ISSN : 2707-6695 (Online) | ISSN : 2707-6687 (Print)

Volume 4 Issue 1

LIFE SCIENCE JOURNAL OF PAKISTAN

LSJP https://www.lifesciencejournal.pk

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

LIST OF CONTENTS

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

Editorial – Issue 1, Volume 4

Life Science Journal of Pakistan (LSJP) is going to publish its fifth issue and become more and more credible among life science researchers who are sending their valuable articles for publication. In the current issue, five manuscripts have been selected for publication, out of which three are original research articles and two review articles. First manuscript focuses on immuno-chromatographic assay-based frequency estimation of most common infectious diseases among the hospital visiting patients of Peshawar, Khyber Pakhtunkhwa, Pakistan. Authors reported almost half of the hospital attending patients (47.01%) were positive for Hepatitis B virus infection followed by *Salmonella typhi* infection among 21.51% of patients. Other reported infections were Hepatitis C virus found in 14.34% of patients, *Helicobacter pylori* infection in 12.35% of patients, and almost every 20th patient was found positive with Plasmodium (4.78%). Both males and females were almost equally screened in over 750 subjects, astonishingly, over 3/4th of screened subjects belonged to the age group 21-30 years. The figures mentioned in this manuscript are alarming for health officials and demand urgent and special attention. The second manuscript accepted for publication was also of great importance in reporting post-operative surgical site infections (POSSIs) to determine the prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) in remote areas of Jacobabad. They highlighted the emergence of a significant fraction of VRSA among MRSA isolates in POSSIs. They also suggested regular monitoring of sensitivity patterns and the necessity of finding new and effective antibiotics sensitivity patterns in order to treat infections in our local settings. The third article, accepted for publication in this issue was regarding plant sciences and reports the impact of hydropriming and halopriming on seed vigor and germination of tomato seeds under heat stress conditions. Authors have devised safe, cost-effective, and easily adaptable protocols for farmers for priming of low-quality tomato seeds and they can achieve seed- germination to several folds by using such protocols.

A mini-review focusing release of the herpes simplex virus from the infected cells was also included in this issue. That review describes the role of different proteins including UL7, UL51, UL 36, UL 37, Pin1, *etc*. in the egress of the herpes simplex virus. Team LSJP is striving and supporting government agencies and healthcare workers, in fighting against COVID-19, by continuously publishing pandemic-related literature. A relevant review was included in this issue focusing on COVID-19 and human physiological systems. Authors have reviewed the effect of COVID-19 on lungs, gut, immune system, brain and mental health, cardiac system, liver, muscular system, *etc*., and reported COVID-19 is a multi-organ affecting unique virus.

LSJP will continue addressing scientific trends related to all disciplines mentioned in the scope of this journal, for that we hope for more active participation of scholars from academia and R&D institutes. Team LSJP made every effort to make the process of manuscript submission, review, and publication very user-friendly and convenient. Efforts of the LSJP team are highly acknowledged for their help and support to bring LSJP to this level of gaining excellence in life sciences publications.

Dr. Yasar Saleem,
Managing Editor,
Life Science Journal of Pakistan

ORIGINAL RESEARCH

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

Frequency Distribution of Most Common Infectious Diseases among the Hospital Visited Patients of Peshawar, Khyber Pakhtunkhwa, Pakistan

Muddasir Khan[1], *, Fazal Akbar[1], Abdullah[2], Syed Hussain Shah[1]

[1]Centre for Biotechnology and Microbiology, University of Swat, Swat, KPK, Pakistan
[2]Department of Health and Biological Sciences, Abasyn University Peshawar, Pakistan
***CORRESPONDING AUTHOR**
Email: mk03025678947@gmail.com, muddasir.khan0302@gmail.com

ABSTRACT

Infectious agents cause serious diseases in humans worldwide and are responsible for the high rate of morbidity and mortality. With this background, the frequency distribution of infectious diseases in the hospital visited patients and their associated risk factors were studied in Peshawar. The data of 753 immune chromatographic technique (ICT) based infected patients were collected during the period of May 2015 to April 2016 from four clinical laboratories of Peshawar. The frequency and percentage of each infectious agent/disease were calculated. The study had approval from the institutional ethical committee. A list of the Top 5 medically most important infectious agents/ diseases were generated. The results showed that 354 (47.01%) patients were positive for Hepatitis B virus infection, 162 (21.51%) were positive for *Salmonella typhi* (causing Typhoid fever) infection, 108 (14.34%) were positive for Hepatitis C virus infection, 93 (12.35%) patients were infected with *Helicobacter pylori*, and 36 (4.78%) were positive for Plasmodium (causing malaria) infection. Among the 753 patients screened for infectious diseases, 378 (50.1%) were males and 375 (49.8%) were females. The prevalence was high in the age group 21-30 and high in married patients (76%). This study provides help in the estimation of the spread of the most common diseases, the calculation of their associated risk factors, and control of the diseases in Pakistan.

Keywords: Infectious agents, Prevalence, Epidemiology, Peshawar

*Life Sci J Pak 2022; 4(01):*03-07 **DOI: https://www.doi.org/10.5281/zenodo.5809438**
(Received 01 July 2021 – Accepted 23 November 2021 – Published March, 2022)

INTRODUCTION

Microbiology deals with the study of microorganisms such as bacteria, fungi, viruses, and parasites. Millions of microscopic organisms live with us, in our environment and cause infections/ diseases in humans (1). Infectious diseases are responsible for the higher rate of morbidity and mortality in the human population worldwide. The global burden of disease study (GBDS) reported that 22% of all deaths and 27% of disabilities were caused by infectious disease during 2000 (2). Infectious diseases are classified based on the infectious agent into bacterial diseases, viral diseases, fungal diseases, and parasitic protozoan diseases, each having different cycles of infection (1).

The common human diseases caused by bacteria are typhoid fever, cholera, and tuberculosis, etc. The important species of bacteria that cause diseases in humans are *Helicobacter pylori*, *Salmonella typhi*, *Streptococcus pneumonia*, *Mycobacterium tuberculosis*, *Vibrio cholera*, *Brucella abortus*, and *Clostridium tetani* (3). Major agents of disease in humans are species of Trypanosomes (causing sleeping sickness or Chagas' disease), Leishmania (causing Kala-azar, oriental sore), Giardia, and Trichomonas. Plasmodium (causing malaria), Toxoplasma, and Cryptosporidium are the potential diseases causing agents. Entamoeba (causing amoebiasis), Naegleria (causing amoebic meningoencephalitis) and Acanthamoeba (causing amoebic encephalitis, keratitis of the cornea) are also pathogenic to humans (4).

Therefore, the aim of this investigation is to point out the frequency distribution of the most common infectious disease and their associated risk factors in hospital visited patients of Peshawar. This investigation provides help in estimation of the spread of most common diseases, the calculation of their associated risk factors, and control of the diseases in Pakistan.

MATERIAL AND METHODS

Data collection and experimental design

Prevalence study-related data of infectious agents were collected from hospitalized infected patients, who have been visited either of the four main clinical laboratories, Sina diagnostic center and laboratory (SDC&L) opposite Hayatabad medical complex Peshawar, Al-Hafiz medical laboratory (AHML) Dabgari garden Peshawar, International medical laboratory (IML) Dabgari garden Peshawar and Frontier medical laboratory (FML) Dabgari garden Peshawar. The data were collected with the proper approval of the authorities from infected patients in the period of May 2015 to April 2016. The data of 753 infected patients were traced and recorded. The infectious diseases were diagnosed by immune chromatographic techniques (ICT). Finally, calculate the infection agents/ diseases frequency and percentage.

Ethical Statement

The study was approved by the ethical committee of the University of Swat, Swat, KPK, Pakistan.

Analysis of data

The overall prevalence of infectious diseases, gender-wise infection/ diseases, month-wise infection/ diseases, infectious disease and patient marital status, and age-wise infection/ diseases were determined. Statistical package for social sciences (SPSS) software version 23 was used for the analysis of data. Each infectious agents/ disease number and the percentage were calculated in the present study. In tables and graphs, the data was arranged and presented.

RESULTS

Overall frequency distribution of infectious diseases

The hospital visited 753 infected patients were traced from May 2015 to April 2016 in the local population of District Peshawar to determine epidemiologically important infectious diseases. The results showed that 354 (47.01%) were positive for Hepatitis B virus infection, 162 (21.51%) were positive for *Salmonella typhi* (causing Typhoid fever) infection, 108 (14.34%) were positive for Hepatitis C virus infection, 93 (12.35%) patients were infected with *Helicobacter pylori* (*H. pylori*), and 36 (4.78%) were positive for Plasmodium (causing malaria) infection (Figure 1).

Gender wise infectious diseases

Out of 753 patients screened for infectious diseases, 378 (50.1%) were males and 375 (49.8%) were females. Thus, in general, both genders were almost equally infected (Figure 2). Among 93 infected patients of *H. pylori*, 48 (51.6%) were females and 45 (48.3%) were males. A total of 162 infected patients with Typhoid fever, 81 (50%) were females and 81 (50%) were males. Among 354 infected patients of Hepatitis B, 180 (58.8%) were males and 174 (49.1%) were females. Out of 108 infected patients of Hepatitis C, 57 (52.7%) were males and females were 51 (47.2%). Total infected patients of Malaria were 36 in which, 21 (58.3%) were females and 15 (41.6%) were males (Figure 2).

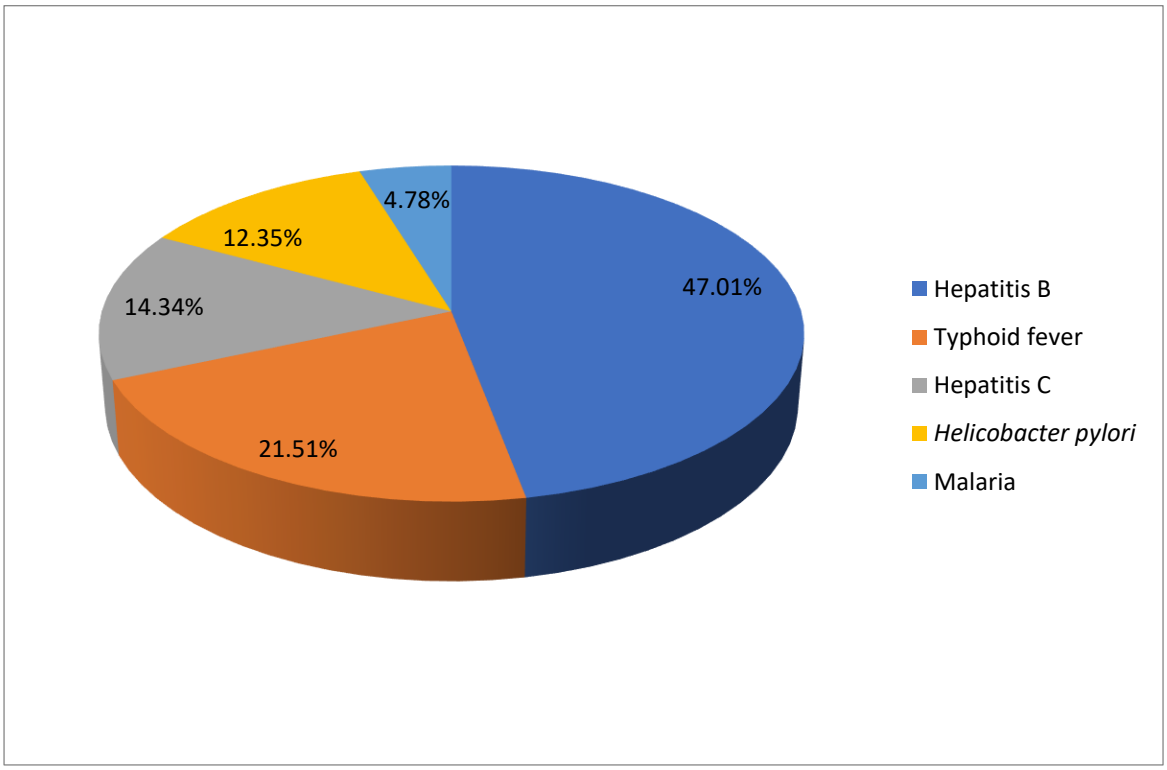

Figure-1: Overall frequency distribution of infectious diseases

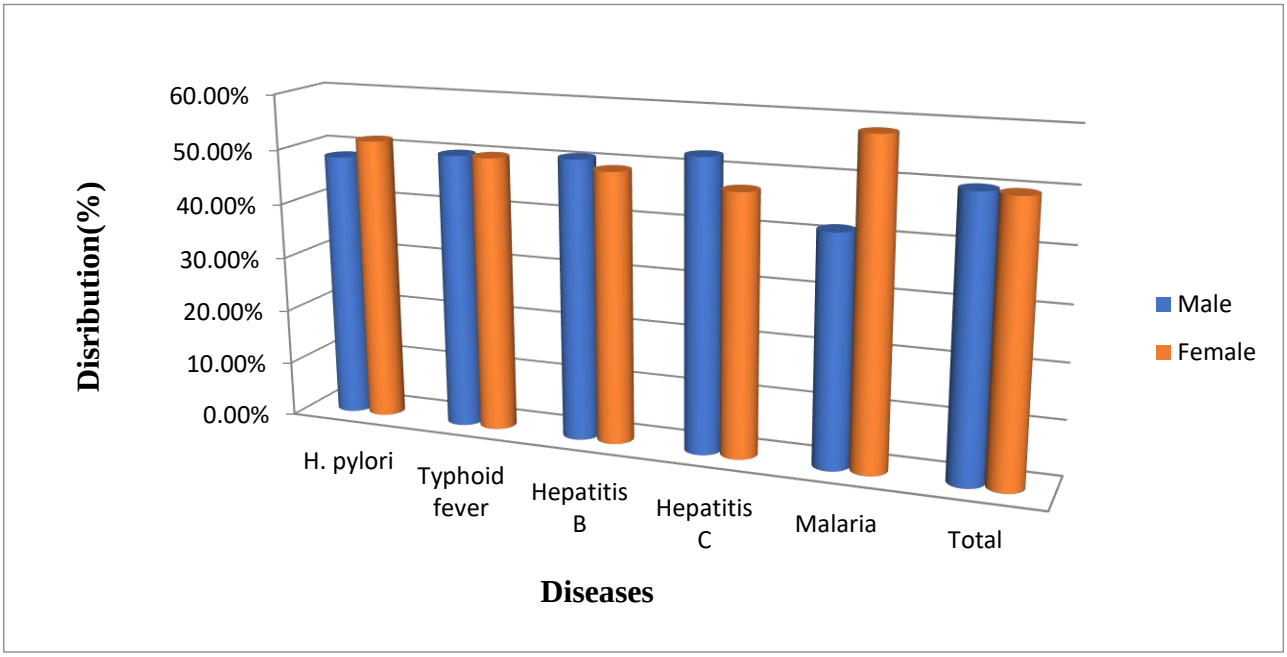

Figure-2: Gender wise frequency of infections/ diseases

Table-1: Age-wise frequency of infections/ diseases

Age Group	*H. pylori*		Typhoid fever		Hepatitis B		Hepatitis C		Malaria	
	Count	%	Count	%	Count	%	Count	%	Count	%
10 to 20	3	3.2%	39	24%	70	19.7%	46	42.5%	9	25%
21 to 30	62	66.6%	70	43.2%	231	65.2%	9	8.3%	9	25%
31 to 40	15	16.1%	18	11.1%	42	11.8%	18	16.6%	6	16.6 %
41 to 50	6	6.4%	12	7.4%	7	1.9%	21	19.4%	8	22.2 %
51 to 60	4	4.3%	21	12.9%	3	0.8%	12	11.1%	1	2.7%
61 to 70	3	3.2%	2	1.2%	1	0.2%	2	1.8%	3	8.3%
Total	93	100%	162	100%	354	100%	108	100%	36	100%

Age-wise infectious diseases

In general, the prevalence of each infectious disease was highest in the age group of 21 to 30 years old patients (Table 1). The prevalence of Hepatitis B was high in the age group of 21-30 years (65.2%), followed by the age group 10-20 years (19.7%). The remaining groups show a rate of Hepatitis B in this order, 31-40 years (11.8%), 41-50 years (1.9%), 51-60 years (0.8%), while the age group 61-70 years shows a low prevalence rate (0.2%) for Hepatitis B. The prevalence of Typhoid fever was high in the age group of 21-30 years (43.2%), followed by 10-20 years (24%). The remaining age groups show a rate of Typhoid fever in this order, 51-60 years (12.9%), 31-40 years (11.1%), 41-50 years (7.4%), while the age group 61-70 years shows a low rate (1.2%) for Typhoid fever. The prevalence of Hepatitis C was high in the age group of 10-20 years (42.5%), followed by the age group 41-50 years (19.4%). The remaining age groups show a prevalence rate of 31-40 years (16.6%), 51-60 years (11.1%), 21-30 years (8.3%), while the age group 61-70 years shows a low rate (1.8%) for Hepatitis C. The prevalence of *H. pylori* was high in the age group of 21-30 years (66.6%), followed by the age group of 31-40 years (16.1%). The remaining age groups show a rate of *H. pylori* in this order, 41-50 years (6.4%), 51-60 years (4.3%), while the age group 10-20 years (3.2%) and 61-70 years (3.2%) show a low rate of *H. pylori*. The prevalence of Malaria was equally high in the age group of 10-20 years (25%) and 21-30 years (25%). The remaining age groups show a prevalence rate in this order: 41-50 years (22.2%), 31-40 years (16.6%), 61-70 years (8.3%), while the low prevalence rate (2.7%) for Malaria was in the age group 51-60 years (Table 1).

DISCUSSION

According to modern scientific belief factors such as gender, age and geography may play an important role in determining their response to antibiotics and other such treatments. In this study five, economically important diseases (Hepatitis B, Typhoid fever, Hepatitis C, *H. pylori*, and Malaria) were reported and highlighted. However, in the studied area and population, the prevalence of Hepatitis B was much high (47%). The prevalence of viral diseases (HBV and HCV) in the studies population/ area was collectively much higher than the diseases caused by other pathogens. The previous findings are inconsistency with these observations (5, 6), they reported HBV and HCV in the top 10 list (ranked first and second, respectively) most economically important virus diseases in Pakistan. The rate of Typhoid fever (21.5%) is higher than the previous finding (7), (8), where this rate was 10%. The frequency of *H. pylori* in the studied area/ population was comparatively less than in the other big cities of Pakistan. The ranges of *H. pylori* were 66% to 84% in the Pakistan northern and central parts (9). In Karachi, the reported prevalence of H. pylori has also been high (80%) (10).

The prevalence of *H. pylori*, Typhoid fever, and Hepatitis B in the present study was almost equivalent in both genders. The previous observations are in controversy with these observations (11), wherein the Swat region the bacterial infections showed the highest prevalence in females. The differences in environmental conditions and immunity of the populations make the difference in the frequency. The prevalence of Malaria in the current notion was higher in females as compared to males. These findings and prevalence in another region of Pakistan were the same (12). In females, however, the high prevalence of Malaria in Peshawar is unclear as compared to males. In contrast, the frequency of Hepatitis C was higher in males as compared to females. The present observations with the previous findings agree (5), where a high prevalence of Hepatitis C in males (78%) vs. females (22%) was reported.

Generally, in the 21 to 30 years age group the highest prevalence of each infectious disease was reported. The previous studies agree with these observations (13), (14), where they studied in Abbottabad the H. pylori and typhoid fever prevalence. However, in the age group 11-20 years the prevalence of Hepatitis C was dramatically high. This is in controversy with the previous findings (5), where the high prevalence was reported in middle-aged people.

CONCLUSION

Among the five most important infectious diseases, results showed that 47.01% were positive for Hepatitis B virus infection, 21.51% were positive for *Salmonella typhi* infection, 14.34% were positive for Hepatitis C virus infection, 12.35% patients were infected with *Helicobacter pylori*, and 4.78% were positive for Plasmodium infection.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

Acknowledgment: Authors appreciation goes to all teachers and staff at the Centre for Biotechnology and Microbiology, University of Swat, Swat, KPK, Pakistan

REFERENCES

1. World health organization. (2001). Infections and infectious diseases, World health organization.
2. World health organization. (2002). Communicable diseases, World health organization.
3. Ali A.P., Patoli B.B., and Mehraj B. (2010). High prevalence of multi-drug resistant Escherichia coli in drinking water samples from Hyderabad. Gomal Journal of Medical Sciences, vol. 8, (pp. 1).
4. Vikarman K. (1992). The diversity and ecological significance of protozoa. Biodiversity Conservation, vol. 1, (pp. 334-341).
5. Ilyas K., and Ahmad I. (2014). Prevalence of Hepatitis B and C infection in Pakistan. Journal of Infection and Molecular Biology, vol. 3, (pp. 35-38).
6. Ullah, A., Attaullah, Ullah, L., Anwar, F., Din, M., Sherwani, S.K., and Ahmad, M. (2020). Incidences of HBV infection among pre-operative patients of elective surgeries in Tehsil Dargai Malakand KPK, Pakistan: Incidences of HBV infection. Life Science Journal of Pakistan, 2(1), 8-13.
7. Ezeigbo O.R., Agomoh N.G., and Chuks N.A. (2015). Laboratory Diagnosis of Typhoid fever using Widal and Blood culture Methods in Aba, Southeastern Nigeria. American Journal of Microbiological Research, vol. 3, (pp. 181-183).
8. Ayub U., Khattak A.A., Saleem A., Javed A., Siddiqui N., Hussain N., and Hayat A. (2015). Prevalence of Typhoid fever in Islamabad, Pakistan. American-Eurasian Journal of Toxicological Sciences, vol. 7, (pp. 220-223).
9. Qureshi T.Z., Bilal R., Saleem K., and Zafar S. (2008). General prevalence of Helicobacter pylori infection in dyspeptic

population of Islamabad, Pakistan. Nucleus, vol. 45, (pp. 157-162).
10. Taj Y., Essa F., Kazmi S.U., and Abdullah E. (2003). Sensitivity and specificity of various diagnostic tests in the detection of Helicobacter pylori. Journal of College of Physicians and Surgeon, Pakistan, vol. 13, (pp. 90-93).
11. Shah F., and Shah A. (2016). Prevalence and epidemiology of bacterial pathogens in Swat region. Paistan, BS Microbiology thesis. Center for Biotechnology and Microbiology, University of Swat, Pakistan.
12. Wubejig A., Kibret M., and Abera B. (2014). Prevalence and Risk Factors of Helicobacter pylori from Dyspeptic Patients in Northwest Ethiopia: A Hospital Based Cross-sectional Study. Asian Pacific Journal of Cancer Prevention, (pp. 15).
13. Arshad H.A., Irfan Z.H., and Sarfraz A. (2011). Helicobacter pylori infection in patients with calcular cholecystitis: A hospital based study. Journal of Ayub Medical College Abbotabad, vol. 23, (pp. 1).
14. Farhat N., Malik S., Afzal S., and Anwar A.S. (2013). Frequency of seropositivity of Helicobacter pylori in patients presenting with dyspepsia. Journal of Ayub Medical College Abbottabad, vol. 25, (pp. 50–54).

ORIGINAL RESEARCH

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

The emergence of vancomycin resistance among methicillin-resistant Staphylococcus aureus isolates from post-operative surgical site infections at District Jacobabad

Rao Muhammad Abid Khan*[2], Aamir Ali[1], Om Parkash[3], Muneer Ahmed Qazi[4], Ali Nadeem[2]

*[1]Pathology Department, Jacobabad Institute of Medical Sciences, Jacobabad
*[2]Sindh Institute of Urology and Transplantation, Karachi
[3]Chandka Medical College, Shaheed Mohtarma Benazir Bhutto University, Larkana, 71770
[4]Institute of Microbiology, Shah Abdul Latif University, Khairpur Mirs-66020

Corresponding author
Rao Muhammad Abid Khan
Address: Sindh Institute of Urology and Transplantation, Karachi
Email: raoabid101@hotmail.com

ABSTRACT

Surgical site infections (SSIs) are the most common post-operative infections and *Staphylococcus aureus* is the leading microbial cause. SSIs contribute significantly to morbidity and healthcare costs. The epidemiology and susceptibility pattern of *S. aureus* is fluctuating over time with the propagation of newer clones and the emergence of highly resistant strains. This cross-sectional study was conducted at Jacobabad Institute of Medical Sciences (JIMS) to determine the prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients suffering from SSIs from January to October 2021. A total of 180 pus aspirate and pus swabs were collected from patients who had undergone surgical procedures. Of these, 146 (81%) specimens were bacterial culture positive. Mono-bacterial growth was isolated from 111 (76%) patients and the remaining 35 (24%) patients revealed growth of either two organisms or mixed bacterial growth. Diabetes mellitus and chronic liver disease were major underlying medical conditions. The most common bacterial isolate was *S. aureus* 88 (79%) of which 38 (43%) were MRSA. The highest sensitivity of MRSA was revealed against vancomycin (87%), followed by Amikacin and Doxycyclin 84% and 82% respectively. Minimum inhibitory concentration (MIC) of vancomycin revealed, 5 (13%) of MRSA isolates were resistant to vancomycin exhibiting three isolates MIC 32 µg/ mL and two isolates MIC 64 µg/ mL. None of the methicillin-sensitive *Staphylococcus aureus* (MSSA) was resistant against vancomycin. Among MRSA and MSSA, the highest rate of resistance was observed against sulfamethoxazole+trimethoprime and ciprofloxacin. This report highlight the emergence of a significant fraction of VRSA among MRSA isolates amongst post-operative SSIs. The findings of this study call for regular monitoring of sensitivity patterns and the necessity of new and effective antibiotics.

Keywords: Emergence, Prevalence, Vancomycin, Surgical site infections, Methicillin resistance, Staphylococcus aureus

Life Sci J Pak 2022; 4(01):08-15. **DOI: https://www.doi.org/10.5281/zenodo.6369323**
(Received 01 November 2021 – Accepted 23 January 2021 – Published March 2022)

INTRODUCTION

Surgical site infections (SSIs) have been estimated the third most common nosocomial infections worldwide and remain a great source of morbidity, a challenge for surgeons, delay wound healing, prolonged hospital stay, pain, increase in medical cost, and long-lasting disability among post-operative patients despite the antimicrobial evolution and advancement in modern aseptic techniques (1,2,3). SSIs frequently occur within 30 days following surgery (4). It has been estimated that there are about 234 million major surgeries worldwide per year. SSIs complicate 2-15% of all patients undergoing inpatient surgical procedures (5). According to the Center for Disease Control and Prevention (CDC), SSIs are of three types, superficial, deep incisional, and organ-space SSIs and among these, superficial incisional SSIs are more

common (6). Risk factors include emergency procedures, patients' age, level of care, and duration of surgery. Appropriate surgical antibiotics prophylaxis (SAP) may reduce the risk of SSIs (5).
Gram-positive cocci are generally considered the most common etiological agents over gram-negative bacteria for SSIs (7). The most common gram-positive cocci causing SSIs is *S. aureus* (7,8). *S. aureus* is usually found in the hospital environment and gains access to the surgical sites by direct contact with contaminated objects. They may also be transmitted through air droplets and aerosols produced by the health care personnel infected with *S. aureus* (9). A variant of *S. aureus* which is more virulent and causes life-threatening invasive infections among post-operative surgical patients is methicillin-resistant *S. aureus* (MRSA) (10). MRSA infections are associated with significant morbidity in both community and hospital environments (11). Since its emergence in 1961, the incidence of MRSA infections are increasing worldwide and a high surge in MRSA especially in the last decade has gotten attention from the medical community and scientists to find out the root causes and to overcome the mishaps (12).
The first choice of treatment for *S. aureus* infections is beta-lactam antibiotics. Resistance against methicillin is the indicator of resistance against most of the members of the beta-lactam group such as penicillin and cephalosporin (13) . The prevalence estimation of MRSA is important to combat the menace of this microorganism (14). The frequency of SSIs due to MRSA varies geographically and from hospital to hospital. Compared to northern Europe, the prevalence of MRSA is higher in Pakistan and India. The prevalence of MRSA in Pakistan has been estimated at 42-51% and in India at 41% (15). Glycopeptides such as vancomycin are frequently the treatment of choice for complicated MRSA infections (16). Infections due to Vancomycin-resistant *S. aureus* (VRSA) have also been reported from various parts of the world. The present study attempts to find the prevalence of MRSA and VRSA and their antimicrobial susceptibility pattern among patients suffering from post-operative SSIs.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the surgery and pathology department of Jacobabad Institute of Medical Sciences (JIMS), Jacobabad, Pakistan from January to October 2021. A total of 180 patients were recruited for this study and monitored for 30 days after surgery. Pus swabs and aspirates were collected from the surgery site of post-operative patients and inoculated on mannitol salt agar, chocolate agar, and McConkey agar. Organisms were identified using conventional methods including colony morphology, gram staining, catalase, coagulase, DNase, pigmentation, and fermentation of different sugars (17). We confined our study to gram-positive cocci and the antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method against amikacin, ciprofloxacin erythromycin, sulphamethoxazole/trimethoprim, clindamycin, vancomycin, tetracycline, fusidic Acid, and doxycycline. Resistance against methicillin was determined using cefoxitin 30 µg disc. *S. aureus* isolates showing resistance against vancomycin disc were further confirmed by determining their level of resistance by minimum inhibitory concentration using the broth dilution method. The interpretation was done according to the CLSI-2018 guidelines. Statistical analysis was done using SPSS version 20.

RESULTS

In this study, a total of 180 samples of pus swabs (n=147) and pus aspirates (n=33) were collected from the surgical site of post-operative patients ranging in age from 11-60 years with a mean age of 12.76 $\pm$ 08.27 years. A total of 146 (81%) specimens were bacterial culture positive. Mono-bacterial growth was isolated from 111 (76%) patients, no growth from 34 (23%) patients, and the remaining 35 (24%) patients revealed growth of either two organisms or poly-microbial growth. The most common isolated organism was *S. aureus* 88 (60.2%), followed by *Escherichia. coli* 44 (30.1%), *Pseudomonas aeruginosa* 10 (6.8%) and *Klebsiella spp*. 4 (2.7%).
Out of 88 *S. aureus*, 42 (48%) were isolated from males and 46 (52%) from females. A large number of *S. aureus* were isolated from age group 21-30 years 25 (28%), followed by 31-40 years 20 (23%), 11-20 and 51-60 years 15 (17% each) and from 41-50 years 13 (15%). The common sites for SSIs were legs 26 (30%), feet 22 (25%), and abdomen 18 (20%) and the least common sites were head 12 (14%), hands 8 (9%), and back 2 (2%). The most common underlying medical condition was diabetes mellitus 28 (32%), followed by chronic liver disease 24 (27%), malignancy 18 (20%), immune-suppression 10 (11%), chronic kidney disease 6 (7%), and heart failure 2 (2%) **(Table: 01).** *S. aureus* showed the highest resistance against sulfamethoxazole-trimethoprim 37 (74%) and ciprofloxacin 25 (50%) followed by clindamycin 16 (32%), fusidic acid 14 (28%), tetracycline 11 (22%), erythromycin 10 (20%), doxycycline 8 (16%), and amikacin 4 (8%) None of the methicillin-sensitive *S. aureus* (MSSA)

Table: 01. Demographic characteristics and distribution of *Staphylococcus aureus* among patients suffering from Surgical site infections (SSIs)

Characteristics	***Staphylococcus aureus* (n=88)**	
	Frequency	%
Gender		
Male	42	48
Female	46	52
Age in (years)		
11-20	15	17
21-30	25	28
31-40	20	23
41-50	13	15
51-60	15	17
Site of infection		
Legs	26	30
Feet	22	25
Abdomen	18	20
Head	12	14
Hands	8	9
Back	2	2
Underlying medical condition		
Diabetes mellitus	28	32
Chronic liver disease	24	27
Malignancy	18	20
Immune-suppression	10	11
Chronic kidney disease	6	7
Heart failure	2	2

was resistant to vancomycin in this study (**Figure: 01).**

We found a very high prevalence of MRSA in our study patients as out of 88 *S. aureus*, 38 (43%) were methicillin-resistant (MRSA). Vancomycin, amikacin, and doxycyclin were found the most effective antibiotic treatments for MRSA as 87%, 84% and 82% of MRSA isolates were sensitive to these antibiotics respectively. MRSA exhibited the highest rate of resistance against sulfamethoxazole+trimethoprime (79%) and ciprofloxacin (66%). MRSA resistance against other antibiotics was fusidic acid (42%), tetracycline (39%), a macrolide (34%), and lincosamide (32%). Notably, in this study we found 5 (13%) of MRSA isolates were resistant to vancomycin, three isolates with MIC 32 µg/ml and the two strains with MIC 64 µg/ml (Figure: 02)

DISCUSSION

Despite the modern surgical techniques and antibiotic prophylaxis, SSIs contribute to a large burden of disease and remains a global challenge for the medical community. Compared to developed countries the rate of SSIs is higher in developing countries (5). The present study reveals a very high SSI rate 146/180 (81%) among post-surgical patients in our hospital. Comparable findings were reported from Mardan, Pakistan where the rate of infection was reported 102/136 (75%) (18). Recently, a study from Islamabad reported the SSIs prevalence (29.85%) (5). In contrast, a very low frequency of SSIs 82/1120 (7.7%) among post-surgical patients was reported from Karachi and Peshawar 25/269 (9.3%) (19) . These variations in the prevalence of SSIs have been attributed to differences in hospital environments, overcrowding, poor sanitation, contaminated water, and lack of infection control policies (20).

S. *aureus* is normally found on the skin or anterior nares of 80% of healthy individuals which makes the *S. aureus* the most common etiological agent of SSIs (21). In this study, the most common isolated organism from SSIs was *S. aureus* (60.2%), followed by *E. coli* (30.1%), *P. aeruginosa* (6.8%), and *Klebsiella spp*. (2.7%). Several other studies have reported similar findings. A study from Pakistan reports the main causative agent of these infections is *S. aureus* (23.80%), followed by *E. coli* (16.66%), *P. aeroginosa*, and *Enterobacter spp.* (14.28% each). In this study, we found a very high prevalence (60.2%) of *S. aureus* among post-surgical infections compared to other studies conducted at Peshawar, Mardan, Attock and Gilgit where the prevalence of *S. aureus*

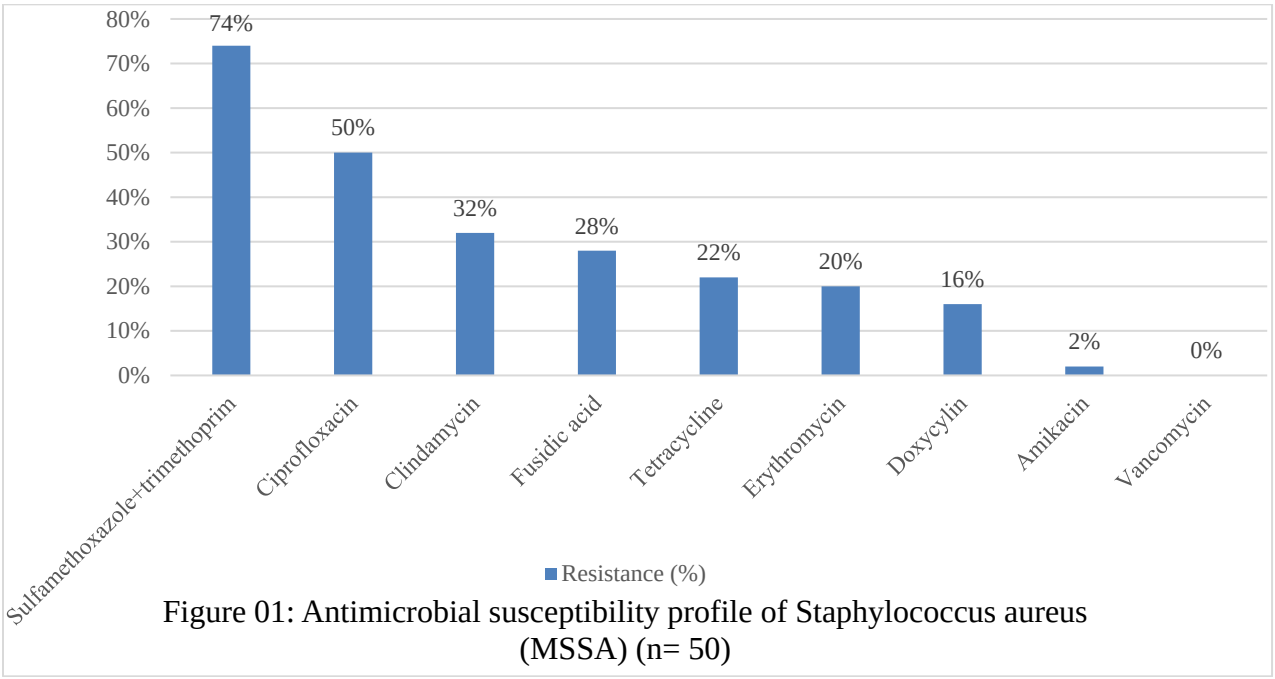

Figure 01: Antimicrobial susceptibility profile of Staphylococcus aureus (MSSA) (n= 50)

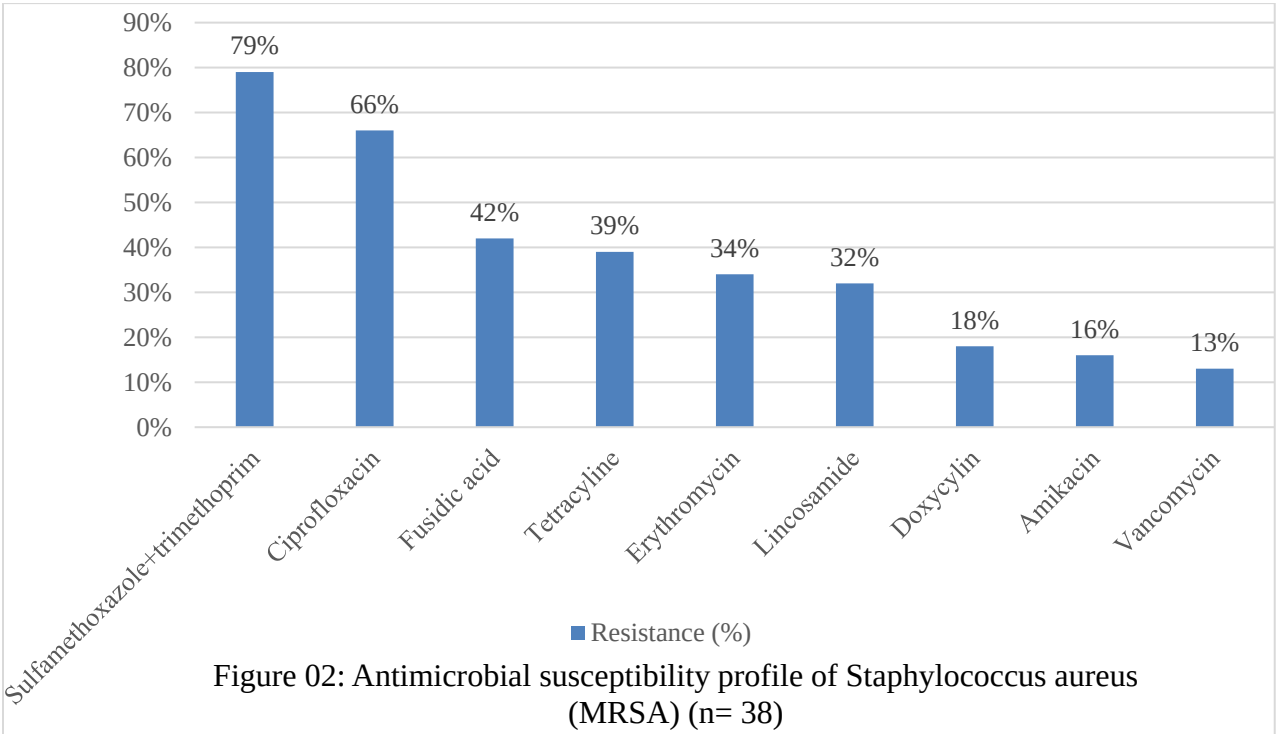

Figure 02: Antimicrobial susceptibility profile of Staphylococcus aureus (MRSA) (n= 38)

among SSIs has been reported ranging from 34-39.3% (22,23) . Our study is comparable to a study from Lahore where the prevalence of *S. aureus* among SSIs has been reported 55% (24). Another study reported the frequency of *Staphylococcus aureus* (43%) (25). Infections due to MRSA complicate the management and treatment of SSIs and beta-lactam antibiotics become no further option for treatment. MRSA has become a global threat due to its resistance to multiple antibiotics (26). In

Karachi, the first case of MRSA was reported in 1989 with a prevalence rate 5% which gradually increased up to 57% in 2002 and then gradually decreased to 38.6% in 2009 (26)**.** In this study, we report 43% of *S. aureus* were resistant to methicillin which is in concordance with several studies from other parts of Pakistan (27). Compared to our study, low prevalence of MRSA has been reported from Rawalpindi (20.3%)(28,29) and high prevalence from Mardan (66%) and Peshawar (62.9%) (30, 18). A very high prevalence of MRSA (78.9%) has also been reported from Iran (25). This variation in MRSA prevalence might be due to differences in quality of post-operative management, types of surgeries, or hospital infection control policies.

Patient's age is frequently recognized as a statistically significant factor for SSIs as decrease in immunity, increase in catabolic processes, low healing power, and co-morbid illnesses make the older age group more susceptible to SSIs (19). In this study, we report 51% of SSIs were among patients ranging in age 21-40 years which is in contrary to several other studies which report a high prevalence of SSIs among older age groups >50 years (31). In our study, we found only 17% of SSIs were among age group >51 years whereas a similar study from Pakistan has reported the highest prevalence (45.23%) in age group ranging from 65-80 years (33). Similar to our finding, few studies also report the maximum number of SSIs in the age group of 21-40 years (33). In this study, we do not find any significant statistical difference in gender prevalence of SSIs as we report 52% of SSIs were among females and 48% were among males. In our study, the rate of SSIs was slightly higher among female patients, in contrast, other studies from Pakistan report higher prevalence among male patients (25,33). In the present study, we report the diabetes mellitus and chronic liver disease major underlying medical conditions (59%) for SSIs whereas a comparable report from Pakistan reports the diabetes mellitus 30.95% and obesity 21.42% the major threat related with these infections (33).

In practice, when beta-lactam antibiotics remain no more effective treatment due to MRSA infections, glycopeptides (vancomycin) are preferably used to treat these complicated MRSA infections (16). Several studies from Pakistan and other countries claim that vancomycin most effective antibiotic treatment for MRSA infections without any resistance (34). Recent reports from several countries and Pakistan regarding the emergence of vancomycin-resistant isolates is another challenge and have further limited the treatment options (22). This alarming situation calls for the necessity to discover new antibiotics for curing MRSA and VRSA infections. Though rate of VRSA is low it has been reported from several parts of the world including Pakistan (22,24). In the present study, we report non of the MSSA isolate was resistant to vancomycin whereas 13% of MRSA isolates were resistant to vancomycin. Recently, similar resistance rate of 14% has been reported from Lahore (35). Vancomycin resistance from other parts of Pakistan has also been reported (36, 18). Recently, cumulative VRSA prevalence among SSIs in various hospitals of Peshawar has been reported 8.33% and from Lahore 2.5 to 9.8% (22, 24, 37). High rate of vancomycin resistance in our patients may be attributed to frequent and misuse of this antibiotic and improper dosage taken by the patient because literacy rate in Jacobabad district is very low.

CONCLUSION

SSIs are the common bacterial infections among post-operative patients. Gram-positive bacteria are the common cause of SSIs. *S. aureus* is the most common culprit responsible for post-operative SSIs. The high prevalence of MRSA has limited the treatment choices. The emergence of vancomycin-resistant *S. aureus* has further complicated the treatment options. Good infection control practices, strict aseptic techniques, and appropriate use of antibiotics may reduce the spread of MRSA in our hospitals. Further multi-center studies are required to determine the actual emergence of vancomycin-resistant *S. aureus* in Pakistan.

REFERENCES

1. Kassavin, D. S., Pascarella, L., & Goldfarb, M. A. (2011). Surgical site infections: incidence and trends at a community teaching hospital. *The American journal of surgery, 201*(6), 749-753.

2. Seni, J., Najjuka, C. F., Kateete, D. P., Makobore, P., Joloba, M. L., Kajumbula, H., Bwanga, F. (2013). Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Uganda. *BMC research notes, 6*(1), 1-7.

3. Shah, S., Singhal, T., & Naik, R. (2015). A 4-year prospective study to determine the incidence and microbial etiology of surgical site infections at a private tertiary care hospital in Mumbai, India. *Am J Infect Control, 43*(1), 59-62.

4. Ullah, H., Siraj, M., Ali, A., Khan, M. A. J., Khan, M. S., & Askar, Z. (2018). Infective Organisms and their Changing Antibiotic Sensitivity Trends in Infections Occurring in Orthopaedics Implant Surgery. *Journal of Pakistan Orthopaedic Association*, *30*(01), 01-04.

5. Khan, F. U., Fang, Y., Khan, Z., Khan, F. U., Malik, Z. I., Ahmed, N., . . . Rehman, A. u. (2020). Occurrence, associated risk factors, and treatment of surgical site infections in Pakistan. *European Journal of Inflammation*, *18*, 2058739220960547.

6. Yang, Z., Wang, J., Wang, W., Zhang, Y., Han, L., Zhang, Y., . . . Zhan, S. (2015). Proportions of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in patients with surgical site infections in mainland China: a systematic review and meta-analysis. *PLoS One*, *10*(1), e0116079.

7. Dessie, W., Mulugeta, G., Fentaw, S., Mihret, A., Hassen, M., & Abebe, E. (2016). Pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, Addis Ababa, Ethiopia. *Int J Microbiol*, *2016*.

8. Shariq, A., Tanvir, S. B., Zaman, A., Khan, S., Anis, A., Khan, M. A., & Ahmed, S. (2017). Susceptibility profile of methicillin-resistant Staphylococcus aureus to linezolid in clinical isolates. *International journal of health sciences*, *11*(1), 1.

9. Naik, G., & Deshpande, S. (2011). A study on surgical site infections caused by Staphylococcus aureus with a special search for methicillin-resistant isolates. *J Clin Diagn Res*, *5*(3), 502-508.

10. Gorwitz, R. J., Kruszon-Moran, D., McAllister, S. K., McQuillan, G., McDougal, L. K., Fosheim, G. E., . . . Kuehnert, M. J. (2008). Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001–2004. *The Journal of infectious diseases*, *197*(9), 1226-1234.

11. Zhao, C., Liu, Y., Zhao, M., Liu, Y., Yu, Y., Chen, H., . . . Liu, Y. (2012). Characterization of community acquired Staphylococcus aureus associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. *PLoS One*, *7*(6), e38577.

12. Kimura, Y., Morinaga, Y., Akamatsu, N., Matsuda, J., Yamaryo, T., Kawakami, K., . . . Hasegawa, H. (2016). Antimicrobial susceptibility and molecular characteristics of methicillin-resistant Staphylococcus aureus in a Japanese secondary care facility. *Journal of Infection and Chemotherapy*, *22*(1), 14-18.

13. Gurusamy, K. S., Koti, R., Toon, C. D., Wilson, P., & Davidson, B. R. (2013). Antibiotic therapy for the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in surgical wounds. *Cochrane Database of Systematic Reviews*(8).

14. Orrett, F. A., & Land, M. (2006). Methicillin-resistant S taphylococcus aureus prevalence: Current susceptibility patterns in Trinidad. *BMC Infect Dis*, *6*(1), 1-6.

15. Ullah, A., Qasim, M., Rahman, H., Khan, J., Haroon, M., Muhammad, N., . . . Muhammad, N. (2016). High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. *Springerplus*, *5*(1), 1-6.

16. Tiwari, H. K., & Sen, M. R. (2006). Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. *BMC Infect Dis*, *6*(1), 1-6.

17. Iqbal, M. S., Saleem, Y., Ansari, F., Qamar, M. U., Mazhar, S., Hassan, A., ... & Syed, Q. (2018). Staphylococcus aureus carrying lukS/F Panton-Valentine Leukocidin (PVL) toxin genes in hospitals of Lahore city. *The Journal of Infection in Developing Countries*, *12*(09), 720-725.

18. Ullah, K., Ahmad, I., & Jalil, F. (2017). BACTERIAL ISOLATES FROM SURGICAL SITE INFECTION AND THEIR PATTERN OF ANTIBIOTICS SENSITIVITY. *Journal of Bacha Khan Medical College*, *1*(2), 8-8.

19. Afzal Khan, A., Mirshad, P., & Mohammed Rafiuddin rAshed, G. B. (2013). A study on the usage pattern of antimicrobial agents for

the prevention of surgical site infections (SSIs) in a tertiary care teaching hospital. *Journal of clinical and diagnostic research: JCDR*, *7*(4), 671.

20. Malik, A. Z. (2015). Surgical site infections after elective surgery in Pakistan: Surgipak Study. *Journal of Rawalpindi Medical College*, *19*(3), 209-214.

21. Anwar, J., Zahoor, M. A., Zahoor, M. K., Siddique, A. B., Nawaz, Z., Rasool, M. H., . Yasmin, A. (2018). Efficacy of Azadirachta indica organic extracts against clinical methicillin resistant Staphylococcus aureus isolates. *Pakistan journal of pharmaceutical sciences*, *31*(4 (Supplementary)), 1485-1488.

22. Ahmad, S., Ahmed, S., Sabir, M. S., Khan, H., Rehman, M., & Niaz, Z. (2020). Frequency and comparison among antibiotic resistant Staphylococcus aureus strains in selected hospitals of Peshawar, Pakistan. *The Journal of the Pakistan Medical Association*, *70*(7), 1199-1202.

23. Ullah, A., Qasim, M., Rahman, H., Khan, J., Haroon, M., Muhammad, N., . . . Muhammad, N. (2016). High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. *Springerplus*, *5*(1), 1-6.

24. Ghias, W., Sharif, M., Yazdani, F. A., & Rabbani, M. (2016). Isolation and identification of Methicillin and Vancomycin resistance Staphylococcus aureus from pus samples of injured skin patients in Lahore, Pakistan. *Biomed Lett*, *2*(2), 103-112.

25. Khorvash, F., Mostafavizadeh, K., Mobasherizadeh, S., Behjati, M., Naeini, A., Rostami, S., . . . Khorvash, F. (2008). Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of surgical site infection (SSI); A 1 year of surveillance. *Pakistan journal of biological sciences: PJBS*, *11*(15), 1940-1944.

26. Ayoub, A., & Iqbal, A. (2018). Past & Current Status of Methicillin-Resistant Staphylococcus aureus & Vancomycin-Resistant Staphylococcus aureus in Pakistan. *Microbiology Research Journal International*, 1-7.

27. Mahmood, A. (2000). Bacteriology of surgical site infections and antibiotic susceptibility pattern of the isolates at a tertiary care hospital in Karachi. *Infection*, *3*, 4.

28. Hubab, M., Ullah, O., Hayat, A., Rehman, M. U., & Sultana, N. (2018). Antibiotic susceptibility profile of bacterial isolates from post-surgical wounds of patients in tertiary care hospitals of Peshawar, Pakistan. *J Pak Med Assoc*, *68*(10), 1517-1521.

29. Ishtiaq, S., & Ahmed, I. (2021). Susceptibility Pattern of Bacterial Isolates from Surgical Site Infections in a Tertiary Care Hospital at Rawalpindi. *Journal of Islamic International Medical College (JIIMC)*, *16*(4), 224-231.

30. Faizan, M., Ullah, I., Ullah, K., Khan, I., Jan, S. U., Ali, F., . . . Hussain, M. (2014). Prevalence and Antibiogram of Hospital Acquired Methicllin Resistant Stapylococcusaureus (HA-MRSA) from a Tertiary Care Hospital in Peshawar. *Pakistan. J Bio Mol Sci*, *2*(2), 28-37.

31. Ki, V., & Rotstein, C. (2008). Bacterial skin and soft tissue infections in adults: a review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. *Canadian Journal of Infectious Diseases and Medical Microbiology*,

32. Zafar, F., Ahmed, K. Z., & Naz, A. (2012). Surveillance Of Surgical Site Infections In Karachi, Pakistan. *Asian J. Pharm. Res. Vol*, *2*(1), 10-15

33. Ranjan, K., Ranjan, N., & Gandhi, S. (2013). Surgical site infections with special reference to methicillin resistant Staphylococcus aureus: experience from a tertiary care referral hospital in North India. *Int J Res Med Sci*, *1*(2), 108-111.

34. Akhtar, R. W., Hannan, A., Saleem, S., Qaisar, A., & Jahan, S. (2018). Frequency of vancomycin resistant Staphylococcus aureus among clinical isolates of MRSA collected from tertiary care hospitals of lahore, Pakistan. *PAFMJ*, *68*(3), 580-584.

35. Saeed, A., Ahsan, F., Nawaz, M., Iqbal, K., Rehman, K. U., & Ijaz, T. (2020). Incidence of vancomycin resistant phenotype of the methicillin resistant Staphylococcus aureus isolated from a tertiary care hospital in Lahore. *Antibiotics*, *9*(1), 3.

36. Shabbir, A. G., Ali, M., Nasir, A., & Malik, K. (2018). Prevalence and Sensitivity of Stayphlococcus Aureus in Sialkot-Pakistan. *PAKISTAN JOURNAL OF MEDICAL & HEALTH SCIENCES*, *12*(1), 18-20.

37. Liaqat, F., Sheikh, A. A., Nazir, J., Hussain, T., Rabbani, M., Shaheen, A. Y., & Muhammad, J. (2015). Isolation identification and control of vancomycin resistant Staphylococcus aureus. *Pak. J. Pharm. Sci*, *28*(3), 997-1004.

ORIGINAL RESEARCH

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

The Impact of Hydropriming and Halopriming on Seed Vigor and Germination of Tomato (*Solanum lycopersicum*) Seeds at High Temperature

Philip J.C. Harris[1], Ibrahim Ahmed Eshkab[2], Salma Assoudani Salem[3]

[1]Centre for Agroecology, Water and Resilience, Coventry University Priory Street, Coventry, CV1 5FB United Kingdom.
[2]Faculty of Agriculture, University of Tripoli, Libya.
[3]Faculty of Science, Bani Waleed University, Libya.

Corresponding Author:
Associate Professor. Dr: Ibrahim Ahmed Eshkab
Faculty of Agriculture, University of Tripoli, Tripoli - Libya.
Tel: +218 919033403
Email: I.Eshkab@uot.edu.ly

ABSTRACT

Seed priming techniques have been widely used to enhance germination, increase germination uniformity and improve seedling establishment. This study was designed to assess the impact of hydropriming or halopriming with 10, 25, 50, or 100 mM NaCI on the seed germination of tomato (*Solanum lycopersicum*) under heat stress conditions. All treatments significantly improved the seed vigor as indicated by increased germination percentage (G%), mean germination time (MGT), germination rate index (GRI), and coefficient velocity of germination (CVG) of primed seeds compared with unprimed ones. All treatments except priming with 10 mM NaCl considerably increased the mean germination rate (MGR) and a germination value (GV) above that of the control. Overall, 100 mM NaCl was superior to the other treatments in improving the measured indices.

Keywords: Germination, Halopriming, Heat stress, Hydropriming, Seed vigor, Tomato seed

Life Sci J Pak 2022; 4(01):16-24. **DOI: https://www.doi.org/10.5281/zenodo.6385333**
Received 01 December 2021 – Accepted 23 December 2021 – Published March 2022)

INTRODUCTION

It is anticipated that the atmospheric temperature will rise up to 4.8°C by 2100 (1). Thus, it may further result in changes in both geographical distribution and growing seasons of crops. (2). Furthermore, temperature and light are both significant environmental factors that regulate seed germination (3). It has also been reported that temperature is crucial in influencing both the capacity and rate of germination of many crops (4) including tomatoes (5, 6). In addition, it has also been asserted that a small rise in the mean atmospheric temperature above a critical threshold may remarkably decrease crop productivity (7). The profound effects of high temperature on vegetative and reproductive phases, the quality and yield of fruit have been reported (8, 9). Heat stress has also been reported to induce a significant reduction in the germination of a number of tomato hybrids (5). Germination inhibition of tomato seeds grown at 37 and 35°C has been observed in previous studies (6, 10). Germination and early seedling establishment are decisive phases in the plant life cycle. Seed vigor is an important aspect that empowers rapid and uniform emergence and growth in a range of environmental conditions (11). Therefore, there have been many attempts to improve seed vigor, seed viability, and seedling establishment under both normal and stressed conditions such as salinity and drought. To enhance seed vigor, several pre-germination treatments, described as seed priming, have been examined and developed (3, 12). Seed priming is a pre-sowing treatment in which seeds are partially hydrated to ensure germination metabolic processes commence, sometimes accelerated by growth regulators, but seeds are held in the lag phase of germination thus preventing radical emergence. This biological technique has been widely practiced due to its desired effects on germination, seedling vigor, plant development, and yield (13). There are numerous priming media utilized; however, hydropriming and halopriming are the most commonly adopted (12).

Hydropriming is a term describing the soaking of seeds in water prior to sowing. Previous works elucidated that hydropriming effectively improved the final germination, germination index, energy of emergence and growth of *Gerbera jamesonii* (14),

and tomato (*Solanum lycopersicum*) (15). Moreover, hydropriming has also proved effective in enhancing the seed vigor of mountain rye (*Secale montanum*) (16) and faba bean (*Vicia faba*) seeds (17). Similarly, beneficial effects of hydropriming on germinability of okra (*Abelmoschus esculentus*) seeds were also reported (18). Halopriming involves the soaking of seeds in an adjusted osmotic potential of an inorganic salt solution. However, specific priming agents, osmotic potential, and length of soaking time are critical factors in successful halopriming protocols (12, 19). It has been reported that NaCl as a halopriming agent resulted in the enhancement of seed germination and seedling vigor of many plants. For instance, halopriming with NaCl significantly increased the germination capacity, mean germination time, and germination index of safflower (*Carthamus tinctorius*) (20) and rice (*Oryza sativa*) (21). Raising the temperature by 10-15°C above the optimum is commonly ascribed as heat stress (22). The optimum temperature for the germination of tomato seeds is reported to be 25°C (6, 23). The objective of this investigation was to elucidate the effects of hydropriming and halopriming with different concentrations of NaCl on the germination vigor of tomato seeds under heat stress conditions (35°C).

METHODOLOGY

Source and Viability of Seeds

The tomato (Solanum Lycopersicum cv. Rio Grande) seed batch used in this experiment was obtained from a local supplier in Bani Walid, which is about 180 km southeast of Tripoli, Libya. These seeds were originally imported from La Semiorto Sementi, Italy by Jebal Atlas Company (Tripoli). According to the label the seeds were produced at the end of 2019 and expire at the end of 2022. However, a preliminary test revealed that unprimed, these seeds have a low germination percentage (35% at 25 °C). The low seed viability could be due to improper storage which is most likely due to long hours of power outage throughout the year.

Priming Protocols

For halopriming, 60 seeds were soaked in 100 ml of 10, 25, 50, or 100 mM NaCl solutions. For hydropriming, 60 seeds were immersed in distilled water. Priming treatments were carried out at room temperature for 24 h. After priming, NaCl primed seeds were rinsed thoroughly with distilled water to eliminate traces of salt. Then, treated seeds were surface dried and left to dry back approximately to their original weight under ambient conditions. Unprimed seeds (UP) served as control.

Germination Test

Primed and unprimed seeds were placed in 9 cm Petri dishes on two sheets of Whatman No. 1 filter paper and watered with 10 ml distilled water. Three replicates with twenty seeds of each treatment were put in an incubator at 35 °C and kept under a 16:8 h day/night regime. The Petri dishes were arranged in a completely randomized design. Seeds were deemed germinated when the radical was at least 2 mm long. Germination was recorded each 24 h for 14 days.

Indices

Germination percentage (GP), mean germination time (MGT), mean germination rate (MGR), coefficient of the velocity of germination (CVG), germination rate index (GRI), germination value (GV), and germination index (GI) were estimated to assess the impact of priming treatments on the germination vigor. The MGR and GV were evaluated utilizing the equations of Ranal and Santana (24). The other germination parameters were computed according to Kader (25).

Statistical Analysis

The data were subjected to one-way analysis of variance using Minitab software (version 16), and comparisons of means made by Tukey's Honestly Significant Difference Test (HSD) at $P < 0.05$ level of confidence.

RESULTS

Variations in germination percentage (GP), mean germination rate (MGR) and germination index (GI), mean germination time (MGT), germination rate index (GRI), germination value (GV) and coefficient velocity of germination (CVG) of tomato seeds, across different priming treatments are presented in Figures 1-7, respectively. Generally, the analysis of variance revealed that there were significant effects of priming treatments on the examined germination indices at $P \leq 0.05$.

GP of unprimed control seeds at 35 °C was 15%, considerably lower than the 35% recorded at 25 °C, confirming the adverse effect of heat stress. All of the priming treatments increased the GP compared with unprimed seeds (Figure 1). However, it is evident that significantly the greatest enhancement of GP was induced by treating seeds with 100 mM NaCl. The GP of these treatments was 95% compared with 15% of untreated seeds.

With regard to MGR (Figure 3), the results revealed that all pre-sowing treatments, except 10 mM NaCl, significantly improved the MGR compared with unprimed seeds. In fact, the MGR of 10 mM NaCl primed seeds did not differ significantly from all pre-sowing seed treatments. It is also evident from Figure 2 that all pre-sowing treatments significantly decreased the MGT compared with the control. However, there was no significant difference between priming treatments.

Germination index was also influenced by priming treatments (Figure 4). Compared with unprimed seeds, all treatments significantly improved the GI. Priming seeds with 100 mM NaCl gave a higher GI than the other three halopriming techniques but was not significantly more effective than hydropriming.

The GI of 100 mM NaCl and hydroprimed seeds was 378.3 and 295, respectively, compared with 25 recorded in untreated seeds. With regard to the GRI of tomato seeds, analysis of variance revealed that both hydropriming and halopriming treatments increased it significantly above that of untreated seeds (Figure 5).

The highest GRI was observed in seeds primed with 100 mM NaCl. However, the GRI value of this regime did not differ significantly from those obtained with 50 mM NaCl and hydroprimed seeds.

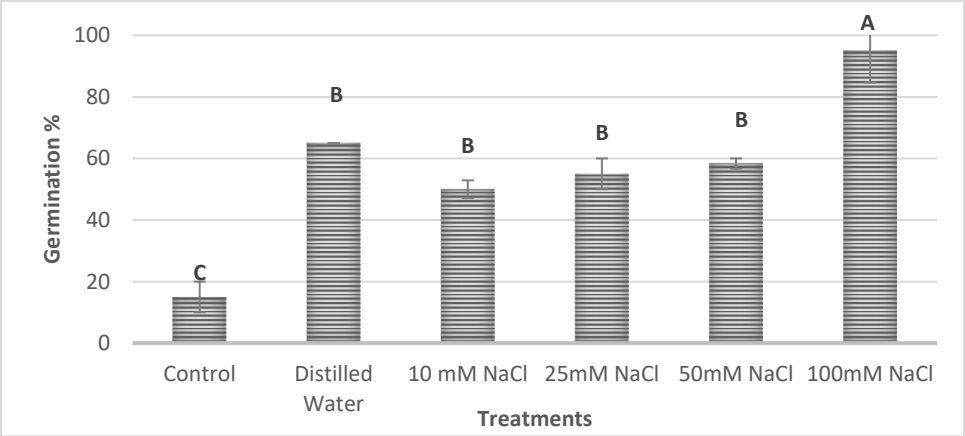

Figure. 1: The Effect of Priming Treatments on the Germination Percentage of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

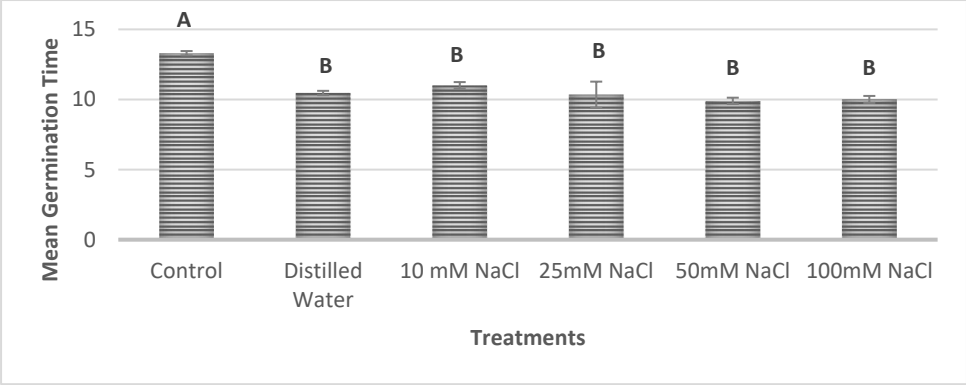

Figure 2: The Effect of Priming Treatments on the Mean Germination Time of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

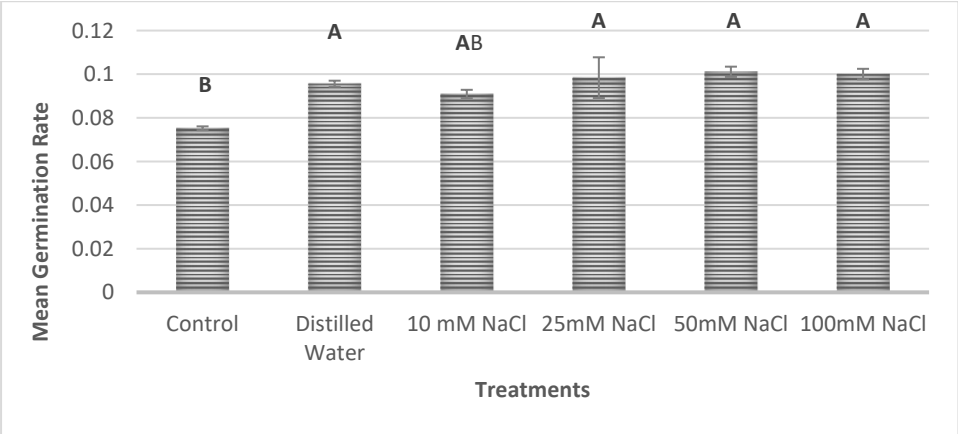

Figure 3: The Effect of Priming Treatments on the Mean Germination Rate of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

In addition, the GRI of the hydroprimed seeds was statistically similar to all four halopriming protocols. With regard to GV (Figure 6), the results revealed that all pre-sowing treatments, except 10 mM NaCl, significantly improved the GV compared with unprimed seeds. The highest GVs were recorded in 100 mM NaCl and hydroprimed seeds and these two treatments did not differ significantly. In fact, the germination value of hydroprimed seeds did not differ significantly from any of the halopriming protocols. The GV of 100 mM NaCl primed seeds was 6.6 compared with 0.011 observed in the control. Pre-sowing seed treatments also considerably enhanced the CVG of tomato seeds compared with untreated ones (Figure 7). Among all treatments, halopriming with 100 and 50 mM NaCl induced a greater CVG than priming with 10 or 25 mM NaCl but did not differ significantly from hydropriming. Furthermore, the CVG of hydroprimed seeds was also statistically similar to that recorded with 10 and 25 mM haloprimed seeds.

DISCUSSION

Exposure of plants to temporary or continuous heat stress may cause a severe decline in productivity through the induction of morphological, molecular, physiological, and biochemical alterations. It influences crops at different stages of development (22, 26), including germination (22). However, the threshold for heat stress-induced symptoms varies among species. Heat stress may induce a significant reduction in the germination speed, and germination capacity may be entirely inhibited (22).

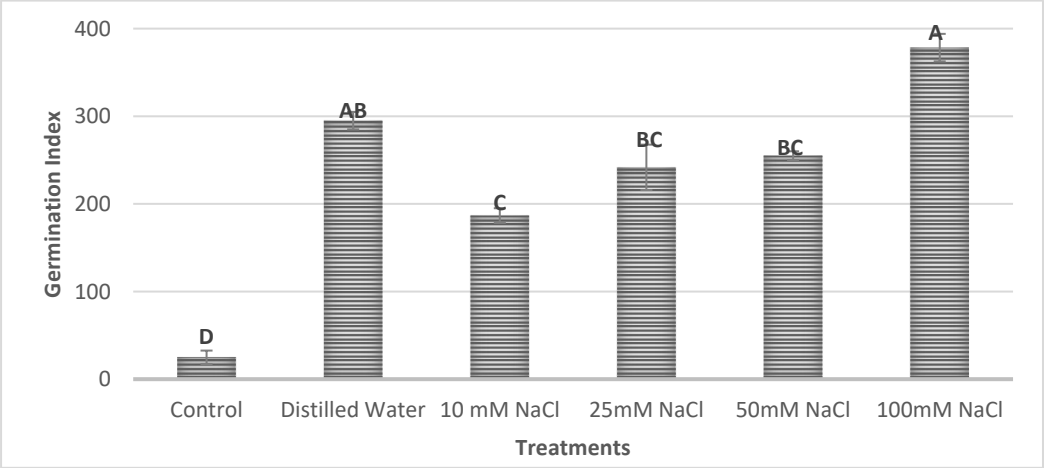

Figure 4: The Effect of Priming Treatments on the Germination Index of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

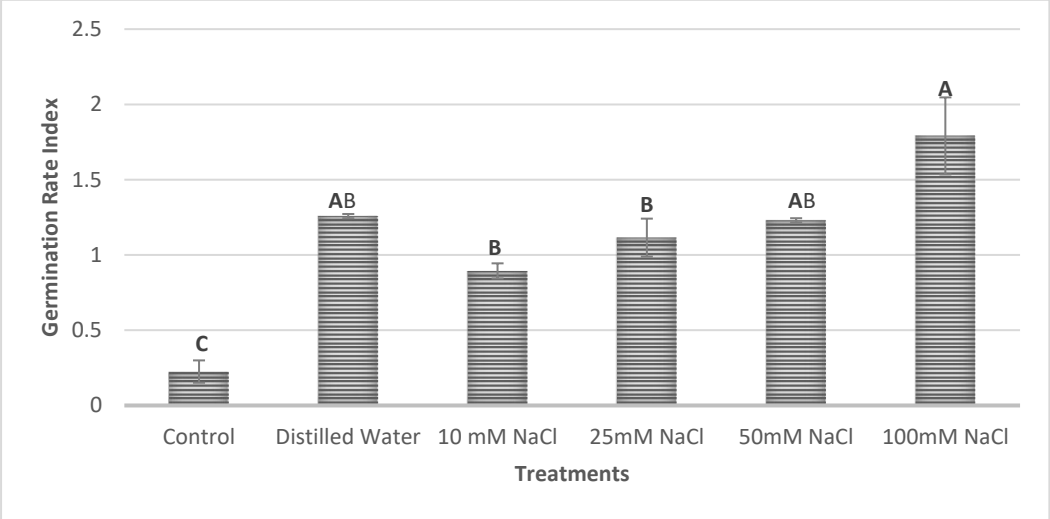

Figure 5: The Effect of Priming Treatments on the Germination Rate Index of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

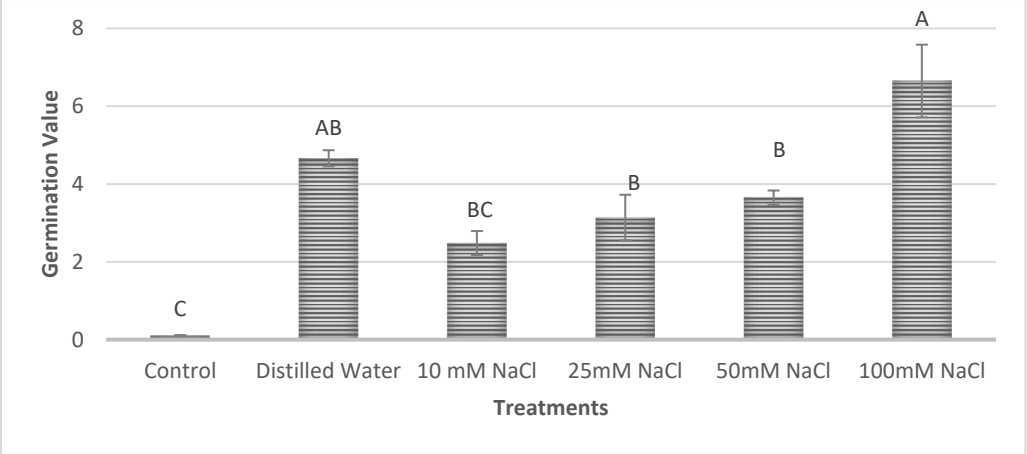

Figure 6: The Effect of Priming Treatments on the Germination Value of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

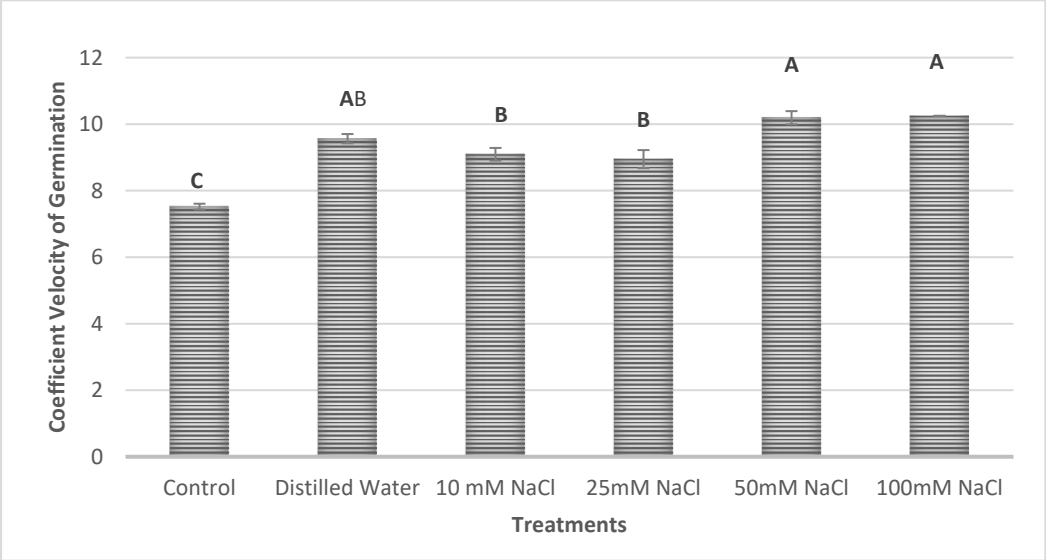

Figure 7: The Effect of Priming Treatments on the Coefficient of Velocity of Germination of Tomato Seeds. Means not followed by the same letter differ significantly at $P \leq 0.05$.

Vigorous seeds and seedlings are crucial for crop establishment because of their contribution to crop growth uniformity, maturity, and productivity. Therefore, enhancing seed vigor is a principal task to ensure successful crop establishment and development (17), particularly under stress conditions including high temperature. This investigation revealed that both hydropriming and halopriming are effective in enhancing seed vigor under heat stress conditions as illustrated by improved germination percentage (GP), germination rate (GR), germination index (GI) coefficient of the velocity of germination (CVG) of tomato seeds. Much research has elucidated that hydropriming advanced germination of several crops resulting in greater germination and seedling vigor and seedling development. The results recorded in this study are in line with Ansari and Zadeh (16) who reported that the germination capacity, normal seedling, GI, MGT, CVG, and seedling vigor index (SVI) of hydroprimed mountain rye seeds were higher than those of untreated controls. Similarly, soaking faba bean seeds in distilled water for 8, 16, or 24 h considerably enhanced their GR, GI and MGT compared with unprimed seeds (17). Furthermore, hydropriming of pepper (*Capsicum annum* cv. Goliath) seeds for 18 or 24 h significantly increased the CVG, coefficient of the velocity of emergence (CVE), emergence %, and coefficient uniformity of germination (CUG), and reduced MGT and T50 of germination and emergence (27). An earlier investigation, (28) also showed that hydropriming rice seeds for 24 h resulted in remarkable enhancement of seedling vigor as indicated by improved time to reach 50% germination, MGT, GR, germination energy (GE), GV, Peak Value (PV), MDG, GI and Relative Growth Index (RGI). Hydropriming also effectively increased the GP, germination rate, GRI, and seedling length of

spinach (*Spinacia oleracea*) under heat stress conditions (30 °C) (29).

The effectiveness of halopriming treatments with NaCl on the germination vigor of several plants has also been widely reported. For instance, NaCl as a priming agent significantly improved the GP, MGT, GI, and CVG of safflower seeds (20). Similarly, 1% NaCl significantly decreased MGT, T50% emergence and coefficient of variation (CV), and improved seedling vigor of two tomato rootstocks (30). Significant enhancement of seed vigor of tomato seeds as depicted by improved GP, GR, GI, germination energy (GE) and decreased MGT has also been observed (31). The beneficial effects of NaCl on germination vigor of *Acacia cyanophylla* seeds as indicated by significant improvement of GI, GRI, and GV have been reported (32). Furthermore, it has also been reported that halopriming seeds of white clover (*Trifolium repens* cv. Ladino) seeds with 0.5, 1, or 2.5 mM NaCl significantly improved the germinability, GI, and GV, and reduced MGT under water stress conditions but not under normal growth conditions (33).

It has been stated that temperature is one of the main aspects that influence the germinability and rapidity of germination. It regulates the metabolism engaged in the process of germination through direct involvement in seed imbibition and in the biochemical reactions (3). However, exposing plants to temporary or continuous heat stress may adversely affect crops at different stages of development including germination (22, 26). High temperature can cause a severe decline in productivity through the induction of morphological, molecular, physiological, and biochemical alterations (22). The poor performance of unprimed seeds observed in this study could be due to hyperaccumulation of reactive oxygen species which can cause inhibition of enzymes, harm the chromatin and encourage early senescence (34), and disturb membrane integrity and metabolic processes (26).

The reduction in MGT, and the enhancement of GI, MGR, GRI, CVG, and GP of primed seeds compared with unprimed controls is an indicator of earlier and synchronized germination and consequently improvement in seed vigor of tomato seeds. It has been claimed that seed vigor determines the capability of rapid, uniform germination and the establishment of robust seedlings in harsh environments (35). Moreover, it has also been asserted that the speedy emergence of seedlings would induce greater resource attainment and better usage consequently ensuing better yield (36). Thus, it is anticipated that halopriming with 100 mM NaCl or hydropriming tomato seeds may result in greater performance under normal and abnormal environmental conditions. In addition, although all NaCl concentrations and hydro priming improved the measured germination indices, overall, 100 mM NaCl was superior in this regard. This observation confirms the assertion that the priming media and the osmotic potential of the priming agents are among the factors affecting the efficacy of seed priming (12, 19). Furthermore, the advantageous impact of priming on germination vigor of many crops has been attributed to several biochemical and physiological changes including enhanced replication of DNA (37, 38, 39), regulation of ROS synthesis (29), repair of cellular and subcellular damage or membrane repair (40, 41), restoration of cellular integrity (42, 43), and activation of antioxidant enzymes (40, 44, 45).

CONCLUSION

It can be concluded that halopriming with 100 mM NaCl and hydro priming is effective regimes in alleviating the deleterious impact of heat stress as indicated by enhanced seed vigor of tomato seeds. It is evident from the results that priming has effectively raised the germination percentage of untreated seed six-folds. This enhancement would improve the seedling establishment of low-quality seeds which is considered as one of the prime targets of crop growers. These protocols are safe, cost-effective, and easily adopted by farmers. Additional investigation should also be conducted to assess the efficacy of these two regimes on the emergence and seedling growth under field seedlings.

REFERENCES

1. IPCC, (2014). Intergovernmental Panel on Climate Change. Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
2. Porter, J. R. (2005). Rising temperatures are likely to reduce crop yields. *Nature*., 436: 174. https://doi: 10.1038/436174b
3. Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). Seeds: physiology of development, germination and dormancy. 3rd ed., Springer, New York, USA. http://dx.doi.org/10.1007/978-1-4614-4693-4
4. Filho, M. J. (2015). Fisiologia de sementes de plantas cultivadas. Londrina: Abrates. Brazil
5. Nascimento, W., Andrade, M., K. P., Freitas, R. A., Silva, G. O., & Boiteux, L. S. (2016). Effects of temperature on tomato seed germination: Phenotypic variability and heterosis. *Hortic. Bras*., 34 (2): 216-222. https://doi.org/10.1590/S0102-053620160000200011

6. Geshnizjani, N., Ghaderi-Far, F., Willems, L. A. J., Hilhorst., H. W. M., & Ligterink, W. (2018). Characterization of and genetic variation for tomato seed thermo-inhibition and thermo-dormancy. *BMC Plant Biol*., 18, 229. https://doi.org/10.1186/s12870-018-1455-6
7. Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thomson, A. M., & Wolfe, D. W. (2011). Climate impacts on agriculture: Implications for crop production. *Agron. J*., 103 (1), 351-370.
8. Srivastava, K., Kumar, S., Prakash, P., & Vaishampaya, A. (2012). Screening of tomato genotypes for reproductive characters under high temperature stress conditions. SABRAO. *J. Breed. Genet*., 44 (2): 263-276.
9. Rajametov, S. N., Yang, E. Y., Jeong, H. B., Cho, M. C., Chae, S. Y., & Paudel, N. (2021). Heat treatment in two tomato cultivars: A study of the effect on physiological and growth recovery. *Horticulturae*., 7, 119. https://doi.org/10.3390/horticulturae7050119
10. Kępczyńska, E., Pikna-Grochala, J., & Kpczyski, J. (2006). Hormonal regulation of tomato seed germination at a supraoptimal temperature. *Acta Physiol Plant*, 28 (3):225-231
11. Ventura, L., Dona, M., Macovei, A., Carbonera, D., Buttafava, A., Mondoni, A., Rossi, G., & Balestrazzi, A. (2012). Understanding the molecular pathways associated with seed vigor. *Plant Physiol. Biochem*.,60:196-206.https://doi.org/0.1016/j.plaphy.2012.07.031
12. Eshkab, I. A., & Harris, P. H. C. (2020). Seed priming: Factors affecting efficacy. *Int. rev. basic appl. sci*., 8 (4): 31-52.
13. Ashraf, M., Athar, H. R., Harris, P. J. C., & Kwon, T. R. (2008). Some prospective strategies for improving crop salt tolerance. *Adv. Agron*., 97: 45-109. https://doi:10.1016/S0065-2113(07)00002-8
14. Ahmad. I., Saleem, A. M., Mustafa, G., Ziaf, K., Afzal I. & Qasim, M. (2017b). Seed halopriming enhances germination performance and seedling vigor of *Gerbera jamesonii* and *Zinnia elegans*. *Sarhad J. Agric*., 33 (2): 199-205. http://dx.doi.org/10.17582/journal.sja/2017/33.2.199.205
15. Maiti, R. K., Vidyasagar, P., Rajkumar, D., Ramaswamy, A., & Rodriguez, H. G. (2011). Seed priming improves seedling vigour and yield of few vegetable crops. *Technol. Dev*., 2 (1): 125-130.
16. Ansari, O., & Zadeh, F. S. (2012). Osmo and hydro priming mediated germination improvement under cold stress conditions in mountain rye (*Secale Montanum*). *Cercet. agron. Mold*., 3 (151): 53-62. Doi: 10.2478/V10298-012-0055-0
17. Damalas, C. A., Koutroubas, S. D., & Fotiadis, S. (2019). Hydro-priming effects on seed germination and field performance of faba bean in spring sowing. *Agriculture (Basel, Switz.)*., 9, 201. https://doi:10.3390/agriculture9090201
18. Saima, S., Tahira, M., & Ahmad, K. M. (2019). Effect of seed priming on seed germination, emergence and seedling growth of okra (*Abelmoschus esculentus* L.). *Int. J. Biosci*., 15 (3): 195-205.
19. Ashraf, M., & Foolad, M. R. (2005). Pre-sowing seed treatment- a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron*., 88: 223-271.
20. Elouaer, M. A., & Hannachi, C. (2012). Effect of NaCl priming duration and concentration on germination behaviour of Tunisian safflower. *J. Stress Physiol. Biochem*., 8 (3): 30-36.
21. Subedi, R., Maharjan, B. K., & Adhikari, R. (2015). Effect of different priming methods in rice (*Oryza sativa*). *J. Agric. Environ*., 16: 156-160.
22. Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. Exp. Bot., 61: 199-223. https://doi.org/10.1016/j.envexpbot.2007.05.011
23. Abdel, C. G., Asaad, S. S., & Mohammad, D. S. (2016). Minimum, optimum, and maximum temperatures required for germination of Onion, Radish, Tomato, and Pepper. *Intl J Farm and Alli Sci*., 5 (1): 26-45.
24. Ranal, M. A., and Santana, D. G. D. 2006. How and why to measure the germination process? *Braz. J. Bot*., 29 (1): 1-11.
25. Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *J. Proc. R. Soc. N. S. W*., 138: 65–75.
26. Bita, C. E., & Gerats, T. (2013). Plant tolerance to high temperature in ac hanging environment: scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci*., https://doi: 10.3389/fpls.2013.00273.

27. Uche, O. J., Adinde, J. O., Omeje, T. E., Agu, C. J., & Anieke, U. J. (2016). Influence of hydropriming on germination and seedling emergence of green bell pepper (*Capsicum annum* cv. goliath.). *Int. J. Sci. Nat*., 7 (1): 70-75.
28. Prasad, S., Prasad, B., & Singh, R. K. (2012). Effect of hydro-priming duration on germination and seedling vigor of rice (*Oryza sativa* L.) cv. *J. crop weed*., 8 (1): 65-71.
29. Neto, A. P. D., Oliveira, G. R. F., Mello, S., Da Silva, M. S., Gomes-Junior, F. G., Novembre, A. D., & Azevedo, R. A. (2020). Seed priming with seaweed extract mitigate heat stress in spinach: effect on germination, seedling growth and antioxidant capacity. *Bragantia*., 79 (4): 377-386. https://doi.org/10.1590/1678-4499.20200127
30. Mavi, K., Ermis, S., & Demir, I. (2006). The effect of priming on tomato rootstocks seeds in relation to seedling growth. *Asian J. Plant Sci*., 5 (6): 940-947. https:/dx.doi.org/10.3923/ajps.2006.940.947
31. Farooq, M., Basra, S. M. A., Saleem, B. A., Nafees, M., & Chishti, S. A. (2005). Enhancement of tomato seed germination and seedling vigor by osmopriming. *Pak. J. Agric. Sci*., 42 (3-4): 36-41.
32. Eshkab, I. A., Shanta, M. B., & El Waer, H. N. (2015). Enhancement of *Acacia cyanophylla* seed germinability and vigour through salt hardening technique. *Alexandria Sci. Exchange J*., 36 (3): 221-225. https://doi:10.21608/asejaiqjsae.2015.2904
33. Cao, Y., Liang, L., Cheng, B., Dong, Y., Wei, J., Tian, X., Peng, Y., & Li, Z. (2018). Pretreatment with NaCl promotes the seed germination of white clover by affecting endogenous phytohormones, metabolic regulation, and dehydrin-encoded genes expression under water stress. *Int. J. Mol. Sci*., 19, 3570. https://doi:10.3390/ijms19113570
34. Banerjee, A. & Roychoudhury, A. (2018). Abiotic stress, generation of reactive oxygen species, and their consequences: an overview. In: Revisiting the Role of Reactive Oxygen Species (ROS) in Plants: ROS Boon or Bane for Plants? V.P. Singh, S. Singh, D. Tripathi, *et al*., pp 23–50. Hoboken, NJ: Wiley.
35. Finch-Savage, W. E., & Bassel., G. (2016). Seed vigor and crop establishment: Extending performance beyond adaptation. *J. Exp. Bot*., 67: 567–591.
36. Murungu, F. S., & Madanzi, T. (2010). Seed priming, genotype and sowing date effects on emergence, growth and yield of wheat in a tropical low altitude area of Zimbabwe. *Afr. J. Agric. Res*., 5: 2341–2349.
37. Sathish, S., Sundareswaran, S., Senthil, N., & Ganesan, K. N. (2012). Biochemical changes due to seed priming in maize hybrid COH(M) 5. *Res. J. Seed Sci*., 5 (3): 71-83. DOI: 10.3923/rjss.2012.71.83
38. Sharma, S. N., & Maheshwari, A. (2015). Expression patterns of DNA repair genes associated with priming small and large chickpea (*Cicer arietinum*) seeds. *Seed Sci. Technol*., 43 (2): 250-261. DOI: 10.15258/sst.2015.43.2.11
39. Mir, H. R., Yadav, S. K., Ercisli, S., Al-Huqail, A. A., Soliman, D. A., Siddiqui, M. H., Alansi, S., & Yadav, S. (2021). Association of DNA biosynthesis with planting value enhancement in hydroprimed maize seeds. *Saudi J. Biol. Sci*., 28 (5): 2634–2640. https://doi.org/10.1016/j.sjbs.2021.02.068
40. Ahmad, I., Basra, S.M.A., Akram, M., Wasaya, A., Ansar, M., & Hussain, S. (2017a). Improvement of antioxidant activities and yield of spring maize through seed priming and foliar application of plant growth regulators under heat stress conditions. *Semina: Cien. Agrar*., 38 (1): 47-56.
41. Iqbal, H., Yaning, C., Hafeez ur Rehman., Waqas, M., Ahmed, Z., Raza, S. T., & Shareef, M. (2020). Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors. *Chil. J. Agric. Res*., 80 (1), 30-40. doi:10.4067/S0718-58392020000100030.
42. Di Girolamo, G., & Barbanti, L. (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. *Ital. J. Agron*., 7 (e25): 178-188.
43. Dawood, M. G. (2018). Stimulating plant tolerance against abiotic stress through seed priming. In: Rakshit, A., and Singh, H. (eds) Advances in Seed Priming. Springer, Singapore. https://doi.org/10.1007/978-981-13-0032-5_10
44. Jiang, W., Zhang, C., & Wei-Hu. (2020). Seed priming improves seed germination and seedling growth of *Isatis indigotica* fort. under salt stress. *J Am Soc Hortic Sci*., 55 (5): 647–650. https://doi.org/10.21273/HORTSCI14854-20
45. Huang, P., He, L., Abbas, A., Hussain, S., Hussain, S., Du, D., Hafeez, M. B.,

Balooch, S., Zahra, N., Ren, X., Rafiq, M., & Saqib, M. (2021). Seed priming with sorghum water extract improves the performance of camelina (*Camelina sativa* (l.) crantz.) under salt stress. *Plants*., 10, 749.https://doi.org/10.3390/plants10040749.

REVIEW

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

The Egress of Herpes Simplex Virus from the Infected Cell

Saba Rani[1], Iqra Zulfiqar[1], Palwasha Rehman[1], Madeha Gohar[1], Farzana Jan[2], Sartaj Ali[1]*

[1]*Department of Biosciences COMSATS University Islamabad (44000), Pakistan
[2]Khyber Teaching Hospital Peshawar Khyber Pakhtunkhwa, Pakistan

***Corresponding Author:**
Sartaj Ali
Address: Department of Biosciences COMSATS University Islamabad (44000), Pakistan
Email Address: sartajali11@gmail.com.

ABSTRACT

Herpes virus is amongst the most complex viruses. It is composed of more than 30 viral as well as cellular proteins. Infection of herpes virus can commonly be observed on genitals and mouth but can appear on other body parts as well. Due to its contagious nature, it can transmit from one person to another through direct contact. Its structure consists of four parts which are morphologically distinct including core, capsid for the protection of viral genome, tegument facilitating the replication of DNA, and envelope for the protection of viral genome. Mostly after the entry of herpes virus into the nucleus of a cell, virus filled up their capsid while being inside the nucleus, and then export or passes their macromolecule outside the nucleus through nuclear pores into the cell's cytoplasm. Various viral and cellular proteins are responsible for the complex process of envelopment and de-envelopment of the herpes virus inside the nucleus, and by fusion with other nuclear membranes. Many viral proteins, as well as cellular proteins, are involved in its regulation process. The current review is aimed to highlight the role of various viral and cellular proteins and their interaction in the egress of the herpes virus facilitating its transmission and thus pathogenicity.

Keywords: Egress; Herpes Simplex Virus, Tegumentation Proteins; Emerin.

Life Sci J Pak 2022; 4(01):25-32. **DOI: https://www.doi.org 10.5281/zenodo.6377012**
Received 01 September 2020 – Accepted 23 August 2021 – Published March 2022)

INTRODUCTION

Egress is a process of production of new viruses inside the infected cells of the host and its release from the cell resulting in its spread within the body and also its transmission to the new host (1). Herpes virus is among the common viral infections (2). If a person has ever suffered from a cold sore or fever, they have chances of acquiring infection from the Herpes virus (3). Symptoms of HSV1 include cold sore, oral herpes, and mouth herpes. Hosts of herpes virion include mollusks and humans (4). 30 diverse viral, as well as cellular proteins, constitute the Herpes virus (5). Herpes virus is composed of 4 distinct structural parts in terms of morphology including core containing the genome, capsid for the protection of genetic material, tegument for aiding in viral replication, and envelope for viral protection (6).

There are various mechanisms of viruses that facilitate their replication and transmission into new host resulting in enhanced virulence (7). Most infections spread from cell to cell, for the virus it is very important to release at that site that is close to the entry area. Every virus is unique in its perspective (8). HSV 1 is a large family of viruses that have double-stranded DNA (dsDNA) in their genome, they have enveloped viruses that infect mostly all invertebrates including mollusks (9). Approximately eight human herpes viruses are involved in causing latent infections (8) at which viruses are usually reactive causing illnesses like skin itching, inflammation of the cornea causing watery painful eyes (10). Herpes virus capsid is very large; it cannot easily quit through the nuclear pore (11). For effective quit of amorphous capsid from nucleus defined as nuclear egress. It requires an encoded nuclear egress complex (NEC) (12). Herpes simplex virus after replication assembles its capsids inside the nucleus and releases its products outside the cell via plasma membrane (13). Nuclear pores are very

small which hinders the diffusion of viruses outside the nucleus. So, the capsid escapes using a budding process through the inner nuclear membrane (14). When herpes virus infections may occur, it involves the transcription of virus (15), replication of DNA (16), capsid formations (17), and viral DNA packs inside the nucleus (6). The nuclear egress of the herpes virus is initiated from budding, through which the capsid is surrounded by a cover made up of the inner side of the nuclear membrane (18).

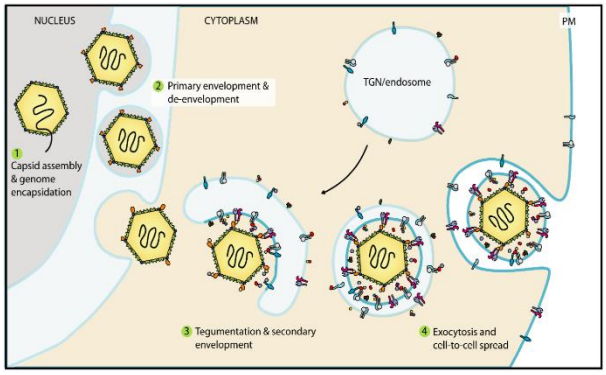

Figure 1.1 Overview of Herpesvirus Egress (19)

Herpes virus can be differentiated into 4 different structural parts (20). The viral set of DNA that is linear and double-stranded is usually surrounded by an icosahedral protein coat which contains four protein coats (21). The nuclear protein coat is inserted in a protein surrounded layer of the tegument that is enclosed by a fat envelope which is derived from the plasma membrane in the process of evolution of virion (22). This outer covering mostly contains glycosylated proteins which are usually encoded by the virus and are responsible for the host's immune response (23). The tegument is divided into two parts that are associated with protein (inner tegument) and cover the central part (outer integument)(24). Different herpes viruses may have different compositions of tegument and are composed of different viral and membrane-enclosed regions (22).

Egress from Nucleus

Budding is the early procedure in the herpesvirus nuclear egression (22). In this procedure, the capsid gains envelope is obtained from the inner part of the nuclear membrane (22).

Before primary development, nucleocapsid encounters the nuclear membrane (25). The movement of the capsid inside the nucleus depends on the actin (26). The contact between the nucleocapsid and nuclear membrane results in softening and partial dissolvement of the nuclear lamina encoded by two viral proteins (27). Two genes UL31 & UL34 genes are involved in this procedure. UL34 gene codes for a membrane protein (Type II c) pUL34 (28). UL31 gene codes for the protein pUL31(29).

In primary envelopment, the formation of the complex of these two proteins is very important. If any of these two proteins is absent the process of egression will be impaired (30). For the phosphorylation of intranuclear lamins, kinases, the cellular protein, are recruited by PUL34 and PUL31 (31). As a result of this phosphorylation, the chromatin and lamin network get dissolved, and by this capsid, it is reached to the inner nuclear membrane (32). Cellular proteins and viral proteins interact in a way that they prepare an environment for primary development (33). To obtain access to the cytoplasm for further process of maturation capsid fuses the primary envelope with nuclear membrane (34).

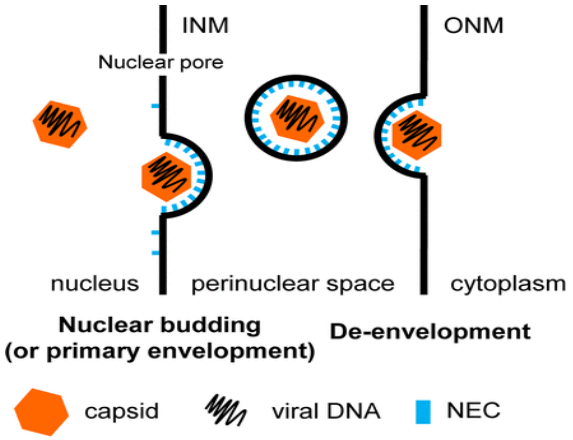

Figure 1.2 Primary Envelopment (Nuclear Budding) (22)

Egress from Perinuclear Space

For the separation of nucleus and cytoplasm, a space known as perinuclear space is located between the inner and outer layer of the nuclear envelope (35). The different steps in the evolution of the herpes virus are sometimes disputed (36). According to model 1, this is suggested for HSV-1 to demonstrate that this perinuclear virion keeps its uniqueness and then quits the cell using the secretory pathway (34). Perinuclear virion contains the complete set of tegument and envelope protein that is an attribute of extracellular virion that is mature (37). One of the other models suggested that the primary envelope intermix with the outwards side of the nucleus envelope and this primary envelope is lost because of this model most probably (38). Central integument of organism and translocation of the coated protein into the protoplasm. At last, into the cytoplasmic compartments, final segmentation and envelopment will occur (39). It has been observed that due to the assemblage of

perinuclear envelope virions mutations may occur that indicate different actions of closely related gene products (40). It was difficult to purify perinuclear virions in homogeneous form and it is not easy to complete the biochemical analysis (41).By using the technique "immunoelectron microscopy" we can distinguish between perinuclear and mature virions (42). No herpes virus is yet to be known to play a role in the intermixing of envelope of virus and cellular membrane resulting in virus egress (43). There are different ways of fusion during egress (44). Most mutations occur in Prv glycoproteins B, in the HSV-1 UL-53 gene product, and glycoprotein-K (45). Two proteins (Prv and HSV-1 US3) are found to play a critical role in the nuclear membrane targeting of UL34 proteins (46). When US3 is present, it increases the perinuclear localization of the protein UL34 (1).

Tegumentation in Cytoplasm

A viral tegument or viral matrix is a network of densely packed proteins in the space between the nucleocapsid and envelopes of herpes viruses that are typically released out into the cytoplasm soon after the infection (47). The teguments formation mostly occurs in the late phase of the infection cycle, most often after the replication of viral genes (48). The proteomics analysis of extracellular HSV-1 virions has identified about twenty-three tegumentation proteins(49), chaperones(50), several host cell enzymes (particularly members of Rab GTPase (51), and Annexin families (52) involved in exocytosis and trafficking) and structural proteins which are integrated into tegument assembly. The determination of the function of each specific protein in the tegument layer has remained difficult to determine owing to the redundancy of their interactions (19). Tegument proteins facilitate various functions in the life cycle of viruses. These include the asymmetrical wrapping of the cytoplasmic capsid to viral membranes during assembly (53), transportation of the virus capsids into the nucleus of a cell during infection which instantly modulates the host cell's environment when entering the cell. Furthermore, suppression of host mRNA transcription, immune response (cellular or extrinsic defenses) evasion by inhibition of immune signals and interferons activation (54), recruitment of host transcription/translation factors, or direct transcription or translation of viral genes (19).

Tegument Formation

Three major events occur in all herpes viruses to ensure accurate glycoprotein assimilation and proper folding of tegument proteins (19). (19)

Incorporation of major UL37 and UL36 tegument proteins

The inner part of the tegument consists of protein UL36 associated with the capsid by UL19-UL25 complex also called as C capsid specific component (CCSC) (55). This complex forms a network with triplexes only located at hexons interfaces or pentons (vertices)(56). Later tegument protein UL37 is added into the tegument layer by binding directly to UL36 (57).

Interaction of UL16 AND UL11 proteins

The Ul16 is crucial for budding, tegument assembly, and viral replication (58). It binds to capsid by recognizing UL11 by an acidic motif, found in the first half of protein (59). The Association of UL11 to the cytoplasmic membrane is helpful in the establishment of a secondary envelope through protein UL16 by bringing capsids to these membranes (34). The interaction of UL16 and UL11 occurs in different herpes viruses such as CMV, HHV-1, and varicella virus UL36 (60).

UL7 and UL51 interaction

The membrane-bounded protein UL51 is essential for recruiting UL7 to the cytoplasm of infected cells. The interaction of tegument proteins UL7 and UL51 interaction is necessary for viral cytoplasmic envelopment and is common for all herpes viruses (61).

Cellular Factors Responsible for Viral Egress

The transport of large nucleocapsids of herpes virus across the nuclear membrane seems to be a complex mechanism, and little is known about its regulation (63). From the infected cells, the egress of the herpes virus involves two different steps: 1. Nuclear budding and 2. Egress from the host cell (64). Initially, it was believed that the budding of the virus is entirely carried out by viral factors but recently it has been reported that cellular factors are also involved in this process (65).

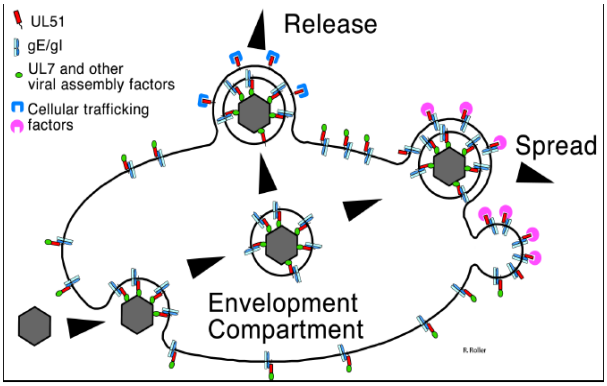

Figure 1.3 UL7 and UL51 interaction (62).

Nucleolin

The egress of HSV-1 is regulated when the viral protein interacts with each other (66). Nucleolin is a marker protein of the nucleolus and is coded by the NLC gene in humans (67). Nucleolin interacts with UL12, and is essential for egress of nucleocapsid in infected cells (63). It might be possible that nucleolin interacts with UL-12 to modulate its role in egress from the nucleus of herpes simplex virus-1 nucleocapsids into the cytoplasm (68). It is generally regarded that nucleolin has role in nuclear egress,

and UL12 may play its role as a cofactor for nucleolin whose mechanism is not yet understood (63). It is also possible that UL12 may have no role in the nuclear egress of nucleocapsids and nucleolin may function independently of UL12 (69). Nucleolin is a protein having multifunction that displays localization in the nucleoplasm, plasma membrane, nucleoli, and cytoplasm (67). It interacts with a diverse variety of viral as well as cellular proteins that affect numerous cellular functions (70). It is also possible that some cellular functions of nucleolin can be involved in the regulation of nucleocapsid egress (71). However, the mechanism of nucleolin interaction and regulation of nuclear egress is still not clear (63).

p32

A major component of HSV-1 NEC is p32, whose important components are UL31 and UL34. p32 regulates the development of HSV-1 during viral egress from the nucleus. It also efficiently disintegrates the nuclear lamina that facilitates the HSV-1 nuclear egress (73). The major viral structural protein interacts with protein p32 of the host cell. It mediates the conscription of p32 into the nuclear envelope in the infected cells (74). ICP34.5 is a viral protein that interacts with p32, a cellular factor and forms a complex (72). This complex is distributed nearly in the nuclear membrane and is linked with the budding of the virus from the nuclei. p32 interacts with ICP34.5 and acts as a mediator for nuclear egress of HSV-1 (73). p32 interacts with pathogen proteins (75). ICP34.5 interacts with p32 and is responsible for the distribution of p32 together with PKCs on the nuclear periphery and forms a complex which phosphorylates the lamina. Consequently, the nuclear lamina disassembles, and viral capsids are released (72).

Emerin and Host Cell Kinases

Protein kinases (PKCs) are known to have a critical role in viral egress (32). In HSV-1 recruitment of PKC-α and PKC-δ occurs into the nuclear membrane (76), indicating the critical role of PKC's recruitment in the egress of Herpes virus (32). Phosphorylation by kinases leads to the breakdown of nuclear lamina (77). In an uninfected cell, emerin remains bound to lamin A & C (78). It is also attached to the inner nuclear membrane by its transmembrane domain. In a cell infected by HSV-1, a complex is made by pUL31 and pUL34. At the inner membrane of the nucleus pUL34 is responsible for attaching a complex through a transmembrane domain (79). This pUL34 and pUL31 complex recruit nuclear membrane sensitive pUS3 and rottlerin (80). Then phosphorylation of lamin A, C, B, and emerin occurs. This phosphorylated emerin detaches from phosphorylated lamin A & C that will result in flexibility due to which the capsid bypasses the lamina and makes its way to the inner membrane of the nucleus. While the inner nuclear membrane is the site of viral envelopment resulting in viral egress from the nuclear membrane (32).

Prolyl Isomerase Pin1

Pin1 is a cellular isomerase that mediates the lamina disassembly by conformational changes of lamins and determines the pathway of nuclear egress (81). The site-specific phosphorylation of lamins is responsible for the disassembly of lamina through a yet unclear mechanism (82). Phosphorylation is proposed to interfere with interactions between lamins and lamin binding proteins, thus leading to lamina disassembly (Liu & Ikegami, 2020). Phosphorylation of lamin can arise by protein kinases of the host cell or herpes virus-encoded or by endogenous kinases. As a result of phosphorylation, Pin1 binds to lamins and mediates isomerization that induces conformational changes in lamins resulting in lamina disassembly. This disassembly is responsible for the process of capsid budding at the inner nuclear membrane (81).

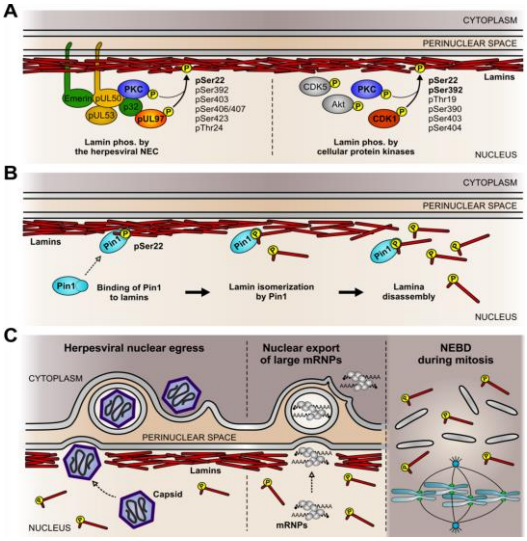

Figure 1.4 Mechanism of Pin1-Induced Nuclear Lamina Disassembly (81).

CONCLUSION

Viruses travel between compartments of the cell during their assembly. Herpes virus assembles in the nucleus and thus migrates into the cytoplasm. Before primary development nucleocapsid interacts with inner nuclear membrane and capsid gain an envelope that results in budding of capsid through INM.

The PEV (primary enveloped virion) is released and its envelope fuses with ONM leading to the release of capsid into the cytoplasm. The cytoplasm is the site for the final tegumentation of the virus. Viral egress is regulated by various cellular and viral factors and their interaction.

ACKNOWLEDGMENT

The authors are very thankful to the faculty at the Department of Biosciences at COMSATS University, Islamabad for their discussion and valuable suggestions.

REFERENCES

1. Mettenleiter TC. (2002). MINIREVIEW Herpesvirus Assembly and Egress. J Virol 76(4), 1537–47.
2. Spear PG., & Longnecker R. (2003). Herpesvirus Entry: An Update. Journal of Virology,77(19),10179–85.
3. Wu YP., Sun DD., Wang Y., Liu W., & Yang J. (2016). Herpes Simplex Virus Type 1 and Type 2 Infection Increases Atherosclerosis Risk: Evidence Based on a Meta-Analysis. Biomed Research International, 2016.
4. Koelle DM., & Corey L. (2003). Recent progress in herpes simplex virus immunobiology and vaccine research. Clinical Microbiology Review, 16(1), 96–113.
5. Copeland AM., Newcomb WW., & Brown JC. (2009). Herpes Simplex Virus Replication: Roles of Viral Proteins and Nucleoporins in Capsid-Nucleus Attachment. Journal of Virology, 83(4), 1660–8.
6. Heming JD., Conway JF., & Homa FL. (2007). Herpesvirus capsid assembly and DNA packaging. Advance in Anatomy Embryology and Cell Biology, 223:119–42.
7. Louten J. (2016). Virus Transmission and Epidemiology. Essential Human Virology, 71–92.
8. MPOC. (2020). Virus Life Cycle Chapter. Malaysian Palm Oil Counc [Internet]. 21(1),1–9. Available
9. Connolly SA., Jackson JO., Jardetzky TS., & Longnecker R. (2011). Fusing structure and function: A structural view of the herpesvirus entry machinery. Nature Reviews Microbiology [Internet], 9(5), 369–81. Available
10. Singh N., & Tscharke DC. (2019). Herpes Simplex Virus Latency Is Noisier the Closer We Look. Journal of Virology, 94(4), 1–7.
11. McElwee M., Vijayakrishnan S., Rixon F., & Bhella D. (2018). Structure of the herpes simplex virus portal-vertex. PLoS Biolgy, 16(6), 1–15.
12. Roller RJ., & Baines JD. (2017). Herpesvirus nuclear egress, 22, 143–69.
13. Rémillard-Labrosse G., Guay G., & Lippé R. (2006). Reconstitution of Herpes Simplex Virus Type 1 Nuclear Capsid Egress In Vitro. Journal of Virology, 80(19), 9741–53.
14. Lin DH., & Hoelz A. (2019). The structure of the nuclear pore complex (An Update). Annual Review of Biochemistry, 88, 725–83.
15. Harkness JM., Kader M., & DeLuca NA. (2014). Transcription of the Herpes Simplex Virus 1 Genome during Productive and Quiescent Infection of Neuronal and Nonneuronal Cells. Journal of Virology, 88(12), 6847–61.
16. Weller SK., & Coen DM. (2012). Herpes simplex viruses: Mechanisms of DNA replication. Cold Spring Harbor Perspective in Biology, 4(9).
17. Brown JC., & Newcomb WW. (2011). Herpesvirus capsid assembly: Insights from structural analysis. Current Opinion in Virology [Internet], 1(2), 142–9. Available (Accessed on 03 June 2011).
18. Crump CM., Yates C., & Minson T. (2007). Herpes Simplex Virus Type 1 Cytoplasmic Envelopment Requires Functional Vps4. Journal of Virology, 81(14), 7380–7.
19. Owen DJ., Crump CM., Graham SC. (2015). Tegument assembly and secondary envelopment of alphaherpesviruses. Viruses, 7(9), 5084–114.
20. Wang J., Yuan S., Zhu D., Tang H., Wang N., Chen W., & et al. (2018). Structure of the herpes simplex virus type 2 C-capsid with capsid-vertex-specific component. Nature Communication [Internet], 9(1), 1–10. Available.
21. Nishiyama Y. (1996). Herpesvirus genes: molecular basis of viral replication and pathogenicity. Nagoya Journal of Medical Science, 59(3–4), 107–19.
22. Bigalke JM., & Heldwein EE. (2015). The Great (Nuclear) Escape: New Insights into the Role of the Nuclear Egress Complex of Herpesviruses. Journalof Virology, 89(18), 9150–3.
23. Yang L., Wang M., Cheng A., Yang Q., Wu Y., Jia R., & et al. (2019). Innate Immune Evasion of Alphaherpesvirus Tegument Proteins. Frontiers of Immunology, 10(September), 1–16.
24. Xu X., Che Y., & Li Q. (2016). HSV-1 tegument protein and the development of its genome editing technology Chunfu Zheng. Virology Journal [Internet], 13(1), 1–7. Available
25. Karasneh GA., & Shukla D. (2011). Herpes simplex virus infects most cell types in vitro: Clues to its success. Virology Journal, 8, 1–11.

26. Forest T., Bernard S., & Baines JD. (2005). Active intranuclear movement of herpesvirus capsids. Nature Cell Biology, 7(4), 429–31.
27. Morrison LA., & DeLassus GS. (2011). Breach of the nuclear lamina during assembly of herpes simplex viruses. Nucleus, 2(4), 271–6.
28. Roller RJ., Zhou Y., Schnetzer R., Ferguson J., & DeSalvo D. (2000). Herpes Simplex Virus Type 1 UL34 Gene Product Is Required for Viral Envelopment. Journal of Virology, 74(1), 117–29.
29. Sherry MR., Hay TJM., Gulak MA., Nassiri A., Finnen RL., & Banfield BW. (2017). The Herpesvirus Nuclear Egress Complex Component, UL31, Can Be Recruited to Sites of DNA Damage Through Poly-ADP Ribose Binding. Scientific Reports, 7(1), 1–18.
30. Newcomb WW., Fontana J., Winkler DC., Cheng N., Bernard Heymann J., & Stevena AC. (2017). The primary enveloped virion of herpes simplex virus 1: Its role in nuclear egress. mBio, 8(3), 1–14.
31. Cibulka J., Fraiberk M., & Forstova J. (2012). Nuclear actin and lamins in viral infections. Viruses, 4(3), 325–47.
32. Leach NR., & Roller RJ. (2010). Significance of host cell kinases in herpes simplex virus type 1 egress and lamin-associated protein disassembly from the nuclear lamina. Virology [Internet], 406(1), 127–37. Available
33. Maginnis MS. (2018). Virus–Receptor Interactions: The Key to Cellular Invasion. Journal of Molecular Biololgy, 430(17), 2590–611. Available
34. Ahmad I., & Wilson DW. (2020). Hsv-1 cytoplasmic envelopment and egress. International Journal of Molecular Sciences, 21(17), 1–34.
35. Shaiken TE., Opekun AR. (2014). Dissecting the cell to nucleus, perinucleus and cytosol. Scientific Reports, 4.
36. Wertheim JO., Smith MD., Smith DM., Scheffler K., Sergei L., Pond K., & et al. (2014). MBE Advance Access published June 10, 2014, 1–26.
37. Farnsworth A., Wisner TW., Webb M., Roller R., Cohen G., Eisenberg R., & et al. (2007). Herpes simplex virus glycoproteins gB and gH function in fusion between the virion envelope and the outer nuclear membrane. Proceeding of the National Academy of Scienc U S A, 104(24), 10187–92.
38. Klupp BG., Granzow H., & Mettenleiter TC. (2011). Nuclear Envelope Breakdown Can Substitute for Primary Envelopment-Mediated Nuclear Egress of Herpesviruses. Journal Virology, 85(16), 8285–92.
39. Hogue IB., Bosse JB., Hu JR., Thiberge SY., & Enquist LW. (2014). Cellular Mechanisms of Alpha Herpesvirus Egress: Live Cell Fluorescence Microscopy of Pseudorabies Virus Exocytosis. PLoS Pathogens, 10(12).
40. Bigalke JM., & Heldwein EE. (2016). Nuclear Exodus: Herpesviruses Lead the Way. Annual Review Virology, 3(July), 387–409.
41. Vijayaragavan KS. (2014). Digital Commons @ Michigan Tech Virus purification, detection and removal.
42. Blanco E. Structure and Physics of Viruses. 2013; 68:631–65. Available
43. Lenard J. (2008). Viral Membranes. Encyclopedia of Virology, 13, 308–14.
44. Mettenleiter TC. (2002). Herpesvirus Assembly and Egress. Journal of Virology, 76(4), 1537–47.
45. Kim I-J., Chouljenko VN., Walker JD., & Kousoulas KG. (2013). Herpes Simplex Virus 1 Glycoprotein M and the Membrane-Associated Protein UL11 Are Required for Virus-Induced Cell Fusion and Efficient Virus Entry. Journal of Virology, 87(14), 8029–37.
46. Ryckman BJ., & Roller RJ. (2004). Herpes Simplex Virus Type 1 Primary Envelopment: UL34 Protein Modification and the US3-UL34 Catalytic Relationship. Journal of Virology, 78(1), 399–412.
47. Pennisi R., Musarra-Pizzo M., Lei Z., Zhou GG., & Sciortino MT. (2010). VHS, US3 and UL13 viral tegument proteins are required for Herpes Simplex Virus-Induced modification of protein kinase R. Scientific Reports, 10(1), 1–14.
48. Cardone G., Heymann JB., Cheng N., Trus BL., & Steven AC. (2012). Procapsid assembly, maturation, nuclear exit: Dynamic steps in the production of infectious herpesvirions. Advances in Experimental Medicine and Biology, 726, 423–39.
49. Loret S, Guay G., (2008). Lippé R. Comprehensive Characterization of Extracellular Herpes Simplex Virus Type 1 Virions. Journal Virology, 82(17), 8605–18.
50. Kyratsous CA., & Silverstein SJ. (2008). The co-chaperone BAG3 regulates Herpes Simplex Virus replication. Proceedings of the National Academy Sciences U S A, 105(52), 20912–7.
51. Raza S., Alvisi G., Shahin F., Husain U., Rabbani M., Yaqub T., & et al. (2018). Role of Rab GTPases in HSV-1 infection: Molecular understanding of viral maturation and egress.

Microbial Pathogenesis [Internet], 118(October 2017), 146–53. Available
52. Paudel N., Sadagopan S., Balasubramanian S., & Chandran B. (2012). Kaposi's Sarcoma-Associated Herpesvirus Latency-Associated Nuclear Antigen and Angiogenin Interact with Common Host Proteins, Including Annexin A2, Which Is Essential for Survival of Latently Infected Cells. Journal of Virology, 86(3), 1589–607.
53. Newcomb WW., & Brown JC. (2010). Structure and Capsid Association of the Herpesvirus Large Tegument Protein UL36. Journal of Virology, 84(18), 9408–14.
54. Melchjorsen J., Matikainen S., & Paludan SR. (2009). Activation and evasion of innate antiviral immunity by herpes simplex virus. Viruses, 1(3), 737–59.
55. Cardone G., Newcomb WW., Cheng N., Wingfield PT., Trus BL., Brown JC., & et al. (2012). The UL36 Tegument Protein of Herpes Simplex Virus 1 Has a Composite Binding Site at the Capsid Vertices. Journal of Virology, 86(8), 4058–64.
56. Yang K., & Baines JD. (2011). Selection of HSV capsids for envelopment involves interaction between capsid surface components pU L31, pU L17, and pU L25. Proceedings of the National Academy Sciences U S A, 108(34), 14276–81.
57. Chouljenko D V., Jambunathan N., Chouljenko VN., Naderi M., Brylinski M., Caskey JR., & et al. (2016). Herpes Simplex Virus 1 UL37 Protein Tyrosine Residues Conserved among All Alphaherpesviruses Are Required for Interactions with Glycoprotein K, Cytoplasmic Virion Envelopment, and Infectious Virus Production. Journal of Virology, 90(22), 10351–61.
58. Gao J., Hay TJM., & Banfield BW. (2017). The Product of the Herpes Simplex Virus 2 UL16 Gene Is Critical for the Egress of Capsids from the Nuclei of Infected Cells. Journal of Virology, 91(10).
59. Chadha P., Han J., Starkey JL., & Wills JW. (2012). Regulated Interaction of Tegument Proteins UL16 and UL11 from Herpes Simplex Virus. Journal of Virology, 86(21), 11886–98.
60. Donczew RSH. (2010). crossm ABSTRACT, 11(3), 1–22.
61. Oda S., Arii J., Koyanagi N., Kato A., & Kawaguchi Y. (2016). The Interaction between Herpes Simplex Virus 1 Tegument Proteins UL51 and UL14 and Its Role in Virion Morphogenesis. Journal of Virology, 90(19), 8754–67.
62. Ryken S., & Ryken S. (2017). Characterization of Herpes Simplex Virus 1 UL51 Self-Interaction.
63. Sagou K., Uema M., & Kawaguchi Y. (2010). Nucleolin Is Required for Efficient Nuclear Egress of Herpes Simplex Virus Type 1 Nucleocapsids. Journal of Virology, 84(4), 2110–21.
64. Lv Y., Zhou S., Gao S., & Deng H. (2019). Remodeling of host membranes during herpesvirus assembly and egress. Protein Cell [Internet], 10(5), 315–26. Available from: https://doi.org/10.1007/s13238-018-0577-9
65. Welsch S., Müller B., Kräusslich HG. (2007). More than one door - Budding of enveloped viruses through cellular membranes. FEBS Letters, 581(11), 2089–97.
66. Guan Y., Guo L., Yang E., Liao Y., Liu L., Che Y., & et al. (2014). HSV-1 nucleocapsid egress mediated by UL31 in association with UL34 is impeded by cellular transmembrane protein 140. Journal of Virology [Internet], 464–465(1), 1–10. Available from: http://dx.doi.org/10.1016/j.virol.2014.06.034
67. Tajrishi MM., Tuteja R., & Tuteja N. (2011). Nucleolin: The most abundant multifunctional phosphoprotein of nucleolus. Communicative and Integrative Biology, 4(3), 267–75.
68. Matthews D., Emmott E., & Hiscox J. (2011). Viruses and the nucleolus. Protein Reviews, 15, 321–45.
69. Lymberopoulos MH., Bourget A., Abdeljelil N Ben., & Pearson A. (2011). Involvement of the UL24 protein in herpes simplex virus 1-induced dispersal of B23 and in nuclear egress. Virology [Internet], 412(2), 341–8. Available from: http://dx.doi.org/10.1016/j.virol.2011.01.016
70. Ginisty H., Sicard H., Roger B., & Bouvet P. (1999). Structure and functions of nucleolin. Journal of Cell Science, 112(6), 761–72.
71. Muriaux D., & Darlix JL. (2010). Properties and functions of the nucleocapsid protein in virus assembly. RNA Biology, 7(6), 744–53.
72. Wang Y., Yang Y., Wu S., Pan S., Zhou C., Ma Y., & et al. (2014). P32 is a novel target for viral protein icp34.5 of herpes simplex virus type 1 and facilitates viral nuclear egress. Journal of Biology Chemistry, 289(52), 35795–805.
73. Liu Z., Kato A., Oyama M., Kozuka-Hata H., Arii J., & Kawaguchi Y. (2015). Role of Host Cell p32 in Herpes Simplex Virus 1 De-Envelopment during Viral Nuclear Egress. Journal of Virology, 89(17), 8982–98.
74. Kato A., Oda S., Watanabe M., Oyama M., Kozuka-Hata H., Koyanagi N., & et al. (2018). Roles of the Phosphorylation of Herpes

Simplex Virus 1 UL51 at a Specific Site in Viral Replication and Pathogenicity. Journal of Virology, 92(18), 1–21.
75. Beatch MD., & Hobman TC. (2000). Rubella Virus Capsid Associates with Host Cell Protein p32 and Localizes to Mitochondria. Journal of Virology, 74(12), 5569–76.
76. Park R., & Baines JD. (2006). Herpes Simplex Virus Type 1 Infection Induces Activation and Recruitment of Protein Kinase C to the Nuclear Membrane and Increased Phosphorylation of Lamin B. Journal of Virology, 80(1), 494–504.
77. Huguet F., Flynn S., & Vagnarelli P. (2019). The Role of Phosphatases in Nuclear Envelope Disassembly and Reassembly and Their Relevance to Pathologies. Cells, 8(7), 687.
78. Demmerle J., Koch AJ., & Holaska JM. (2012). The nuclear envelope protein emerin binds directly to histone deacetylase 3 (HDAC3) and activates HDAC3 activity. Journal of Biological Chemistry, 287(26), 22080–8.
79. Schuster F., Klupp BG., Granzow H., & Mettenleiter TC. (2012). Structural Determinants for Nuclear Envelope Localization and Function of Pseudorabies Virus pUL34. Journal of Virology, 86(4), 2079–88.
80. Leach N., Bjerke SL., Christensen DK., Bouchard JM., Mou F., Park R., & et al. (2007). Emerin Is Hyperphosphorylated and Redistributed in Herpes Simplex Virus Type 1-Infected Cells in a Manner Dependent on both UL34 and US3. Journal of Virology, 81(19), 10792–803.
81. Milbradt J., Hutterer C., Bahsi H., Wagner S., Sonntag E., Horn AHC., & et al. (2016). The Prolyl Isomerase Pin1 Promotes the Herpesvirus-Induced Phosphorylation-Dependent Disassembly of the Nuclear Lamina Required for Nucleocytoplasmic Egress. PLoS Pathogrns, 12(8), 1–30.
82. Torvaldson E., Kochin V., & Eriksson JE. (2015). Phosphorylation of lamins determine their structural properties and signaling functions. Nucleus, 6(3), 166–71.

REVIEW

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

Covid-19 And Human Physiological Systems-A Review Based Study

Shafaq Razi[1], Ishrat Perveen[2]*, Sumbal Nazir[3], Sajida Naseem[1], Saba Sabahat[4], Abeer Qasim[2], Hafiza Najma Naeem[5]

[1]Department of Zoology, University of Education, Lower Mall Campus, Lahore, Pakistan.
[2] Food and Biotechnology Research Center, PCSIR Laboratories Complex, Ferozepur Road, Lahore, Pakistan.
[3]Department of Zoology, Faculty of Science, University of Central Punjab, Lahore, Pakistan.
[4]Rashid Latif Medical College of Pharmacy, Lahore, Pakistan.
[5]Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

Corresponding Author
Dr. Ishrat Perveen
Address: Food and Biotechnology Research Center, PCSIR Laboratories, Ferozepur Road, Lahore, Pakistan.
Email Address: drishratperveen51214@outlook.com

ABSTRACT

COVID-19 is commonly recognized as a respiratory infection. Effects of COVID-19 are, however, not only limited to the human respiratory system but all other physiological systems are also prone to COVID-19. The virus can attack many different cells of the body via angiotensin-converting-2 (ACE2) receptors. COVID-19 impact on the human body has been considered a multi-organ response, causing a range of physiological symptoms. Age-related chronic diseases coupled with a hyperactive inflammatory response can lead to the severity of the infection and death. The risk of physiological complications is higher in comorbidity, weak, and aged patients. Acknowledgment of the various physiological effects of the disease and its complications is fundamental for the proper clinical management of patients. This review intends to provide a detailed perspective on the possible physiological impacts of COVID-19 and adds to the ever-emerging knowledge of human physiology.

Keywords: Contagiousness, Coronavirus, Transmission, Respiratory system, Pandemic

Life Sci J Pak 2022; 4(01):33-44. DOI: https://doi.org/ https://doi.org/10.5281/zenodo.6374110
Received 01 September 2020 – Accepted 23 August 2021 – Published March 2022)

INTRODUCTION

The Coronavirus Disease (widely known as COVID-19) originated in Wuhan, China, later turned into a pandemic, heavily wrecking the physiological and mental health of humans (1, 2). COVID-19 induced by chronic respiratory complex coronavirus 2 (SARS-CoV-2), reported to have been arising from the group of bats known as Rhinolophus bats, leads to myriad ranges of various organ dysfunctions (2). COVID-19 has been found to affect more than two hundred million people worldwide and caused 5,316,286 global deaths (3). The above-mention pandemic has been reported to affect the physiological, social, economic, and psychological systems of human beings (4). COVID-19 is transferred to the human body through respiratory droplets in the air or through direct contact with an infected person (5). It primarily affects the respiratory system by attacking the lungs and causing pneumonia (6). The physiological effects of COVID-19 are manifold, which confirms that it is not merely a respiratory infection. Along with the respiratory system, COVID-19 also affects the gastrointestinal, nervous, cardiovascular, and musculoskeletal systems (6, 7, 8, 9). Inflammation is an obvious outcome of COVID-19 on human physiological systems which may result in an overactive immune response (10,11). Although COVID-19 affects people of all ages, it is deadly and more complicated in older people, especially in those who are already sick and have a compromised immune system (12, 13).

This paper aims to point out the possible effects of COVID-19 on human physiological systems. These effects can get complicated in severely ill patients. Although SARS-CoV-2 directly attacks the pulmonary system, its pathological and inflammatory pathway in other organ systems has not been properly diagnosed and established

Table- 1. A Tabular representation of a range of physiological conditions related to COVID-19 Disease

Cluster No.	Physiological Manifestations of COVID-19	Countries/Regions	Key Findings	Reference/s
1. COVID-19 and human lungs	- COVID-19 and lungs of comorbidity patients	USA	Lung injury Inflammation Hypoxia	(15)
	-COVID-19 and lungs in comparison with influenza	Switzerland, Germany, Massachusetts, and Boston	Perivascular T-cell infiltration and alveolar damage in both COVID-19 and influenza-associated respiratory failure. Endothelial injury and damaged cellular membranes Micro-thrombi, which is an alveolar-capillary is nine times more prevailing in the patients of COVID-19 than influenza	Than
	-long-term pulmonary consequences of the COVID-19	Los Angeles, CA	Difficulties in breathing Abnormalities in the diffusion of gasses in the blood.	(19)
	-To study the physiological effects of COVID-19 associated ARDS	Italy	Increased lung weight Increased dead space in the lungs Micro-thrombi and emboli in the pulmonary vascular bed.	(18)
2. COVID-19 and gut	-COVID-19 and gut microbiota	Houston, Texas, and New York	Intestinal damage Enterocyte dysfunction	(6)
	-gut dysbiosis and COVID	Turkey	Altered gut microbiota Increased gut permeability Increased inflammation	(20)
	-Fecal viral activity and changes in the patient's gut microbiota	Hong Kong	Alterations in fecal microbiome Gut dysbiosis	(21)
3. COVID-19 and the human immune system	-COVID-19 and immune responses	Bangladesh, USA	Cytokine storm Increased inflammation Impaired immune response	(24, 12)
4. COVID-19 and brain	-Neurological effects of COVID-19 on humans	USA, Belgium, and Sweden	Damage to CNS Hypoxic injury Encephalopathy Encephalitis Thrombosis Neuroinflammatory responses related to COVID-19 may lead to neurodegenerative problems and neuropsychiatric symptoms Long term neurological complications such as strokes Psychiatric problems such as anxiety, depression, and PTSD	(26, 27, 41)
	-To study the neurological sequence of the COVID-19	US, Mexico, and Canada	In the central nervous system: Viral entry into the brain Adverse immune responses Respiratory stress In the peripheral nervous system: Chemosensory dysfunction	(5)
	-To study how COVID-19 can damage the brain	United Kingdom	Damage to brain blood supply in most patients can lead to stroke and hemorrhage The altered mental state of patients leads to confusion and prolonged unconsciousness Encephalitis is rare Less common complications include PNS damage, which can cause anxiety, PTSD, and loss of smell	(28)

	-To study brain injury in comorbidity patients and neurological effects of severe COVID-19 infection	USA and Italy	Brain injury in comorbidity patients due to: Encephalitis Ischemia Oxidative stress	(8, 15)
	-To study the neurological involvement in COVID-19	USA	Cytokine storm can lead to neurological injury Apoptosis and necrosis in brain parenchyma in the medial temporal lobe Cerebral hemorrhage PNS damage Loss of taste and smell	(40)
	-Neurological and neuropsychiatric complications	UK	Altered mental state Encephalopathy Encephalitis	(29)
5. COVID-19 and Cardiac System	-The effect of COVID-19 on the heart in comorbidity patients	USA	Plaque rupture Myocardial injury Arrhythmia Coronary thrombosis Venous thromboembolism Heart injury Ischemia	(15)
	- COVID-19 and heart	Italy, India	Deterioration of previously existing cardiovascular disorders Inflammatory heart disease Conduction disturbances Disseminated intravascular coagulation Myocardial injury Pre-existing cardiovascular diseases increase the danger of the COVID-19 infection Cardiovascular complexions in comorbidity patients	(7, 34, 35)
6. COVID-19 and liver	-Study the impacts of COVID-19 on liver function and hepatic injury	India, Singapore	COVID-19 patients indicate mild hepatic injury Common hepatic injury is related to low albumin levels in the blood More common in patients who require mechanical ventilation and intensive care and	(36, 37)
7. COVID -19 and musculoskeletal system	-To study the impact on the musculoskeletal consequences	New York	Musculoskeletal dysfunction In skeletal muscle: Muscle pain, muscular atrophy, fatigue, and weakness In joints and bones: Joint pain and immense loss in bone minerals	(9)
8. COVID-19 and the mental wellness	-Study the impact of COVID-19 on mental wellness	Italy, Brazil, and Paraguay	Stress, depressive symptoms, anxiety, denial, insomnia, fear, and anger may Emerging mental health issues could get evolve into some long-lasting health conditions, isolation, and stigma.	(1)
	-COVID-19 and mental health	Ohio	Health care workers, people with pre-existing medical or psychological problems, those who contract it, and also those who are in quarantine are at an increased risk for adverse psychological problems such as irritability, insomnia, fear, confusion, frustration, boredom, anger, stigma, stress, mood swings and depression Suicidal thoughts may emerge	(33, 42, 43, 46)
	- COVID-19 and the mental health of females	UK and Germany	Changes in hormone levels increase emotional sensitivity to pre-existing stressful situations related to COVID-19	(45)
9. Age-related impact of COVID-19	-COVID-19 and age-linked comorbidities	USA	Number of fatalities according to age; Age >85 years old: 10-27% (the highest fatality rate) Age 65-84 years old: 3-11% Age 55-64 years old: 1-3%	(12, 13)

			Age 20-54 years old: <1% Age 19 years old: No fatalities Age and age-related chronic diseases lead to increased inflammation and mitochondrial dysfunction Inflammation and mitochondrial dysfunction can lead to weakened immune response and increased severity of COVID-19	
10. COVID-19 and comorbidity patients	-COVID-19 and comorbidity patients	India, Italy	People with pre-condition of hypertension, diabetes, and cardiovascular diseases are more prone and may lead to heart failure.	(34, 35)
	-The impact of COVID-19 on the heart and heart of the patients in comorbidity	USA	In comorbidity patients (i.e. diabetic patients): Severe complications High mortality rate High ICU admissions Inflammatory responses Hepatic, Cardiac, and renal coagulopathy	(15)
	-Study impacts of COVID-19 on pre-existing cardiovascular disorders	Italy	Deterioration of pre-existing medical conditions	(7)

EFFECTS ON THE LUNGS (Initial and Progressive Phases of COVID-19)

Epidemiological studies marked various phases indicating the COVID-19 development. COVID-19 symptoms tend to develop during the period of 2 to 14 days. However, these symptoms were not diagnosed as identical in every infected person. COVID-19 Symptoms range from mild (*i.e.,* flu or cold-like condition) to severe (*i.e.,* fever, cough, and breathing difficulties). Other symptoms include digestive issues and/or loose stools (14). Lung injury, inflammation, and hypoxia are predominant respiratory symptoms of COVID-19 (15). When get compared with influenza patients, the patients with COVID-19 indicated the state of angiogenesis and the presence of microthrombi in alveolar capillaries (16). The COVID-19 virus enters the lung cells by getting attached to its angiotensin-converting enzyme 2 (ACE2) receptors. Following the attachment, ACE2 activity decreases in the cells which, on the other hand, increases ACE1 activity. The increase in ACE1 activity produces more angiotensin 2 which is a naturally occurring vasoconstrictor peptide hormone recognized for raising blood pressure by triggering aldosterone production. High levels of angiotensin-2, provide attachment sites for SARS-COV-2 in the human lungs, which may thus lead to the COVID-19 severity. ACE2 normally reduces the levels of angiotensin 2 in the body by consuming it. As COVID-19 downregulates ACE2, build-up of angiotensin-2 occurs in the body, which may result in causing acute respiratory distress syndrome (ARDS) and cardiac injury in human beings (Figure 1) (17). COVID-19 along with the ARDS may injure lungs in the same pattern as the classical ARDS. The manifestations of classical ARDS include increased lung weight and decreased compliance which can get complicated by increased dead space and thrombi in the lungs (18). It has been observed that the majority of survivors may live their normal lives. However, persistent and prolonged pulmonary abnormalities like residual ventilation and irregular diffusion of blood gasses have been observed in a significant number of survivors (19).

EFFECTS ON THE GUT

The earliest symptoms of the disease involve a major effect on the gut and fecal microbiomes (6, 20, 21, 22). During the initial phases, the patients with COVID-19 are marked with high amounts of viral load were faced with gastrointestinal problems (Figure 6). Viral attachment with Angiotensin-Converting Enzyme 2 (ACE2) receptors is followed by the infection of gastrointestinal cells with SARS-CoV-2 which may lead to inflammation and diarrhea (6). Prolonged and persistent exposure to the virus in the guts of the survivors even after the pulmonary removal of the virus (21).

There are two different possible routes of viral infection in the gastrointestinal tract (GIT):

1. The ACE2 receptors are exceedingly expressed in epithelial cells of the GIT which leads to the receptor-mediated entrance of the virus into the cells of the host (23).

2. Pulmonary infection of COVID-19 induces an inflammatory immune response which leads to viral translocation from the lungs to the circulatory system. Once in the circulatory system, the virus stimulates the activation of cytokines resulting in an exaggerated immune response and hence, further augments the inflammation. In response to this, gut permeability may increase which could result in alteration of the gut microbiome. The change in gut microbiota known as gut dysbiosis leads to the transfer of infectious agents and toxic substances into the circulation resulting in the development of severity of COVID-19 and multiple organ failure. Other physiological conditions such as pre-existing medical disorders and increased age contribute to the severity of the disease (**Figure**. 2) (20, 23).

EFFECTS ON IMMUNE SYSTEM

The immune system in human beings is meant for safety against various xenobiotics and infections. The early phase of COVID-19 is defined by the initiation of innate cytokine response. The later phase of COVID-19 conversely can be characterized by a 'cytokine storm' and inflammation (24) (Figure 5).

Cytokines are, however, small molecules of protein that are secreted by the cells of the immune system in blood or directly into the tissues. They regulate different functions of the immune system by looking for target immune cells to bind and interact with, to activate specific immune responses (Figure 5). The immune system of COVID-19 patients may produce both anti-inflammatory and pro-inflammatory cytokines. Lung damage, dysfunction, and decreased lung capacity can occur as a result of an uncontrolled or overactive immune response (17).

Cytokine storm is a serious disorder characterized by cytokine overproduction, over-activation of immune cells, systemic inflammation, and multiple organ damage (25). A hyperactive immune response has an obvious physiological impact in COVID-19 patients (26). However, people with having strong immune systems are less prone to serious COVID-19 infection (12).

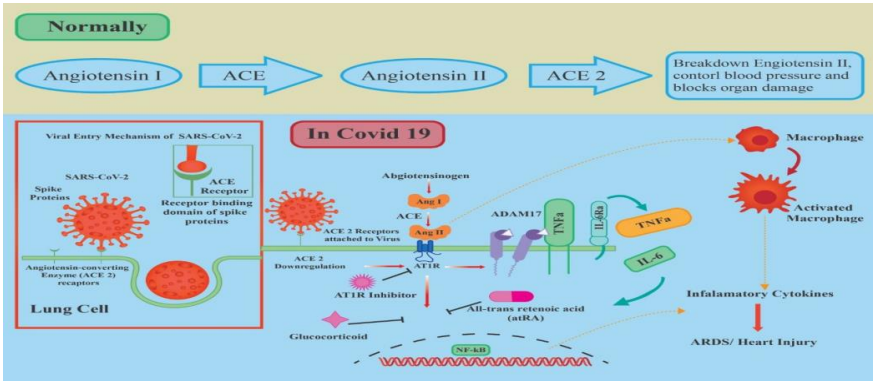

Fig. 1. Schematic Representation of the Role of ACE2 Receptors and Angiotensin 2 in COVID-19 (17).

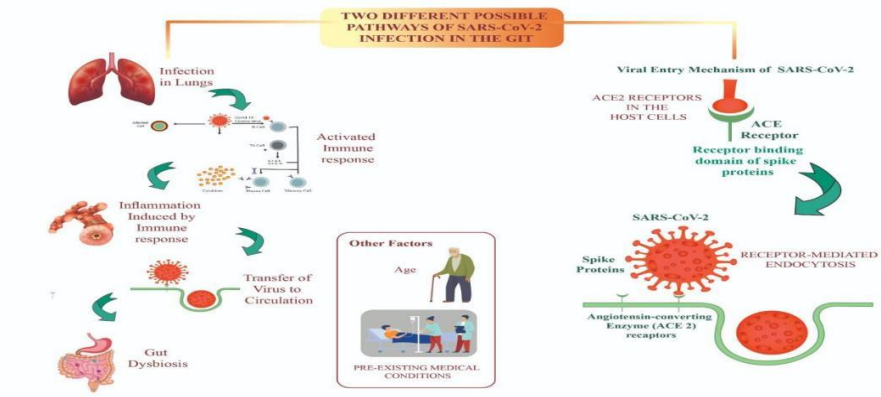

Figure. 2. Schematic Description of the Development of Secondary COVID-19 Infection Along the Gut and Lung Pathway (20, 23).

EFFECTS ON THE NERVOUS SYSTEM

COVID-19 can cause a range of neurological symptoms for instance loss of smell, headache, unconsciousness, and stroke (27). Such symptoms can be the result of COVID-19 induced immune responses, respiratory stress in the Central Nervous System (CNS), and chemosensory dysfunction in the Peripheral Nervous System (PNS) (5). Along with psychosis in a few patients, COVID-19 has also been observed to cause mental disturbances (31% of patients), strokes, and hemorrhages (62% of patients). Some people with COVID-19, however, also experience confusion, agitation, and disorientation during hospitalization (28, 29). There are three different possible scenarios for the damage to Central Nervous System (CNS) (5, 26). The manifestation of damage in the Peripheral Nervous System (PNS) is characterized by chemosensory dysfunction. The pathway of PNS damage may involve the entry of a virus into the brain cells following the same pathways as CNS. This triggers an inflammatory immune response in the PNS leading to anosmia and ageusia (5). The pathway for nervous system infection by SARS-CoV-2 may involve three different pathways *i.e.* olfactory pathway, ACE2 receptors, and blood circulation pathway.

Olfactory Pathway: SARS-CoV-2 might vitiate the CNS which may initiate from the olfactory bulb, then move to brain's inner parts such as the brainstem and thalamus with the help of trans-synaptic transfer. From there on, this virus could target the respiratory center of the brain. It is the common pathway for both CNS and PNS as the virus may also affect the chemosensory function of the brain (30, 5).

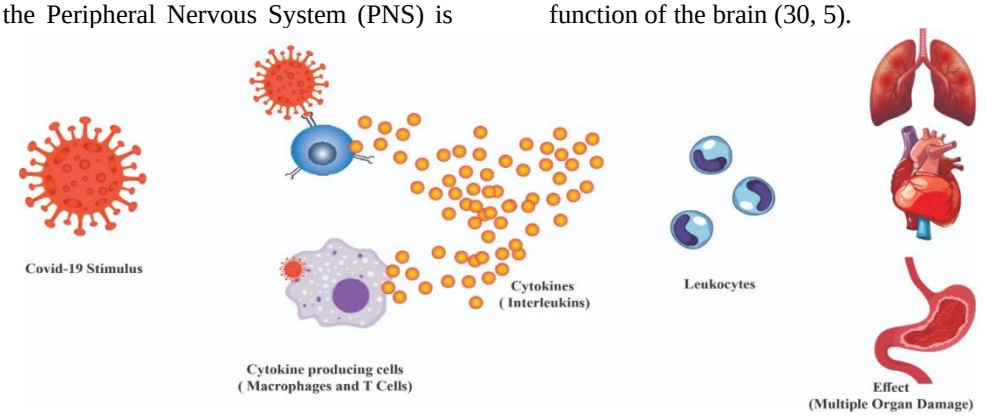

Figure. 3. Representation of the Role of Cytokines in Immune Response (17, 25).

THREE DIFFERENT POSSIBLE SCENARIOS OF DAMAGES IN CENTRAL NERVOUS SYSTEM (CNS) (5, 26)	1. SARS-CoV-2 may enter CNS through nasal passageways, gain access to the olfactory bulb, and mucosa as well as the cerebral circulation. The ACE2 receptors found in endothelial cells and blood vessels are identified by the virus which can further lead to apoptosis of brain cells. Consequently, cerebral edema occurs which causes the compression of brain stem. Moreover, entry of the virus and recognition of ACE2 receptors on the endothelial brain cells can also damage the blood-brain barrier. This is because the virus is transported across the blood vessel into the neurons.
	2. COVID-19 also causes an overactive immune response in the body due to the cytokine storm. This can result in apoptosis of brain cells which ultimately cause Acute Necrotizing Encephalopathy (ANE) and hemorrhage.
	3. The third plausible scenario of CNS damage involves the respiratory stress caused by the depletion of oxygen in the lungs. This hypoxic condition can result in brain cells injury.

Figure. 4. Three different possible scenarios of damage in the central nervous system (CNS) (5, 26).

1. **ACE2 Receptors:** SARS-CoV-2 recognizes ACE2 receptors present on the surface of neurons, neuroglia, and capillary epithelium. The ACE2 receptors express itself on these cells which makes them susceptible for SARS-CoV-2 attack. Through these cellular receptors, the virus may damage the capillary endothelium and gain access to the brain (31). This pathway involves CNS only.
2. **Cerebral Blood Circulation Pathway**

   The viruses that affect the CNS do so by directly affecting the nerve endings found in the tissues or by targeting the cells of the circulatory system. This would further result in the transfer of virus through the blood-brain barrier into the CNS (32). SARS-CoV-2 and cytokines could target the endothelial cells which can result in inflammation and damage to the blood-brain barrier. This pathway explains the CNS invasion by the virus (26) (Figure 4).

   As the neurological symptoms become more serious with strokes, hemorrhages, and memory loss, the main question is, why is the brain impacted at all? Moreover, whether the neurological symptoms are the result of a hyperactive immune system or the virus directly invades the cells of the brain is still not obvious and requires further research (28).

EFFECTS ON MENTAL HEALTH

This pandemic has been reported to cause mental and health issues among humans *i.e.* depressive symptoms, stress, denial, anxiety, anger, and insomnia. These developing mental health issues may result in long-lasting human health issues like isolation and stigma (1). Some people may be more exposed than others to the psychological and social effects of a pandemic. Quarantine, loss of freedom, boredom, and self-isolation create a negative impact on mental health (42). People with weak immune responses, living or caring in congested places, and with pre-existing medical or psychological issues are more prone and vulnerable to developing the disease. Health-care workers are particularly more susceptible to psychological issues and emotional stress in the pandemic since they are regularly exposed to the virus, work for longer hours and care for the sick (33,43). Due to the speedy outspread of COVID-19, emigrants and immigrants have been reported to face hostility, stigmatization, and discrimination (43). The mental health of females is considered more susceptible and vulnerable to stress as compared to males. It may be due to the fluctuations in ovarian hormone levels which change the emotional sensitivity to various stress stimuli (45). It is analyzed that younger age groups have a high tendency of stimulation against surrounding stressors of COVID-19 (44).

COVID-19 pandemic is also a major contributor to domestic violence, loneliness, and child abuse. It may be the increase in isolation during lockdowns due to which closeness of abusers and victims has taken place and escape is not possible. Fear and stress are major contributing factors in the widespread dissemination of mental health conditions. During this pandemic, anxiety and depression show their peak increase in their symptoms with an increase in a low mood, limited interest, and a decrease in energy in daily activities (46).

EFFECTS ON THE HEART

The cardiac physiological symptoms in COVID-19 sufferers may include hypoxia, myocardial injury, plaque rupture, arrhythmia, coronary thrombosis, venous thromboembolism, dispersed intravascular coagulation, and inflammation (7, 15). Several patients with myocardial damage can develop serious cardiovascular issues which may include heart failure (34).

SARS-CoV-2 may cause myocardial injury in three possible ways,

1. The virus may directly attack cardiac cells by attaching to ACE2 receptors.
2. Arterial hypotension leading to oxygen dysregulation in the heart may also be the reason for myocardial injury.
3. Cytokine storms can lead to an impaired immune response which may eventually result in myocardial injury (Figure 5) (15, 25, 35).

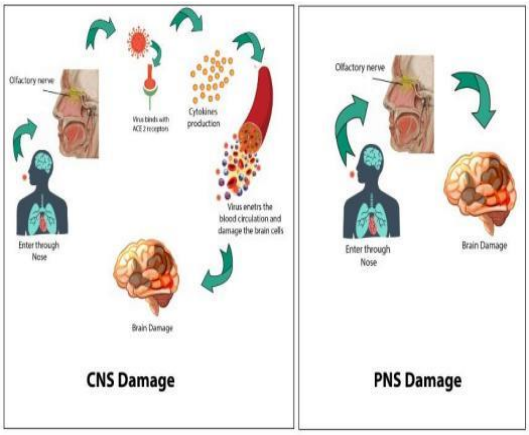

Figure. 5. Schematic Representation of the Pathway of Nervous System Damage in COVID-19 (5, 26, 30, 31).

A bidirectional association has been found between COVID-19 and cardiovascular diseases (CVD). On one hand, people with pre-existing CVD are supposed to be at a high risk of becoming infected with COVID-19. Thus are considered more likely to acquire a severe form of the disease. On the other hand, some people infected with COVID-19 develop cardiovascular symptoms in addition to other complications associated with the COVID-19 (35).

LIVER DYSFUNCTION AND INJURY

Liver injury is prominent in severe disease groups of COVID-19 (36, 37). The Insufficient level of albumin in the blood and cirrhosis are the main contributors to developing severity of COVID -19. This is considered to be due to immune dysregulation which may be traced in tests of abnormal liver function i.e. alanine transferase (ALT) and aspartate transferase (AST). The mechanism of COVID-19 effect on liver dysfunction may be multifactorial but still is not fully understood. ACE2, is the host cell receptor of SARS-CoV-2. The entry of SARS-CoV-2 involves transmembrane serine protease 2 (TMPRSS2). Minimal or no Expression of ACE2 messenger RNA (mRNA) has been reported in hepatocytes. The expressions of TMPRSS2 mRNA were found in hepatocytes. Hepatocytes were reported to contain no ACE2 protein. All these findings have suggested that SARS-CoV-2 has not been found to directly cause the cytopathic damage of hepatocytes. Hepatocellular Carcinoma cell line Huh-7 has been found to show SARS-CoV-2 replication (26). The cytoplasm of hepatocytes has been found to contain particles of SARS-CoV-2 without any membrane-bounded vesicles (49). Factors like liver enzymes, liver steatosis, and liver fibrosis have been found as contributing factors in developing the severity of COVID-19 (49, 50, 51). However, no significant change in the transaminases and bilirubin levels of COVID-19 diagnosed patients has been observed (52). Patients with cirrhosis have been reported to be at high risk of mortality. This danger may happen through many converging pathways, which may include systemic inflammatory response, acute hepatic decompensation, and cirrhosis-associated immune dysfunction. Cirrhosis-associated immune dysfunction can also lead to defective immune responses followed by the future SARS-CoV-2 vaccination (53). A decline in liver functioning may further quadruple the risk of developing severe corona infection. Patients thus having chronic hepatic diseases and/or cirrhosis are considered more susceptible to the COVID-19 severity (54).

The direct effects of COVID-19 mean the process of active infection of a virus on a patient's musculoskeletal system. First, COVID-19 hits the respiratory tract, especially the alveoli epithelium which accesses the virus to the bloodstream, the coronavirus disseminates throughout the body.

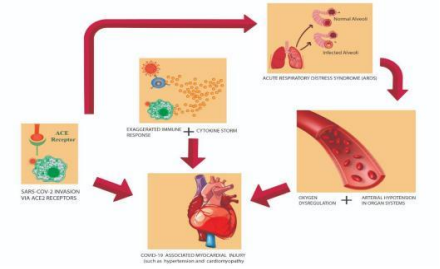

Figure. 6. A schematic representation of the possible causes of myocardial injury in COVID-19 (15, 25, 35)

This damages the other systems such as myalgias, sarcopenia, muscle loss, Cachexia, myasthenias, and fatigue. The indirect effect on the musculoskeletal system has been found more prevalent in those patients who face prolonged exposure to inactivity such as in ICU and because of strict lockdowns. During the lockdown, people are limited to exercise at home. This creates a negative impact on muscles due to unused muscle allocated energy. This may be reallocating the metabolic substrates in the liver which leads to an increase in obesity (anthropogenic lipoprotein production) and atherosclerosis (increasing the risk of lipids collecting in blood vessels). Most ICU patients require the use of invasive ventilation to maintain a constant airflow which may be the cause of bone and muscle frailty (55).

The inflammatory response is produced as a result of the primary stage of pulmonary infection, which causes an integral impact on the human musculoskeletal system. Various kinds of musculoskeletal cells show the ACE2 and TMPRSS2 genes, that are necessary for direct viral infection. But the way these genes' are infected is still unclear (9). Myalgia means muscle pain and aches. Primarily, immune-mediated mechanism theory is considered the most extensively accepted theory of muscle damage in corona patients. Secondly, the inflammatory response with cytokine storming mechanism and immune cells activation was also accepted for muscle damage. These suggested mechanisms cause myotoxic cytokine release, immune complex deposition, and injury that is secondary to homology between the viral antigens and human muscle cells. Myositis (inflammation of muscles), Rhabdomyolysis (a disease of myositis involving myoglobinuria and myonecrosis), Virus-induced arthritis Diaphragm muscle dysfunction was also reported in COVID-19 patients (56).

EFFECTS ON THE MUSCULOSKELETAL SYSTEM

The corona infection has been found to cause weakness and fatigue in the skeletal muscle. In the bones and joints, it causes loss of bone tissue and cartilage. However, it is not known whether these symptoms are the result of systemic inflammation alone or the virus directly infects the cells of the musculoskeletal system (9). During the first four days of infection, a rapid twenty percent loss in the body occurs.

AGE-RELATED EFFECTS OF COVID-19

In the case of patients having age-related chronic diseases, increased inflammation and mitochondrial dysfunction occur which leads to a weakened immune response causing a severe form of COVID-19 (12). Older people also have decreased diversity of gut microbiota which could be the reason for the severe form of infection. Center for disease control and prevention (CDC) revealed through a published report that the highest mortality rate was found in people as aged as eighty-five (85) or older and no casualties were reported among the individuals aged 19 or younger (13). Adolescents and children were found affected by aggressive behavior and depression due to COVID-19 lockdown measures (58). High mortality risks in older adults have been reported due to the increased level of anxiety, fear of death, optimism, and social isolation (59). Social vulnerabilities such as low income and unemployment also are the major factors creating mental issues in many developing countries with financially downtrodden situations (60).

EFFECTS ON COMORBIDITY PATIENTS

COVID-19 may appear as asymptomatic, or with mild and severe pneumonia-like symptoms. It was found that patients diagnosed as suffering from coronavirus were mostly reported with a pre-condition of diabetes, cardiovascular diseases (CVD), chronic obstructive pulmonary disease (COPD), hypertension, HIV, malignancies, and some other diseases which may also tend to develop some life-threatening situations. ACE-2 receptors are responsible for the entry of SARS-COV-19 into the host cell. Certain pro-protein convertases are released due to the expression of ACE-2 receptors which can further improve the entry of the virus into host cells. These comorbidities may further lead these coronavirus patients into a vicious and infectious cycle. In addition, they are also substantially associated with a significant level of mortality and morbidity (61). Hypertension, renal disease, cancer, HIV, dementia, chronic pulmonary disease, and diabetes are the predominant comorbidities that show high mortality rates in patients affected by COVID-19 (62). In addition, it was found that populations with pre-existing diseases like cardiovascular and cerebrovascular, renal infections, and hypertension are considered more prone to coming under the attack of coronavirus. This article further provides ample evidence to indicate the effect of certain comorbidities on the COVID-19 intensity. Comorbidities including diabetes and other age-linked diseases may lead to increased inflammation and abnormal mitochondrial function which can further weaken the immune response resulting in COVID-19 severity (12). A comparative hospital-based study on non-diabetic and diabetic patients having COVID-19 has shown high shifting ratios of diabetic patients into ICU *i.e.* Intensive Care Unit and/or high mortality ratio. This can further be safely concluded that COVID-19 patients with pre-condition of diabetes mellitus have shown severe inflammatory immune responses as compared to non-diabetic patients (39).

CONCLUSION

During its course, SARS-COV-19 has been found to target many vital organs which may cause life-threatening physiological complications (26). Being a respiratory infection, COVID-19 has been found to mainly cause pneumonia. Gut dysbiosis is the major effect on the gastrointestinal system which also occurs in the early phase of the disease. Neurological complications include strokes, hemorrhages, and impaired consciousness. Cardiovascular manifestations are observed in severely ill patients and the situation of people with pre-existing CVD can get worse during COVID-19. Low levels of albumin in blood and weakness in muscles and joints are also observed. Hyperactive immune system and cytokine storm syndrome may quadruple the severity. Moreover, the negative influences on the economy, mental and physical human health along with social interactions are considered damaging globally. A global comprehensive response regarding a focus on COVID-19 patients' mental health has also been considered an essential factor and should be encouraged worldwide. Moreover, COVID-19 related news dispensed through social media and other platforms must closely be monitored and other community relief programs regarding human mental health must be encouraged worldwide. Some common diseases like hypertension, coronary artery, and diabetes-like diseases are considered the most common comorbidities among COVID-19 patients. People who are weak, aged, and sick are considered most prone to develop severe complications and may require intensive care and mechanical ventilation. It should be noted that the majority of people recover from the disease and continue with their normal lives but some people may have long-term effects on their health.

REFERENCES

1. Torales, J., O'Higgins, M., Castaldelli-Maia, J. M., & Ventriglio, A. (2020). The outbreak of COVID-19 coronavirus and its impact on global mental health. International Journal of Social Psychiatry, 66(4), 317-320.
2. Das, S., Das, S., & Ghangrekar, M. M. (2020). The COVID-19 pandemic: biological evolution, treatment options, and consequences. Innovative Infrastructure Solutions, 5(3), 1-12.
3. Johns Hopkins University. Coronavirus resource centre. Accessed December 14, 2021. https://coronavirus.jhu.edu
4. Lwin, M. O., Lu, J., Sheldenkar, A., Schulz, P. J., Shin, W., Gupta, R., & Yang, Y. (2020). Global sentiments surrounding the COVID-19 pandemic on Twitter: analysis of Twitter trends. JMIR public health and surveillance, 6(2), e19447.
5. Fiani, B., Covarrubias, C., Desai, A., Sekhon, M., & Jarrah, R. (2020). A contemporary review of neurological sequelae of COVID-19. Frontiers in Neurology, 11, 640.
6. Villapol, S. (2020). Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. Translational Research.
7. Mai, F., Del Pinto, R., & Ferri, C. (2020). COVID-19 and cardiovascular diseases. Journal of cardiology.
8. Nuzzo, D., & Picone, P. (2020). Potential neurological effects of severe COVID-19 infection. Neuroscience research.
9. Disser, N. P., De Micheli, A. J., Schonk, M. M., Konnaris, M. A., Piacentini, A. N., Edon, D. L., ... & Mendias, C. L. (2020). Musculoskeletal consequences of COVID-19. JBJS, 102(14), 1197-1204.
10. Liu, W., & Li, H. (2020). COVID-19: Attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. 2020. ChemRxiv. DOI, 10.
11. Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., & Manson, J. J. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. The lancet, 395(10229), 1033-1034.
12. Holder, K., & Reddy, P. H. (2020). The COVID-19 effect on the immune system and mitochondrial dynamics in diabetes, obesity, and dementia. The Neuroscientist, 1073858420960443.
13. Covid, C. D. C., Team, R., COVID, C., Team, R., COVID, C., Team, R., ... & Sauber-Schatz, E. (2020). Severe outcomes among patients with coronavirus disease 2019 (COVID-19)—United States, February 12–March 16, 2020. Morbidity and mortality weekly report, 69(12), 343.
14. Osler, S. (2019). Coronavirus Outbreak.
15. Suri, J. S., Puvvula, A., Biswas, M., Majhail, M., Saba, L., Faa, G., ... & Naidu, S. (2020). COVID-19 pathways for brain and heart injury in comorbidity patients: A role of medical imaging and artificial intelligence-based COVID severity classification: A review. Computers in biology and medicine, 103960.
16. Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., ... & Jonigk, D. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. New England Journal of Medicine, 383(2), 120-128.
17. Mercola, J., Grant, W. B., & Wagner, C. L. (2020). Evidence regarding vitamin D and risk of COVID-19 and its severity. Nutrients, 12(11), 3361.
18. Grasselli, G., Tonetti, T., Protti, A., Langer, T., Girardis, M., Bellani, G., ... & Seccafico, C. (2020). Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. The Lancet Respiratory Medicine, 8(12), 1201-1208.
19. Salehi, S., Reddy, S., & Gholamrezanezhad, A. (2020). Long-term pulmonary consequences of coronavirus disease 2019 (COVID-19): what we know and what to expect. Journal of thoracic imaging, 35(4), W87-W89.
20. AKTAŞ, B., & Aslim, B. (2020). Gut-lung axis and dysbiosis in COVID-19. Turkish Journal of Biology, 44(SI-1), 265-272.
21. Zuo, T., Liu, Q., Zhang, F., Lui, G. C. Y., Tso, E. Y., Yeoh, Y. K., ... & Ng, S. C. (2021). Depicting SARS-CoV-2 fecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut, 70(2), 276-284.
22. Gu, S., Chen, Y., Wu, Z., Chen, Y., Gao, H., Lv, L., ... & Li, L. (2020). Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. Clinical Infectious Diseases, 71(10), 2669-2678.
23. Wong, S. H., Lui, R. N., & Sung, J. J. (2020). Covid-19 and the digestive system. Journal of gastroenterology and hepatology, 35(5), 744-748.

24. Chowdhury, M. A., Hossain, N., Kashem, M. A., Shahid, M. A., & Alam, A. (2020). Immune response in COVID-19: A review. Journal of Infection and Public Health.
25. Fajgenbaum, D. C., & June, C. H. (2020). Cytokine storm. New England Journal of Medicine, 383(23), 2255-2273.
26. Iadecola, C., Anrather, J., & Kamel, H. (2020). Effects of COVID-19 on the nervous system. Cell.
27. Wood, H. (2020). New insights into the neurological effects of COVID-19. Nature Reviews. Neurology, 1.
28. Marshall, M. (2020). How COVID-19 can damage the brain. Nature, 585(7825), 342-343.
29. Varatharaj, A., Thomas, N., Ellul, M. A., Davies, N. W., Pollak, T. A., Tenorio, E. L., ... & Plant, G. (2020). Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. The Lancet Psychiatry, 7(10), 875-882.
30. Gandhi, S., Srivastava, A. K., Ray, U., & Tripathi, P. P. (2020). Is the collapse of the respiratory center in the brain responsible for the respiratory breakdown in COVID-19 patients?. ACS chemical neuroscience, 11(10), 1379-1381.
31. Baig, A. M., Khaleeq, A., Ali, U., & Syeda, H. (2020). Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. ACS chemical neuroscience, 11(7), 995-998.
32. Koyuncu, O., Hogue, I., & Enquist, L. (2013). Virus infections in the nervous system. Cell host & microbe, 13(4), 379-393.
33. Pfefferbaum, B., & North, C. S. (2020). Mental health and the Covid-19 pandemic. New England Journal of Medicine, 383(6), 510-512.
34. Harikrishnan, S., Mohanan, P. P., Chopra, V. K., Ambuj, R., Sanjay, G., Bansal, M., ... & Das, M. K. (2020). Cardiological Society of India position statement on COVID-19 and heart failure. Indian heart journal, 72(2), 75.
35. Russo, V., Bottino, R., Carbone, A., Rago, A., Papa, A. A., Golino, P., & Nigro, G. (2020). Covid-19 and heart: from clinical features to pharmacological implications. Journal of clinical medicine, 9(6), 1944.
36. Kumar, A., Kumar, P., Dungdung, A., Gupta, A. K., Anurag, A., & Kumar, A. (2020). The pattern of liver function and clinical profile in COVID-19: A cross-sectional study of 91 patients. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 14(6), 1951-1954.
37. Wong, Y. J., Tan, M., Zheng, Q., Li, J. W., Kumar, R., Fock, K. M., ... & Ang, T. L. (2020). A systematic review and meta-analysis of the COVID-19 associated liver injury. Annals of Hepatology, 19(6), 627-634.
38. McCray Jr, P. B., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L., ... & Perlman, S. (2007). Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. Journal of virology, 81(2), 813-821.
39. Yan, Y., Yang, Y., Wang, F., Ren, H., Zhang, S., Shi, X., ... & Dong, K. (2020). Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. BMJ open diabetes research and care, 8(1), e001343.
40. Aghagoli, G., Marin, B. G., Katchur, N. J., Chaves-Sell, F., Asaad, W. F., & Murphy, S. A. (2020). Neurological involvement in COVID-19 and potential mechanisms: a review. Neurocritical care, 1-10.
41. Pero, A., Ng, S., & Cai, D. (2020). COVID-19: a perspective from clinical neurology and neuroscience. The Neuroscientist, 26(5-6), 387-391.
42. Javed, B., Sarwer, A., Soto, E. B., & Mashwani, Z. U. R. (2020). The coronavirus (COVID-19) pandemic's impact on mental health. The International Journal of health planning and management, 35(5), 993-996.
43. Yuan, Y., Zhao, Y. J., Zhang, Q. E., Zhang, L., Cheung, T., Jackson, T., ... & Xiang, Y. T. (2021). COVID-19-related stigma and its sociodemographic correlates: a comparative study. Globalization and Health, 17(1), 1-9.
44. Al Dhaheri, A. S., Bataineh, M. A. F., Mohamad, M. N., Ajab, A., Al Marzouqi, A., Jarrar, A. H., ... & Cheikh Ismail, L. (2021). Impact of COVID-19 on mental health and quality of life: Is there any effect? A cross-sectional study of the MENA region. PloS one, 16(3), e0249107.
45. Schäfer, S. K., Sopp, M. R., Schanz, C. G., Staginnus, M., Göritz, A. S., & Michael, T. (2020). Impact of COVID-19 on public mental health and the buffering effect of a sense of coherence. Psychotherapy and Psychosomatics, 89(6), 386-392.
46. Haider, I. I., Tiwana, F., & Tahir, S. M. (2020). Impact of the COVID-19 pandemic

on adult mental health. Pakistan Journal of Medical Sciences, 36(COVID19-S4), S90.
47. Martinez, M. A., & Franco, S. (2021). Impact of COVID-19 in liver disease progression. Hepatology Communications.
48. Wu, Z. H., & Yang, D. L. (2020). A meta-analysis of the impact of COVID-19 on liver dysfunction. European Journal of Medical Research, 25(1), 1-9.
49. Sharma, A., Jaiswal, P., Kerakhan, Y., Saravanan, L., Murtaza, Z., Zergham, A., ... & Malik, P. (2021). Liver disease and outcomes among COVID-19 hospitalized patients–a systematic review and meta-analysis. Annals of hepatology, 21, 100273.
50. Bzeizi, K., Abdulla, M., Mohammed, N., Alqamish, J., Jamshidi, N., & Broering, D. (2021). Effect of COVID-19 on liver abnormalities: a systematic review and meta-analysis. Scientific reports, 11(1), 1-18.
51. Marjot, T., Webb, G. J., Barritt, A. S., Moon, A. M., Stamataki, Z., Wong, V. W., & Barnes, E. (2021). COVID-19 and liver disease: mechanistic and clinical perspectives. Nature Reviews Gastroenterology & Hepatology, 18(5), 348-364.
52. Cichoż-Lach, H., & Michalak, A. (2021). Liver injury in the era of COVID-19. World Journal of Gastroenterology, 27(5), 377.
53. Sullivan, W. (2020). EFFECTS OF COVID-19 ON THE HUMAN MUSCULOSKELETAL SYSTEM.
54. Ramani, S. L., Samet, J., Franz, C. K., Hsieh, C., Nguyen, C. V., Horbinski, C., & Deshmukh, S. (2021). Musculoskeletal involvement of COVID-19: a review of imaging. Skeletal Radiology, 1-11.
55. Greve, J. M. D. A., Brech, G. C., Quintana, M., Soares, A. L. D. S., & Alonso, A. C. (2020). Impacts of COVID-19 on the immune, neuromuscular, and musculoskeletal systems and rehabilitation. Revista Brasileira de Medicina do Esporte, 26, 285-288.
56. Schmidt, S. J., Barblan, L. P., Lory, I., & Landolt, M. A. (2021). Age-related effects of the COVID-19 pandemic on the mental health of children and adolescents. European journal of psychotraumatology, 12(1), 1901407.
57. Pasion, R., Paiva, T. O., Fernandes, C., & Barbosa, F. (2020). The AGE effect on protective behaviors during the COVID-19 outbreak: Sociodemographic, perceptions and psychological accounts. Frontiers in Psychology, 11, 2785.
58. Mistry, S. K., Ali, A. M., Akther, F., Yadav, U. N., & Harris, M. F. (2021). Exploring fear of COVID-19 and its correlates among older adults in Bangladesh. Globalization and Health, 17(1), 1-9.
59. Ejaz, H., Alsrhani, A., Zafar, A., Javed, H., Junaid, K., Abdalla, A. E., ... & Younas, S. (2020). COVID-19 and comorbidities: Deleterious impact on infected patients. Journal of infection and public health.
60. Osibogun, A., Balogun, M., Abayomi, A., Idris, J., Kuyinu, Y., Odukoya, O., ... & Akinroye, K. (2021). Outcomes of COVID-19 patients with comorbidities in southwest Nigeria. PloS one, 16(3), e0248281.
61. Cho, S. I., Yoon, S., & Lee, H. J. (2021). Impact of comorbidity burden on mortality in patients with COVID-19 using the Korean health insurance database. Scientific reports, 11(1), 1-9.
62. Fernández Villalobos, N. V., Ott, J. J., Klett-Tammen, C. J., Bockey, A., Vanella, P., Krause, G., & Lange, B. (2021). Effect modification of the association between comorbidities and severe course of COVID-19 disease by age of study participants: a systematic review and meta-analysis. Systematic reviews, 10(1), 1-15.