patients' survival time is significantly varied with sex of patient, marital status of patients, family income, patient type (HIV/ HCV) and CD4 count. Age of patient is screened out to be lowest insignificant effect in the 2nd model from the 1st model carrying the β-value of -0.002 with absolute t-value, 0.02 (P>0.05). The transition of 3rd model from 2nd model can screen out the number of children in the family with β- value of -0.029 (t= 0.34, P>0.05) along with patient's age (β = 0.003, t = 0.04, P>0.05). In this advancement of each model the amount of covariates' effects on survival time duration are also changes. In this way, six less influencing independent variables can be screened out in the last fitted 7th model.

In the best fitted 7th model, the duration of patient's survival that is the time duration from date of detection of HIV/AIDS to the survey date is estimated to be at least six years (β = 6.23) assuming constant the joint effects of five covariates – sex of patient, marital status, family income, patient type (HIV/ both HIV and HCV) and CD4 count. In the similar way, the survival time of male patient can extend about two years (β = 1.99) from that of female while controlled the joint effects of other four covariates. It means that duration of survival time is significantly influenced by the sex of patient as witnessed by statistics value (t = 2.39, P<0.05). While controlled the joint effects of four variables the survival duration of currently married patient may be reduced by 1 year and 3 months from those of others say single, widow, separated etc. (β = -1.24). Keeping constant the

joint effects of sex of patient, marital status, type of patient and CD4 count, the survival time is also reduced by seven months while increasing of one member in the patient's family (β = -0.59). This reduction is found to be statistically significant (t= 2.37, P<0.05). It is noted that the survival time of patient with HIV can extend at least 3 years (β = 3.19) from the patient with both HIV and HCV while controlled joint effect of four other covariates. This enhancement of survival time is highly significant as witnessed by t - statistics, 4.29 (P<0.01). Lastly, the survival time may also be increased by 2 years 5 months (β = 2.42) to each increment of one level in CD4 count when adjusted the joint effects of four other variables – sex of patient, marital status of patient, family size and type of patient.

Table 2. *Step-Wise Regression Coefficients of on Patient's Survival Time*

Model	Factors	β	t	P-value	95%CI for b	
					Lower	Upper
1	Constant	6.912	2.178	0.031	0.652	13.172
	Sex of patient	1.545	1.458	0.147	-0.546	3.636
	Age of Patient	-0.001	-0.022	0.982	-0.106	0.103
	Marital status of patient	-1.167	-1.549	0.123	-2.652	0.319
	Family size	-0.553	-1.740	0.083	-1.179	0.074
	Number of children	-0.107	-0.337	0.736	-0.735	0.521
	Respondents employment	-0.363	-0.391	0.696	-2.189	1.464
	Monthly income	-0.129	-0.445	0.657	-0.702	0.443
	Type of patient	3.439	4.281	0.000	1.854	5.023
	Mode of transmission	-0.713	-0.693	0.489	-2.743	1.316
	CD4 count	2.531	1.996	0.040	-0.004	5.066
	Supplementary medicine	0.425	0.544	0.587	-1.116	1.966
2	Constant	6.866	2.866	0.005	2.140	11.592
	Sex of patient	1.545	1.461	0.146	-0.540	3.629
	Marital status of patient	-1.169	-1.575	0.117	-2.633	0.295
	Family size	-0.553	-1.755	0.081	-1.175	0.069
	Number of children	-0.106	-0.339	0.735	-0.723	0.511
	Respondents employment	-0.364	-0.395	0.693	-2.181	1.453
	Monthly income	-0.128	-0.447	0.655	-0.695	0.438
	Type of patient	3.437	4.315	0.000	1.866	5.008
	Mode of transmission	-0.712	-0.695	0.488	-2.735	1.310
	CD4 count	2.529	1.997	0.040	0.006	5.052
	Supplementary medicine	0.423	0.547	0.585	-1.103	1.948
3	Constant	6.992	2.961	0.003	2.335	11.650
	Sex of patient	1.556	1.477	0.141	-0.523	3.635
	Marital status of patient	-1.218	-1.676	0.095	-2.651	0.215
	Family size	-0.616	-2.416	0.017	-1.119	-0.113
	Respondents employment	-0.384	-0.418	0.676	-2.193	1.425
	Monthly income	-0.125	-0.438	0.662	-0.691	0.440
	Type of patient	3.416	4.312	0.000	1.853	4.978
	Mode of transmission	-0.738	-0.723	0.471	-2.750	1.275
	CD4 count	2.580	2.036	0.043	0.080	5.080
	Supplementary medicine	0.412	0.534	0.594	-1.109	1.932
4	Constant	6.725	2.965	0.003	2.251	11.200
	Sex of patient	1.489	1.433	0.154	-0.561	3.538
	Marital status of patient	-1.231	-1.699	0.091	-2.660	0.198
	Family size	-0.604	-2.390	0.018	-1.103	-0.106

	Monthly income	-0.136	-0.478	0.633	-0.698	0.426
	Type of patient	3.395	4.303	0.000	1.839	4.951
	Mode of transmission	-0.731	-0.718	0.473	-2.739	1.277
	CD4 count	2.570	2.033	0.043	0.077	5.064
	Supplementary medicine	0.408	0.531	0.596	-1.109	1.925
5	Constant	6.337	2.997	0.003	2.167	10.508
	Sex of patient	1.531	1.482	0.140	-0.507	3.569
	Marital status of patient	-1.214	-1.681	0.094	-2.638	0.210
	Family size	-0.594	-2.362	0.019	-1.089	-0.098
	Type of patient	3.425	4.364	0.000	1.877	4.973
	Mode of transmission	-0.733	-0.721	0.472	-2.736	1.271
	CD4 count	2.515	2.002	0.047	0.037	4.994
	Supplementary medicine	0.408	0.531	0.596	-1.106	1.922
6	Constant	6.832	3.606	0.000	3.096	10.569
	Sex of patient	1.463	1.429	0.155	-0.556	3.481
	Marital status of patient	-1.267	-1.774	0.078	-2.675	0.142
	Family size	-0.586	-2.339	0.020	-1.080	-0.092
	Type of patient	3.401	4.349	0.000	1.858	4.943
	Mode of transmission	-0.870	-0.887	0.376	-2.804	1.064
	CD4 count	2.404	1.944	0.053	-0.035	4.843
7	Constant	6.226	3.525	0.001	2.743	9.709
	Sex of patient	1.989	2.388	0.018	0.346	3.633
	Marital status of patient	-1.237	-1.735	0.048	-2.643	0.169
	Family size	-0.594	-2.374	0.019	-1.087	-0.101
	Type of patient	3.188	4.286	0.000	1.721	4.655
	CD4 count	2.423	1.961	0.041	-0.014	4.860

Discussion

In the multiple regression models, out of eleven variables only two were found to have their significant influences on patient's survival time. They were patient type that is patients with HIV and the patients with both HIV and HCV ($P<0.01$) and CD4 count ($P<0.05$) when the joint effects of ten remaining variables are controlled. Despite statistically insignificance, the patients' survival time may also visibly enhance 1.5 years more in male patient than those of female irrespective of joint effects of other ten variables. This view is in association with findings of previous study (Paranjape and Challacombe 2016). In the same manner, the survival time may be reduced eight and half months (0.7 year) in sexual transmission of the disease than those of IUD, blood, vertical, unknown etc. In the last regression model in 7^{th} step, the duration of patient's survival is estimated to be at least six years keeping constant the joint effects of five covariates namely sex of patient, marital status of patients, family income, patient type and CD4 count. The survival time of male patient is extended to two years from that of female while controlled the joint effect of other four covariates – marital status of patient, family size, type of patient and CD4 count. It means that survival time of the patient after detection of disease is significantly influenced by the sex of patient. While controlled the joint effects of four variables, the survival duration of currently married patient may be reduced by 1 year and 3 months from those of others say single, widow, separated etc. Keeping constant the joint effects of sex of

patient, marital status, type of patient and CD4 count, the survival time is also significantly reduced by seven months when increasing of one member in the patient's family. It is incorporated with the past findings. (Woods et al. 2006, Saydah and Lochner 2010, Hawkins et al. 2012, Collazos et al. 2009) In this analysis, the survival time of the patient with HIV can extend at least 3 years from the patient with HIV+HCV in the same manner. As similar as in previous findings (Mohammadi-Moein et al. 2013), the survival duration of patients may also be increased by 2 years 5 months corresponding to each increment of one level in CD4 count when adjusted the joint effects of four other variables.

Conclusion

To the present authors' knowledge, these effects of socio-economic and health and allied factors on duration of survival time after HIV diagnosed patients have not been previously provided in India's North Eastern Region particularly in Manipur, the easternmost state internationally bordering with Myanmar. Considering the eleven explanatory variables, the present findings could have health practitioners' prediction to patients about their life expectancies after detection of HIV/AIDS and also policy makers to estimate expenses and allocate government funds thereon. Besides, the present results can be helpful for patients planning and establishing general norms of their expectations or so called survival duration of time after diagnosis.

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Impact of Obesity-Related Social Media Contents on Urban Men: Application of the Health Belief Model

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Health situations across the world triggered by obesity are evidence that obesity has become a global epidemic disease. India had the third-highest number of people with obesity in the world. In this digital era, a key source of health information is social media. There are visible studies on obesity awareness obtained from social media among people with obesity and women. So this study focused on urban men who are more prone to obesity than rural men and who commonly seek health information through social media. Health belief model (HBM) was adopted for the study to understand the impact of social media obesity contents in bringing health behaviour change. Sample data (N=530) were collected using a digitally circulated survey questionnaire. The study found that Facebook, YouTube and Instagram are the top three most used social media among urban men to look for health information. The study found the respondents' attention to social media obesity contents influence their knowledge on HBM constructs which in turn influence their health behaviour change to treat obesity. Study findings suggest that well-informing posts about obesity from health experts should be shared over social media frequently to create enough obesity awareness to bring a health behaviour change.

Keywords: *obesity, social media, health communication, health belief model, health behaviour, urban men*

Introduction

Obesity

Obesity, a global epidemic, is causing a surge in health issues around the world. It ranks as the fifth leading cause of global deaths, as per the World Health Organization (WHO) in 2004. In 2017, approximately 30% of the world's population, totaling around 2 billion people, were overweight or obese (Sifferlin 2017). The health repercussions of obesity contribute to almost 2.8 million preventable deaths annually (Ahirwar & Mondal 2019). Several evident factors contribute to this escalating obesity crisis, including the consumption of energy-dense foods, sedentary lifestyles, inadequate access to healthcare services, and limited financial support. Developing countries are particularly vulnerable to the adverse effects of obesity. In India, a nation experiencing significant economic growth, white-collar workers such as engineers, technicians, scientists, and teachers exhibit higher Body Mass Index (BMI) values compared to their blue-collar counterparts

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like farmers and laborers. BMI classification, following WHO standards, categorizes individuals as underweight if their BMI is below 18.5 kg/m², normal if it falls between 18.5 and 25 kg/m², overweight if it ranges from 25 to 30 kg/m², and obese if it surpasses 30 kg/m². By 2019, India ranked third globally in terms of the number of obese individuals, with 20% being adults and 11% adolescents (Ahirwar & Mondal 2019, Raibagi 2019). Obesity prevalence in India varies widely, ranging from 8% to 38.2% in rural areas and from 13% to 50% in urban areas. For instance, in Tamil Nadu, 28.2% of men and 30.9% of women are obese, while in urban Tamil Nadu, these figures rise to 30.6% of men and 36.2% of women, and in rural areas, they are at 25.6% for men and 25.4% for women (ICF 2017).

Social Media for Health Communication

In this age of digital connectivity, a wealth of essential information is readily accessible online. The healthcare sector is no exception, as it has embraced the digital realm for disseminating health-related information (Vitak & Ellison 2012). Among the primary sources of health information, social media stands out. Unlike traditional media, social media offers a wide spectrum of health information, transcending demographic boundaries (Scanfeld et al. 2010). Surprisingly, not only millennials but also a significant 90% of older adults who engage with social media actively seek and share health-related information through these platforms (Sanford 2010). Social media platforms serve various purposes in health communication, including providing access to information about a diverse range of health conditions, establishing connections between patients and healthcare professionals, and fostering peer, social, and emotional support networks. It's important to note that while social media's potential for health communication is immense, there are commonly reported limitations. These limitations encompass concerns regarding the reliability of information, issues of confidentiality and privacy, and worries about information quality (Moorhead 2017). However, it's worth highlighting that a well-designed and informed social media page dedicated to healthcare information has the potential to bring about positive changes in health behavior within a short-term follow-up period (Parker & Thorson 2009).

Social Media for Obesity Awarness

Jennifer and her colleagues conducted a comprehensive review of multiple articles, leading to the conclusion that social media and related electronic technologies serve as viable components and effective delivery channels for weight management programs. They found that motivational content related to obesity, disseminated through social media, has the potential to support the sustained adoption of healthy behaviors such as regular exercise and balanced eating habits (Li et al. 2013). Additionally, social media platforms offer valuable knowledge about the characteristics and severity of health consequences associated with obesity (Kent et al. 2016). In specific contexts, such as families with infants at high risk of obesity, engaging group chats on social media have demonstrated a

significant impact on promoting healthier eating behaviors (Fiks et al. 2017). However, it's important to note that while media can provide healthcare information, its ability to induce actual changes in health behavior is variable. The frequency of healthcare-related posts on social media platforms also plays a role in influencing public behavior (Kitzinger 2004). Given this backdrop, it is pertinent to investigate whether posts related to obesity on social media platforms effectively contribute to changes in health behavior among consumers. Previous research has explored obesity awareness campaigns on social media, primarily among obese individuals and women (Banerjee 2020) (George et al. 2016, Holmberg 2016). Therefore, it becomes relevant to extend this inquiry to assess the impact of obesity awareness campaigns on social media, specifically targeting urban men.

Urban men, particularly in Chennai, India, face a higher risk of obesity compared to their rural counterparts, and they frequently seek health-related information through social media channels (Express News Service 2018). Hence, the primary objective of this study is to investigate the level of obesity awareness and assess the influence of obesity-related social media content in promoting positive health behavior changes among urban men in Chennai.

Research Questions and Hypotheses

The primary aim of this study is to assess the level of obesity awareness among urban men in Chennai as gathered from their exposure to social media. To investigate this, a research question was formulated to gauge the extent to which urban men engage with obesity-related content on social media.

> Research Question 1 (RQ1): What is the extent of exposure and attention that urban men give to obesity-related content on social media platforms?

For the purpose of this study, we have adopted the widely recognized Health Belief Model (HBM) as a framework to analyze the impact of obesity-related content on social media in influencing health behavior changes among urban men. The HBM comprises several key constructs, including:

Perceived Susceptibility: This construct relates to an individual's belief regarding their likelihood of developing a particular disease or condition (Strecher & Rosenstock 1997, Champion & Skinner, 2008).

Perceived Severity: Perceived severity encompasses an individual's belief concerning the seriousness of a condition and its potential consequences (Strecher & Rosenstock 1997, Champion & Skinner 2008).

It is essential to note that the concept of perceived threat, which involves eliciting fear-related emotions about a disease or condition, is influenced by both perceived susceptibility and perceived severity (Saghafi-Asl, et al. 2020, Pearlman et al. 2020). Therefore, to explore the knowledge levels among urban men who pay attention to obesity-related content on social media concerning the threats associated with obesity, a research question has been formulated.

Research Question 2 (RQ2): To what extent does the attention of urban men to obesity-related social media content impact their perception of the associated threats?

Perceived Barriers: Perceived barriers refer to an individual's assessment of the obstacles and difficulties involved in adopting a recommended health behavior (Strecher & Rosenstock 1997, Champion & Skinner 2008). In light of this, a research question has been formulated to investigate whether urban men who pay attention to obesity-related content on social media possess an understanding of the barriers associated with combating obesity.

Research Question 3 (RQ3): Does the attention of urban men to obesity-related social media content influence their perception of the barriers hindering efforts to combat obesity?

Cue-to-Action: This construct entails identifying the triggers or stimuli necessary to prompt an individual to engage in a particular health behavior (Strecher & Rosenstock 1997, Champion & Skinner 2008). To explore whether urban men who give attention to obesity-related content on social media receive the necessary cues to take action and address obesity, a research question has been posed.

Research Question 4 (RQ4): Does the attention of urban men to obesity-related social media content provide them with the cues needed to initiate health behavior changes aimed at overcoming obesity?

Perceived Benefits: This refers to an individual's belief in the effectiveness of a specific health behavior in reducing the risk of a disease or condition (Strecher & Rosenstock 1997, Champion & Skinner 2008). To explore whether urban men who pay attention to obesity-related content on social media possess knowledge about the effectiveness of health behaviors in addressing obesity, a research question has been formulated.

Research Question 5 (RQ5): To what extent does the attention of urban men to obesity-related social media content influence their perception of the benefits associated with health behaviors aimed at combating obesity?

Self-efficacy: This pertains to an individual's belief in their own capacity to successfully embrace a specific health behavior (Strecher & Rosenstock 1997, Champion & Skinner 2008). To examine whether urban men who engage with obesity-related content on social media have an understanding of their own capabilities in adopting health behaviors to combat obesity, a research question has been posed.

Research Question 6 (RQ6): To what degree does the attention of urban men to obesity-related social media content influence their self-efficacy in adopting health behaviors aimed at overcoming obesity?

An additional research question was introduced to investigate whether urban men who actively engage with obesity-related content on social media possess

adequate knowledge of the Health Belief Model (HBM) constructs, and if this knowledge, either directly or indirectly, impacts their adoption of health behaviors, specifically related to maintaining a healthy diet and engaging in regular exercise.

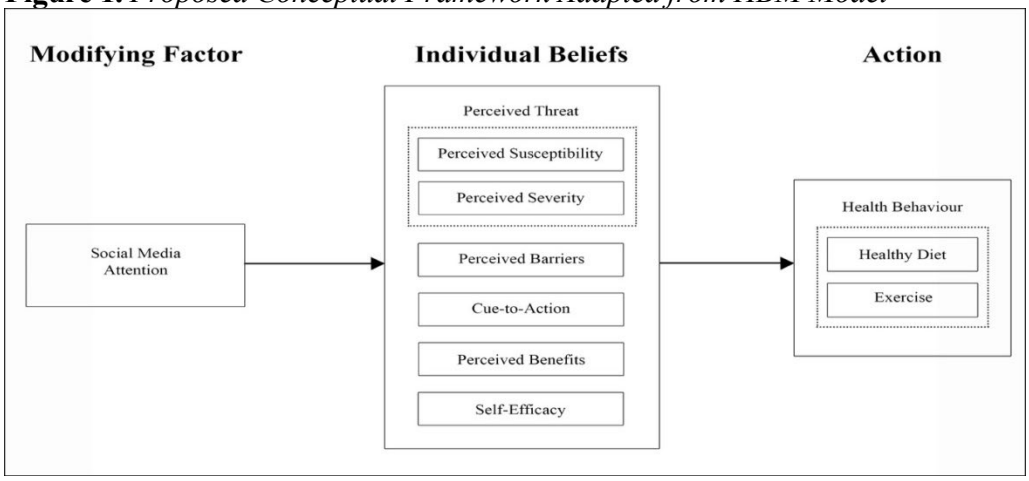
> Research Question 7 (RQ7): To what extent does the knowledge of urban men regarding the HBM constructs influence their adoption of health behaviors, particularly with respect to maintaining a healthy diet and engaging in regular exercise?

As a result, the following hypotheses have been formulated:

> Hypothesis 1 (H1): Paying attention to obesity-related social media content has a significant influence on the perception of the associated threats related to obesity.
>
> Hypothesis 2 (H2): Paying attention to obesity-related social media content has a significant influence on the perception of barriers to addressing obesity.
>
> Hypothesis 3 (H3): Paying attention to obesity-related social media content has a significant influence on the motivation and cues to take action toward addressing obesity.
>
> Hypothesis 4 (H4): Paying attention to obesity-related social media content has a significant influence on the perception of the benefits associated with adopting health behaviors aimed at overcoming obesity.
>
> Hypothesis 5 (H5): Paying attention to obesity-related social media content has a significant influence on an individual's self-efficacy in adopting health behaviors to combat obesity.
>
> Hypothesis 6 (H6): Knowledge of the Health Belief Model (HBM) constructs significantly influences the adoption of health behaviors related to maintaining a healthy diet and engaging in regular exercise among urban men.

Therefore, this study employed a conceptual framework encompassing all the constructs of the Health Belief Model (HBM) to assess whether the attention of urban men to obesity-related content on social media influences these HBM constructs and subsequently affects the adoption of health behaviors, specifically focusing on maintaining a healthy diet and engaging in exercise. Figure 1 illustrates the conceptual framework derived from the HBM model (Champion & Skinner 2008).

Figure 1. *Proposed Conceptual Framework Adapted from HBM Model*



Materials and Methods

Digital Questionnaire

The primary research methodology employed in this study is a survey, which serves as a systematic approach for gathering data from a sample of respondents to gain insights into a larger population to which these respondents belong (Groves et al. 2009). Drawing inspiration from the literature of similar studies utilizing the Health Belief Model (HBM), we created a survey questionnaire (Riggs 2017). To facilitate distribution and participation, this questionnaire was developed using Google Forms. It was strategically designed so that individuals who read and consented to the research details provided in the description section could proceed to complete the form. This consent mechanism aimed to ensure the trustworthiness and fidelity of the respondents (Sala et al. 2012).

Data Collection

The digital survey questionnaire was distributed during the final week of September and throughout October 2019. To align with the World Obesity Day, which was observed on October 11, 2019, only responses received within the month of October 2019 were included in the study (World Obesity Organization 2019). A total of 792 responses were collected from men who actively use social media and express interest in obtaining health information through these platforms. After excluding entries from locations outside the designated study area of Chennai and eliminating outliers, a total of 530 responses were deemed suitable for inclusion in the study.

Statistical Analysis

The study initially presents demographic data pertaining to age and BMI, as well as information regarding respondents' engagement with social media, exposure to, and attention toward obesity-related content. This data is presented in

terms of frequency and percentage distributions. Subsequently, the collected data underwent reliability and normality tests. To assess the relationships within the dataset, Pearson's correlation analysis was employed to examine the associations between the independent variable (attention to obesity-related social media content) and the dependent variables (perceived threat, perceived barriers, cue-to-action, perceived benefits, and self-efficacy). Furthermore, to explore potential linear relationships between the dependent variables and health behavior, multiple linear regression analysis was applied.

Results

Demographic Data

In the respondent demographic breakdown, it is observed that the majority, accounting for 70%, fall within the age range of 19 to 25 years. Additionally, 25% of the respondents are aged between 26 and 35 years, while a smaller percentage, specifically 5%, are above 35 years old. Regarding BMI distribution, approximately 4% of the respondents are classified as underweight, while the majority, constituting 58%, fall within the normal BMI range. Furthermore, 29% of the respondents are categorized as overweight, and 9% are identified as obese.

Social Media Consumption

The researchers conducted a preliminary survey involving 100 randomly selected men from Chennai to identify the most commonly utilized social media platforms for obtaining health-related information. The results of this survey indicated that Facebook, YouTube, and Instagram ranked as the top three social media platforms among Chennai men when seeking health information. Specifically, among the respondents in the main study, it was found that 82% of them use a combination of Facebook, YouTube, and Instagram for this purpose. Additionally, 6% use both Facebook and YouTube, 2% use both Facebook and Instagram, 4% use both YouTube and Instagram, 3% solely utilize Facebook, 1% exclusively use YouTube, and 2% exclusively rely on Instagram. Please refer to Table 1 for further details on the duration of social media consumption by the respondents.

Table 1. *Social Media Consumption Duration of the Respondents*

Social Media	**Not necessarily use everyday**	**Use every day for**			
		Less than 15 minutes	**At least 30 minutes**	**At least one hour**	**More than one hour**
Facebook (495 respondents)	3%	34%	22%	22%	19%
YouTube (500 respondents)	3%	27%	29%	19%	22%
Instagram (470 respondents)	1%	31%	21%	18%	29%

Exposure and Attention to Obesity-related Content

Media exposure refers to the degree to which individuals using media platforms come across messages related to a particular topic (de Vreese & Neijens 2016). In this study, respondents were requested to employ a five-point Likert scale (ranging from 1=Never to 5=Frequently) to express the frequency with which they encounter information about obesity on social media. The mean exposure of the respondents to obesity-related content across the three social media platforms is presented in Table 2.

Table 2. *Exposure of Respondents to Obesity-Related Social Media Content*

Social Media	Total Responses	Minimum (Never)	Maximum (Frequently)	Mean
Facebook	495	1	5	3.16
YouTube	500	1	5	3.09
Instagram	470	1	5	3.06

Media attention refers to the degree of focus or concentration that individuals using media platforms devote to specific information they come across (de Vreese & Neijens 2016). In this study, respondents were instructed to employ a five-point Likert scale (ranging from 1=No attention to 5=Most attention) to express the level of attention they allocate to information about obesity on social media. Table 3 presents the average attention levels of the respondents regarding obesity-related content across the three social media platforms.

Table 3. *Attention of Respondents to Obesity-Related Social Media Content*

Social Media	Total Responses	Minimum (Never)	Maximum (Frequently)	Mean
Facebook	495	1	5	2.29
YouTube	500	1	5	2.57
Instagram	470	1	5	2.37

Though the respondents had considerably good exposure to obesity related social media contents, their attention to those obesity related contents were less comparatively.

Findings Based on HBM

Constructs of HBM

The answers provided for each item within the Health Belief Model (HBM) constructs, including Perceived Susceptibility, Perceived Severity, Perceived Barriers, Perceived Cue-to-action, Perceived Benefits, and Perceived Self-efficacy, were averaged. Table 4 presents the mean values derived from the responses for each of the HBM constructs.

Table 4. *Mean Responses for Constructs of HBM.*

Factor of HBM	Minimum	Maximum	Mean (M)
Perceived Susceptibility	1	5	2.69
Perceived Severity	1	5	2.53
Perceived Barrier	1	5	2.28
Perceived Cue-to-action	1	5	2.61
Perceived Benefits	1	5	2.91
Perceived self-efficacy	1	5	2.71

In general, the respondents indicated that they perceive health behaviors, specifically maintaining a healthy diet and engaging in exercise, as highly effective (with an average rating of M=2.91) in reducing the risk of obesity. Furthermore, they expressed a strong belief in their own capacity to adopt these health behaviors (with an average rating of M=2.71).

Health Behaviour

Healthy diet and regular exercise are two widely recognized and widely accepted health behaviors for preventing and addressing obesity (Abdel-Hamid 2003). The mean value for respondents' behavioral intention regarding a healthy diet is notably higher (M=3.49) than their behavioral intention toward exercise (M=2.00).

Statistical Analysis

Normality Test

The results of the normality test, as presented in Table 5, indicate that the skewness and kurtosis values of the variables fall within the range of +2. The lowest kurtosis value observed is -1.439 for item EE11, while the highest kurtosis value recorded is 0.571 for item C2. Additionally, the highest skewness value detected is 1.076 for item C6, and the lowest skewness value observed is 0.036 for item EE10. These results from the normality test demonstrate that the items exhibit a normal distribution, as the skewness and kurtosis values for each item fall within the acceptable ranges, as established by Kit et al. (2014).

Table 5. *Normality Statistics*

Constructs	Items	Skewness	Kurtosis
Susceptibility	A1	.827	.200
	A2	.312	-1.269
	A3	.376	-1.034
	A4	.050	-1.294
	A5	.344	-.988
	A6	.243	-.992
Severity	B1	.485	-.665
	B2	.244	-.727
	B3	.106	-1.231
	B4	.821	.014
	B5	.190	-.980
	B6	.856	.474
	B7	.349	-.540
	B8	.491	-.401
	B9	.516	-.518

	B10	.365	-1.006
	B11	.750	-.139
	B12	.845	-.007
	B13	.513	-.550
	B14	.449	-.471
	B15	.422	-.697
	B16	.527	-.586
	B17	.969	.361
	B18	.807	.217
	B19	.402	-.883
Barriers	C1	1.037	.439
	C2	.922	.571
	C3	.843	.193
	C4	.886	.266
	C5	.889	.018
	C6	1.075	.543
	C7	.646	-.460
	C8	.690	-.622
	C9	.461	-.557
	C10	.621	-.750
	C11	.716	-.421
	C12	1.035	.379
	C13	.654	-.201
	C14	.707	.051
	C15	.610	-.629
	C16	.720	-.079
	C17	1.059	.554
	C18	.712	-.664
	C19	.528	-.678
	C20	.693	-.118
Cue-to-actions	D1	.543	-1.031
	D2	.431	-.592
	D3	.238	-.961
	D4	.258	-.980
	D5	.268	-.912
	D6	.465	-.831
	D7	.322	-.784
	D8	.295	-.989
	D9	.151	-1.028
	D10	.141	-.807
	D11	.364	-.865
	D12	.308	-.880
	D13	.252	-1.123
Benefits	E1	.194	-1.406
	E2	.289	-1.223
	E3	.173	-1.389
	E4	.254	-1.210
	E5	.345	-1.026
	E6	.089	-1.333
	E7	.115	-1.259
	E8	.170	-1.314
	E9	.179	-1.273
	E10	.036	-1.415

	E11	.099	-1.439
	E12	.137	-1.268
	E13	.203	-1.106
	E14	.085	-1.234
	E15	.245	-1.128
Self-efficacy	F1	.143	-1.379
	F2	.298	-.828
	F3	.160	-.894
	F4	.155	-1.035
	F5	.197	-1.174
	F6	.055	-.890
	F7	.213	-.879
	F8	.130	-.994
	F9	.296	-.937
	F10	.115	-1.026
	F11	.178	-.748
	F12	.148	-1.101
	F13	.249	-.726
	F14	.233	-.696

Relationship between Attention and Perceived Threat

Hypothesis 1 (H1) suggests that paying attention to obesity-related social media content influences consumers' perception of the threat posed by obesity. To assess this hypothesis, items measuring perceived susceptibility and perceived severity were adapted from a similar study conducted among college students (Riggs 2017). Respondents were asked to express their level of agreement using a five-point Likert scale (ranging from 1=Not at all susceptible/severe to 5=Extremely susceptible/severe) with various statements related to perceived susceptibility and perceived severity regarding obesity. These statements covered factors such as genetics, lack of physical activity, consumption of sugary beverages, consumption of junk foods, mental illness, lifestyle changes, and the health and social consequences of obesity.

The 25 items used for measuring perceived susceptibility and perceived severity demonstrated high internal reliability (α=0.963) when combined.

To test H1, a bivariate Pearson correlation analysis was conducted. The results revealed a statistically significant correlation between attention to obesity-related social media content and perceived threat (R=.206, p=.034). Table 6 presents the results of a simple regression analysis, indicating that attention to obesity-related social media content significantly influenced perceived threat [F(1,528)=4.613; p=.034], explaining 4.2% of the variance. Therefore, H1 was supported. It's important to note that the degree of correlation is considered low, as the R value (R=.206) falls below the threshold of 0.3 (Ratner 2009).

Table 6. *Simple Regression Testing the Attention to Obesity Content on perceived Threat*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.206[a]	.042	.033	22.04658

a. Predictors: (Constant), Attention

ANOVA[a]

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	2242.125	1	2242.125	4.613	.034[b]
	Residual	50549.385	528	486.052		
	Total	52791.509	529			

a. Dependent Variable: Threat
b. Predictors: (Constant), Attention

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	53.767	5.344		10.061	.000
	Attention	1.548	.721	.206	2.148	.034

a. Dependent Variable: Threat

Relationship between Attention and Perceived Barriers

Hypothesis 2 (H2) posits that paying attention to obesity-related social media content influences consumers' perceived barriers to treating obesity. To examine this hypothesis, respondents were asked to use a five-point Likert scale (ranging from 1=Not a barrier to 5=Most important barrier) to express their agreement with various statements related to perceived barriers associated with obesity. These statements encompassed categories such as awareness, emotional/mental health, internal and external factors affecting physical activity, and several related sub-components.

The 20 items used for measuring perceived barriers demonstrated strong internal reliability (α=0.933) when aggregated.

To test H2, a bivariate Pearson correlation analysis was conducted. The results revealed that attention to obesity-related social media content and perceived barriers were not statistically correlated (R=.122, p=.213). Therefore, H2 was not supported.

Relationship between Attention and Cue-to-Action

Hypothesis 3 (H3) posits that paying attention to obesity-related social media content influences consumers' cue-to-action to address obesity. To investigate this hypothesis, respondents were asked to use a five-point Likert scale (ranging from 1=Strongly disagree to 5=Strongly agree) to express their level of agreement with various statements related to cue-to-action in the context of obesity. These statements encompassed factors such as personal dissatisfaction with one's body, seeking expert advice, receiving advice from friends and family, health problems

related to weight, perceptions of unfair judgment based on weight, and other relevant factors.

The 13 items used for measuring cue-to-action demonstrated high internal reliability (α=0.970) when combined.

To test H3, a bivariate Pearson correlation analysis was conducted. The results indicated that attention to obesity-related social media content and cue-to-action were statistically correlated (R=.229, p=.018). Table 7 presents the results of a simple regression analysis, demonstrating that attention to obesity-related social media content significantly influenced cue-to-action [F(1,528)=5.747; p=.018], explaining 5.2% of the variance. Therefore, H3 was supported. It's important to note that the degree of correlation is considered low, as the R value (R=.229) is less than 0.3 (Ratner 2009).

Table 7. *Simple Regression Testing the Attention to Obesity Content on Cue-to-Action*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.229[a]	.052	.043	13.45659

a. Predictors: (Constant), Attention

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1040.612	1	1040.612	5.747	.018[b]
	Residual	18832.303	528	181.080		
	Total	19872.915	529			

a. Dependent Variable: Cuetoactions
b. Predictors: (Constant), Attention

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	26.864	3.262		8.236	.000
	Attention	1.055	.440	.229	2.397	.018

a. Dependent Variable: Cuetoactions

Relationship between Attention and Perceived Benefits

Hypothesis 4 (H4) posits that paying attention to obesity-related social media content influences consumers' perception of the benefits associated with treating obesity. To investigate this hypothesis, respondents were asked to use a Likert scale ranging from 1, signifying 'not at all beneficial,' to 5, signifying 'extremely beneficial,' to indicate their level of agreement with various statements related to the perceived benefits of addressing obesity. These statements covered a wide range of factors, including improved health, prevention of chronic diseases, ease of daily life, attractiveness to others, social reactions, increased energy, mental well-being, personal and professional goal achievement, enhanced physical appearance, long-term health, physical fitness, self-esteem, comfort in social situations, and better sleep quality.

The 15 items used for measuring perceived benefits demonstrated strong internal reliability (α=0.988) when aggregated.

To test H4, a bivariate Pearson correlation analysis was conducted. The results indicated that attention to obesity-related social media content and perceived benefits were statistically correlated (R=.249, p=.010). Table 8 presents the results of a simple regression analysis, revealing that attention to obesity-related social media content significantly influenced perceived benefits [F(1,528)=6.869; p=.010], explaining 6.2% of the variance. Therefore, H4 was supported. It's noteworthy that the degree of correlation is considered low, as the R value (R=.249) is less than 0.3 (Ratner 2009).

Table 8. *Simple Regression Testing the Attention to Obesity Content on Perceived Benefits*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.249[a]	.062	.053	18.98427

a. Predictors: (Constant), Attention

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2475.637	1	2475.637	6.869	.010[b]
	Residual	37481.873	528	360.403		
	Total	39957.509	529			

a. Dependent Variable: Benefits

b. Predictors: (Constant), Attention

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	32.667	4.602		7.099	.000
	Attention	1.627	.621	.249	2.621	.010

a. Dependent Variable: Benefits

Relationship between Attention and Self-Efficacy

Hypothesis 5 (H5) posits that paying attention to obesity-related social media content influences consumers' self-efficacy. To investigate this hypothesis, respondents were asked to use a Likert scale ranging from 1, signifying 'strongly disagree,' to 5, signifying 'strongly agree,' to indicate their level of agreement with various statements related to self-efficacy in the context of addressing obesity. These statements covered a wide range of factors, including refraining from sedentary lifestyles, avoiding sweets and fatty foods, regular exercise, balanced eating, mindful eating, and resisting external temptations to eat.

The 14 items used for measuring self-efficacy demonstrated strong internal reliability (α=0.963) when combined.

To test H5, a bivariate Pearson correlation analysis was conducted. The results indicated that attention to obesity-related social media content and self-efficacy were statistically correlated (R=.296, p=.002). Table 9 presents the results of a simple regression analysis, revealing that attention to obesity-related social

media content significantly influenced self-efficacy [F(1,528)=9.953; p=.002], explaining 8.7% of the variance. Therefore, H5 was supported. It's noteworthy that the degree of correlation is considered low, as the R value (R=.296) is less than 0.3 (Ratner 2009).

Table 9. *Simple Regression Testing the Attention to Obesity Content on Self-Efficacy*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.296[a]	.087	.079	13.55605

a. Predictors: (Constant), Attention

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1829.058	1	1829.058	9.953	.002[b]
	Residual	19111.706	528	183.766		
	Total	20940.764	529			

a. Dependent Variable: Selfefficay

b. Predictors: (Constant), Attention

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	28.549	3.286		8.688	.000
	Attention	1.398	.443	.296	3.155	.002

a. Dependent Variable: Selfefficay

Relationship between HBM Constructs and Health Behaviour

Hypothesis 6 (H6) posits that knowledge of the Health Belief Model (HBM) constructs, obtained through attention to obesity-related social media content, influences consumers' health behavior. To test H6, a multivariate regression analysis was conducted. The results indicated that the HBM constructs can be used to explain health behavior change [F(1,528)=2.325; p=.038], contributing to 12.4% of the variance. Thus, H6 was supported. However, it's important to note that the degree of correlation between the HBM constructs and health behavior is considered low, as the R value is .378 (Ratner 2009).

According to Table 10, the results show that cue-to-action is the only significant predictor with a positive influence on health behavior change. Conversely, perceived susceptibility, perceived barriers, perceived benefits, and self-efficacy do not have a significant influence on the adoption of health behavior because their p-values are greater than 0.05. Additionally, perceived severity was found to negatively influence health behavior.

Table 9. *Multiple Regression Testing the Knowledge of HBM Constructs on Health Behaviour*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.351[a]	.124	.070	1.76592

a. Predictors: (Constant), Selfefficacy, Barriers, Susceptibility, Benefits, cuetoaction, Severity

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	43.508	6	7.251	2.325	.038[b]
	Residual	308.727	523	3.118		
	Total	352.236	529			

a. Dependent Variable: HealthBehaviour

b. Predictors: (Constant), Selfefficacy, Barriers, Susceptibility, Benefits, cuetoaction, Severity

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	5.232	.603		8.675	.000
	Susceptibility	.058	.045	.202	1.295	.198
	Severity	-.047	.017	-.445	-2.813	.006
	Barriers	.002	.017	.019	.135	.893
	Benefits	-.020	.019	-.150	-1.043	.299
	cuetoaction	.035	.015	.378	2.392	.019
	Selfefficacy	.001	.018	.008	.055	.956

a. Dependent Variable: HealthBehaviour

Discussion

The Health Belief Model (HBM) provides a structured framework for comprehending individuals' healthcare decision-making processes. This study aimed to assess the attitudes of urban men in Chennai towards health behavior change, taking into account their exposure to obesity-related content on social media. The findings indicate that the attention participants paid to obesity-related information on social media significantly influenced their perceptions of obesity as a threat, their belief in the benefits of treating obesity, their confidence in their ability to adopt health behaviors to address obesity, and their initiation of actions to tackle this issue. However, the impact of their attention to social media obesity content was not significant enough to influence their assessment of the barriers to adopting health behavior changes for obesity treatment.

Furthermore, the study revealed that, overall, the HBM constructs (Perceived Susceptibility, Perceived Severity, Perceived Barriers, Cue-to-action, Perceived Benefits, and Self-Efficacy) had a marginal influence on participants' health behavior changes for treating obesity. Notably, cue-to-action emerged as the only significant predictor with a positive impact on health behavior change. This suggests that the presence of a compelling trigger is essential for urban men to initiate and maintain health behavior changes to address obesity. Conversely, perceived severity was found to have a negative influence on health behavior,

suggesting that an excessive emphasis on the seriousness of obesity and its consequences in social media content could deter urban men from adopting health behavior changes to treat obesity.

Participants' responses indicated that they had a reasonable awareness of how obesity could negatively affect various aspects of health-related quality of life, including physical, mental, and social functioning (Kushner & Foster 2000). However, their awareness of the sexual health consequences of obesity appeared to be limited, as only a small percentage believed that obesity could lead to erectile dysfunction (7.5%) and a decreased quality of sexual life (10.4%) (Kolotkin et al. 2012).

Nevertheless, it's important to acknowledge the limitations of this study. It was conducted in a single city within a specific cultural context, and India's diverse culture may limit the generalizability of the results. The survey questions related to health behavior (healthy eating and exercise) were asked at a broad level, and variations in food habits and lifestyles across different geographical regions could influence the propensity to adopt healthy eating and exercise habits. Additionally, participants' knowledge about obesity, aside from the information they obtained from social media content, could have influenced the study's outcomes.

Future research endeavors should take these limitations into account and consider conducting studies in diverse cultural contexts to gain a more comprehensive understanding of the influence of social media on health behavior change regarding obesity.

Conclusion

In conclusion, this study has delved into the intricate relationship between social media, health behavior change, and obesity awareness among urban men in Chennai. Employing the Health Belief Model (HBM) as a guiding framework, the research aimed to elucidate how individuals' engagement with obesity-related content on social media platforms influenced their perceptions and subsequent health behavior decisions.

The findings of this investigation unveiled a nuanced connection between social media exposure and health behavior transformation. Notably, participants who exhibited a higher level of attention to obesity-related content on social media tended to have heightened perceptions of obesity as a significant threat. They also displayed a stronger belief in the benefits of addressing obesity and an increased self-efficacy in adopting healthier behaviors. Moreover, these individuals were more inclined to take actionable steps toward treating obesity, as indicated by their cues-to-action.

However, it is important to note that the influence of social media was not uniform across all facets of the Health Belief Model. While it positively impacted perceived threat and cues-to-action, it did not significantly sway participants' assessments of the barriers to health behavior change. Interestingly, the study also revealed that excessive emphasis on the severity of obesity and its consequences in

social media content could potentially deter individuals from engaging in health behavior change, highlighting the need for a balanced and informative approach in online health communication.

Nonetheless, this research contributes valuable insights into the role of social media in shaping health-related perceptions and behaviors among urban men. Future studies should consider diverse cultural contexts and broader geographical areas to enhance the generalizability of findings. Additionally, exploring the long-term effects of social media engagement on sustained health behavior change would provide a more comprehensive understanding of its impact in combating the global obesity epidemic.

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