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EDITORIAL

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Life Science Journal of Pakistan (LSJP) is publishing its fourth issue and becoming popular among life science researchers who are sending their valuable manuscripts for publication. Although due to strict publication ethics and higher standard of acceptance we find it difficult to accept many even good research topics to be published.

In this issue, four manuscripts will be published out of which three are original research articles and one is mini-review. One manuscript accepted for publication focused on frequency of drug-resistant *Mycobacterium Tuberculosis* in remote area of Tehsil Chiniot, Pakistan. Authors reported, high incidence rate of TB and a substantial number of RR-TB in males versus females. This article is unique as authors investigated over 6000 suspected individuals where 900 (14.7%) were diagnosed with TB (8.3% - males, 6.3% - females). Middle aged persons of 45-54 years showed highest resistant TB frequency (18.4%). The figures mentioned in this manuscript are alarming for health officials and demand urgent and special attention. Second manuscript accepted for publication was also of great importance as they carried out a survey depicting prevalence of a hygiene related disease scabies. They reported very high prevalence of scabies and concluded that low personal and environmental hygiene and personal direct or indirect contact with the infected persons and sharing of household accessories as means of scabies spread. This manuscript is worth reading to understand communicability of scabies in our local settings. Third article, accepted for publication in this issue, mentioned *in-silico* studies related to protein-protein interaction between collagen and peptide degrading protease, hypodermin C. Authors mentioned three important amino acids involved in docking and based on inhibitory studies they reported their role in protein degradation. This study revealed the characteristic structure of HyA and HyB of warble fly larvae, functionally evolved to proteolysis of skin collagen of its host, essential for parasitic activity, thereby possibility of developing vaccines against HyA and HyB antigens. Team LSJP is striving and supporting government agencies and healthcare workers, fighting against COVID-19, by continuously publishing pandemic related literature. A relevant review was included in this issue regrading current COVID-19 situation. This review encompassed meteorological parameters and established direct and indirect relationships between various climatic patterns, environmental factors including air quality index (AQI), humidity, temperature, wind speed and transmission of COVID-19. Authors also reviewed COVID-19 emergence, survival, stability, and transmission ratios due to environmental, meteorological and climatic factors. They recommended to improve the quality of life by reversing the necessary climatic and meteorological parameters.

This journal will continue addressing scientific trends of life science related all disciplines mentioned in the scope of LSJP, for that we hope more and 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, for that we used a similar model as that of journals of repute. Efforts of LSJP team working tirelessly is highly acknowledged for their help and support for bringing LSJP at this level towards gaining excellence in life sciences publications.

Dr. Yasar Saleem,

Managing Editor,

Life Science Journal of Pakistan

ORIGINAL RESEARCH

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Frequency of drug-resistant *Mycobacterium Tuberculosis* in Chiniot, Pakistan

Muhammad Daood Khan[1], Bilal Ahmad[2], Abid Hussain,[2] Tahir Ishaq[1], Orangzaib Haider[4], Hafiz Ghulam Murtaza Saleem[2],*
[1]University Institute of MLT, Faculty of Allied Health Sciences, The University of Lahore
[2]Department of Medical Lab Technology Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, QIE Campus Lahore Pakistan
[3]King Edward Medical University, Lahore Pakistan
[4]Punjab TB Control Program, Pakistan

*__Corresponding author:__
Dr.Hafiz Ghulam Murtaza Saleem
Address: Department of MLT, Faculty of Rehabilitation and Allied Health Sciences
Riphah International University, QIE Campus Lahore' Pakistan
Email Address: ghulam.murtaza@riphah.edu.pk

ABSTRACT

Introduction: Tuberculosis is one of the major causes of morbidity and mortality among developing countries, and conditions are getting worse with the emergence of antibiotic-resistant strains of *Mycobacterium tuberculosis* (MTB). Pakistan is trying to deal with TB but a substantial number of cases are still recorded every year.

Materials and methods: A cross-sectional study was designed and sputum samples from the suspected individuals were tested for acid-fast bacilli (AFB) and samples were further evaluated for the frequency of rifampicin-resistant MTB by Cepheid Xpert® MTB/Rif assay. The diagnosis of patients for TB Frequency was made based on clinical signs and symptoms with the lab results.

Results: The present study enrolled 6110 suspected individuals, out of which 900 (14.7%) were diagnosed with TB of which 511(8.3%) were males and 389(6.3%) were females. The highest (18.4%) frequency of TB cases was found in ages 45-54 (years) and substantially high (16.5%) TB cases were observed in ages 15-24 (years). Results indicated that 18 (0.3%) patients have been established with rifampicin-resistant TB (RR-TB) and among those cases, 13(0.21%) and 5(0.08%) were males and females respectively.

Conclusion: The current study concluded that a substantial number of rifampicin-resistant TB (RR-TB) cases were existing more in males than females from different areas of Chiniot, Pakistan and Xpert® MTB/Rif assay was observed to be an accurate and rapid tool to diagnose MTB.

Keywords: Tuberculosis, frequency, antibiotics, rifampicin resistance
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INTRODUCTION

Tuberculosis (TB) is the 9th leading cause of death throughout the globe and about 10 million new cases of TB are notified every year. In 2016, the world witnessed 1.3 million deaths of people affected by HIV-negative TB and the loss of 374,000 HIVpositive people (1). TB is an airborne infection caused by *Mycobacterium tuberculosi*s (MTB). Poor socioeconomic situation and overcrowding in developing countries are a major cause of MTB aerosol spread and it is more prevalent in males than females (2, 3).

Mycobacterium can infect any site in the body (lymph nodes, pleura, GIT, bones, CNS, and Urogenital track) with up to 85% of pulmonary TB (4) and up to 15% cases were of extrapulmonary TB and sites can (5). MTB-contaminated aerosols get access to alveolar macrophages and tubercle bacilli multiply, survive within macrophages, and spread via bloodstream or lymphatics to any part of the body. About 2 to 8 weeks later a cellular immune response is elicited and cellular immunity encapsulates or kills bacteria. When encapsulated, bacteria cannot further spread and cause infection, this condition is known as latent TB, in latent TB body is immunologically active but there are no signs and symptoms of active TB. At any point in life if the person becomes immune compromised these enclosed bacteria start multiplying which leads to Active TB (6, 7).

Patients presented with a cough lasting for two weeks or more along with fever of no proper etiology and weight loss are suspected of TB. The diagnosis is made using chest X-ray, Ziehl-Neelsen staining of sputum smear, and examining it under light microscopy, culturing on LJ media, histological biopsies, and by molecular assays like Xpert® MTB/Rif (8). The diagnosis of TB in the lab is mostly made through ZN staining of a sputum sample, but it has the lowest sensitivity among all the diagnostic tools. Culture is considered a gold standard, but this method takes a long time to generate results. The best diagnostic tool for respiratory samples is Xpert® MTB/Rif assay, it's highly specific, and reports result in just 2 hours, along with Rifampicin resistance status (9). The first-line drugs are rifampicin and isoniazid (10). Globally antibiotic resistance is becoming a crucial problem. *Mycobacterial* resistance to both of the first-line drugs is called multidrug resistance TB (MDR-TB) and resistance to fluoroquinolone and at least one of the second-line injectable drugs like amikacin, Kanamycin, and Capreomycin is called extensively drug-resistant TB (XDR-TB) (11, 12).

Drug resistance in MTB is caused by a mutation in a gene that code for the target site of the drug. It has been evident that MTB encodes for many drug multidrug transporters that depend upon proton motive force (PMF) or ATP, these transporters efflux the drug out of the cell, and evading bacterium from the lethal antimicrobial activity of drugs (13). Rifampicin targets the RNA polymerase, mutation in the gene that codes for the beta subunit of RNA polymerase leads to the development of resistant bacterial strains against the drug (14). Pakistan ranks fifth among high burden TB countries and ranks the fourth among high burden drug-resistant TB countries (15). The Frequency of bacteriologically positive TB in > 15-year-old population is 389/100,000 (16).

The objective of the current study is to observe the frequency of MTB and the incidence of Rifampicin-Resistance TB in the suspected population of district Chiniot, Pakistan.

METHODOLOGY

Study design

An observational study was conducted which based on the data of tuberculosis suspected individuals that visited eleven different Tehsil Headquarter and District Hospitals in different areas of Chiniot Punjab, Pakistan from April 2019 to September 2019. Individuals having signs and symptoms (cough, weight loss, and fever, blood in sputum, and spots in chest X-ray) of TB that visited the hospital first time and relapse TB cases were included in the study.

Lab analysis

Two sputum samples, on alternate days, were collected from the TB suspected individuals that didn't have a history of the previous infection with TB, and one sample was collected from relapse TB cases. Samples were analyzed for AFB screening (0.5% carbon fuchsin, 25% Sulphuric acid, 0.3% methylene blue or malachite green) and TB final diagnosis was made on MTB Gene Xpert (Cepheid) analysis in respective labs of eleven different Tehsil Headquarter and District Hospitals in different areas of Chiniot Punjab (17, 18). The study was approved by the ethical committee of the University Institute of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Lahore. Study subjects were diagnosed based on AFB screening and clinical signs & symptoms. AFB smear-positive cases were characterized as 'bacteriological MTB' and other AFB smear-negative were categorized 'clinical MTB' with the detection of the spot on chest X-ray. The data was precisely tabulated in Microsoft Excel to limit the risk of error and the frequencies (%) were calculated.

RESULTS

In the present study, 900 (14.7%) of total 6110 suspected individuals were found positive for the frequency of MTB, and 545 out of a total 900 (60.5%) positive TB cases were prevailing in Chiniot city, 155 (17%) were in Bhuwana, 112 (12.4%) in Lalian, 39 (4.3%) in Ahmed Nagar, 29 (3.2%) in Barana, and 20 (2.2%) in Chak No. 14 (Figure 1).

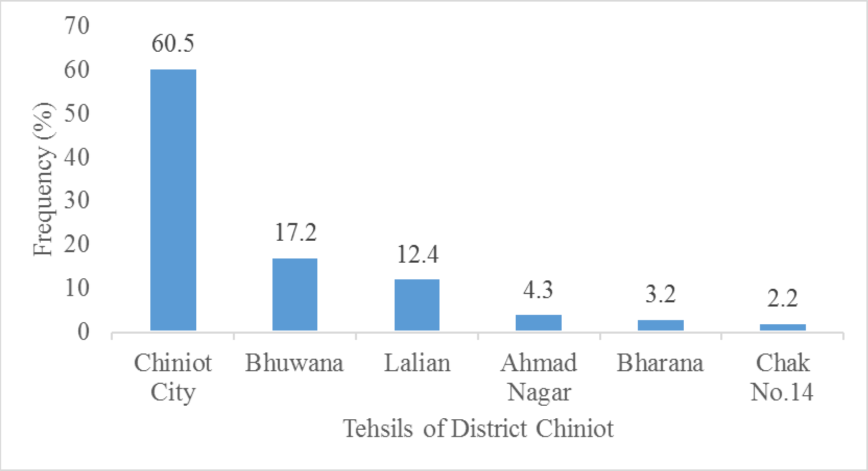

Figure 1: Frequency of Tuberculosis in different tehsils (Towns) of Chiniot district

Among 900 TB diagnosed patients 511(56%) were males and 389(43%) were females. Study did not found significant relationship (*p-value*=0.523) between gender and MTB infection. The highest number of infected TB male patients was 103 (11.5%) from the 45-54years age group. The age group 15-24 indicated the highest incidence of females 70 (8%) in table 1.

Eight hundred and fifteen (90.56%) from a total of 900 patients were newly diagnosed with pulmonary TB and 83 (9.2%) cases were diagnosed with extrapulmonary TB in table 2.

Table 1: Age and gender-wise distribution of all TB diagnosed patients

Age (Years)	Gender		Total No. (%)
	Male No. (%)	Female No. (%)	
0-4	1 (0.1)	7(0.7)	8 (0.8)
5-14	19 (2.1)	60(6.6)	79 (8.7)
15-24	79 (8.7)	70(7.7)	149 (16.5)
25-34	59 (6.5)	53(5.8)	112 (12.4)
35-44	75 (8.3)	44((4.8)	119(13.)
45-54	103 (11.5)	63(7)	166 (18.4)
55-64	78(8.6)	48(5.3)	126 (1)
>65	97 (10.7)	44(4.8)	141 (15.6)
Total	**511 (56.7)**	**389(43.23)**	**900(100)**

Table 2. Distribution of confirmed TB Cases based on diagnostic procedures

Type of tuberculosis (TB)	Confirmation on diagnostic procedures	Cases of TB		Total
		New No. (%)	Relapse No. (%)	
Pulmonary	Clinically	366 (40.7)	21 (2.4)	387(43)
	Bacteriologically	371(41.2)	59(6.5)	430(47.8)
Extrapulmonary	Clinically	54(6)	5(0.5)	59(6.6)
	Bacteriological	24(2.7)	-	24(2.7)
Total		**815(90.5)**	**85(9.4)**	**900(100)**

Among the total 900 TB cases, 430 (47.78%) were bacteriologically confirmed with 371 (41.12%) newly diagnosed cases, and 59(6.5%) relapse TB infection. Clinically diagnosed individuals were 387(43%) where 366 (40.67%) were newly diagnosed for TB and 21 (2.34%) patients had a relapse infection as in table 2. A total of 24 (2.67%) bacteriologically diagnosed cases were confirmed for MTB and 59 (6.5%) cases were diagnosed with extrapulmonary on clinical ground.

It has been established from the results that 18 (2%) out of 900 MTB patients had an infection of Rifampicin-resistant MTB strains. Thirteen (72%) out of these 18 patients were males and 5(27.78%) were females with the highest percentage (28%) of 5 patients from 15-24 and 25-34 years of age. The highest frequency 2 (11%) of RR-MTB was observed in 25-34 years of females and males were frequent 4 (22%) in age group (15-24 years) for resistance MTB infection with a non-significant relationship of TB frequency and gender (table 3).

Table 3: Age and gender-wise distribution of Rifampicin resistance in TB

Age (Years)	Gender		Total No. (%)
	Male No. (%)	Female No. (%)	
5-14	-	1(5.6)	1(5.6)
15-24	4(22.3)	1(5.6)	5(27.8)
25-34	3(16.7)	2(11.2)	5(27.8)
35-44	-	-	-
45-54	2(11.2)	-	2(11.2)
55-64	1(5.6)	1(5.6)	2(11.2)
>65	3(16.7)	-	3(16.7)
Total	**13(72.3)**	**5(27.8)**	**18(100)**

DISCUSSION

About 10.2 million cases of new and relapse TB were recorded worldwide in 2015, which reduced to 9.02 million in 2016. A drop in TB cases has been noticed in developed countries because health organizations are being equipped with modern tools to detect TB in the early stage with great accuracy, treating the disease in a juvenile state, and regular follow-ups of patients are being implemented (19, 20). The rate of new TB cases is very low among developed countries. In the United States, the incidence rate of TB is 0.002% as compared to the current study from different areas of Punjab, Pakistan in which 6110 suspected individuals were enrolled and the Frequency recorded was 14% (21). A substantial number of TB cases were recorded in developing countries. A study from 2006-2012 in India reported 0.17%-0.52% of TB burden (22). A high Frequency of TB in Madhya

Pradesh, India was reported in 2010 of 0.423% (23). Not all areas of India are overwhelmed by TB there is a low Frequency recorded in Faridabad district Haryana and a north Indian district of 0.101% and 0.025% respectively in contrast to India's national estimate. In Haryana specifically, the low Frequency is because of the successful execution of TB control measures in the area as stated by Ashutosh, N.A. et al (24, 25). Surveillance data from Dhaka's urban slum, Bangladesh reports the incident rate of TB, 0.253%, which is quite high as compared to developed countries (26).

WHO, in 2000 implemented directed observed treatment and short courses (DOTS), in Pakistan. Adopting DOTS remarkably increased the productivity of health care centers in Pakistan as a result, 85% of positive patients were cured successfully (27). The National TB control program in 2011 attained a TB detection rate of 64% and established an incident rate of 0.23%, but it seems that there is a lag in the record of an unknown number of patients regarding private sectors (28). Pakistan is also overwhelmed by TB, the incident figures for bacteriologically and smear-positive TB is 0.398%, and 0.27%. Another finding of the national TB survey was that 61% of TB patients were positive on sputum smear, as compared to the current study in which 43.89% of smears were positive of total TB patients. The mortality rate was recorded at 0.023% in Pakistan (16).

The incidence rate of TB is higher among males than females globally, male is 1.8 times more probable to TB infection than females (29). In the present study, the incidence rate of TB was more among males than females, 57% and 43% respectively which are relatable to a global report. Similar results from Balochistan, Pakistan are observed among males 57% and females 43%. (30). An unusual spectrum of results is observed in Lower Dir Valley, KPK, Pakistan, in which the frequency of females was slightly higher than males0, 49.5%, and 50.4% respectively (31).

Extrapulmonary tuberculosis (EPTB) represents a minority of TB cases. Global TB report states that EPTB makes up 15% of the total TB cases (32). A study from East Sudan informs the incidence rate of 22% of EPTB, as compared to the current study which reports 9.2% (33). The reason for this high Frequency lies in the ignorance of people to the consequences of the diseases, a study conducted in Lahore, Pakistan, reports that 2/3 of patients fail to follow treatment after they are diagnosed with TB (34). These untreated patients then become the culprits who spread infection, a single person infected with TB can infect 10-15 persons annually (35). Failure to receive treatment is not the only case, medical practitioners lack sufficient knowledge about the diagnosis of the disease (36). Furthermore, the lab personals fail to follow SOPs regarding AFB smear microscopy, she argues that there is a crucial need to edify the health worker's circle (37). The temperature has a relation to TB, as the number of cases diagnosed peaks in summer. A study conducted in Lahore, Pakistan reported that the infection transmission was higher in winter, which lead to a higher number of patients diagnosed in summer (38). Two identified factors escalate the infection transmission in winter these are Vitamin D deficiency and overcrowded rooms (39). These factors are relatable to District Chiniot's environment as some areas are overcrowded.

The frequency of MDR-TB is increasing, according to WHO only half the patients diagnosed with MDR-TB are successfully treated (40). A study carried out in Congo from 2012-2017 has reported 11% RR-TB patients out of 1535 TB patients, in comparison to the current study in which the incident rate was 2% (41). A study reported in the light of their national survey 2012-2014, the Frequency of MDR-TB was 2.1% and the Frequency of RR-TB was 4.6% (42). The results of their study were higher than previously recorded data regarding RR-TB, which was 2.8% in 2001-2002, they extrapolated that the Frequency of RR-TB has doubled among new cases of TB (43). In India, the highest incident rate of MDR-TB is reported in the western states, which is 39.9% (44). Drug resistance in Pakistan is an emerging problem, A study conducted in Punjab, enlighten that 11.5% of isolates from TB patients were resistant to at least one drug, used to treat TB and 9.3% of isolates were multidrug-resistant (45). The present study provides evidence for the high incidence rate of TB and a substantial number of RR-TB in District Chiniot, Pakistan. While developed countries are on the verge to eradicate TB from their states, Pakistan is still overwhelmed by TB even though a lot of work is being done with the assistance of international funding bodies to eradicate TB in South Asia. However, a poorly executed program can lead to development of drug resistant strains. There is intense need to improve the quality of health by health departments in order to eradicate the TB and to get rid of resistance that is emerging in TB strains.

CONCLUSION

The present study provides evidence for the high incidence rate of TB and a substantial number of RR-TB in males to females from district Chiniot Pakistan. While developed countries are on the verge to eradicate TB from their states, Pakistan is still overwhelmed by TB even though a lot of work is being done, with the assistance of international funding. However, a poorly executed program can lead to the development of drug-resistant strains. We need to improve the quality of the health care system if we are to win this war against TB.

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ORIGINAL RESEARCH

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Socio-demographic Patterns, Perceptions, Prevalence and Communicability of Scabies in Islamabad, Pakistan

Tallat Anwar Faridi[1*], Arif Munir [2], Sohaib Ali Hassan[3], Ishrat Perveen[4], Muhammad Saleem Rana[1]

[1]Institue of Public Health, Department of Allied Health Sciences, University of Lahore, Pakistan.
[2] Pakistan medical research council, Islamabad, Pakistan.
[3] Malaria Directorate, Rawalpindi.
[4] Department of Zoology, University of the Punjab, Lahore, Pakistan.

***Corresponding author**
Tallat Anwar Faridi
Address: Institute of Public Health, Department of Allied Health Sciences, University of Lahore, 54590, Pakistan.
Email Address: tallat.anwar@ pht.uol.edu.pk

ABSTRACT

Even though there is a high prevalence of scabies in various impoverished communities, no profound research work has been done so far on its contagiousness, communicability, and risk assessments in Islamabad, Pakistan. Scabies has been enlisted by the World Health Organization as the most neglected contagious tropical disease and its high prevalence in various underprivileged, impoverished and resource-poor communities, no profound research work has been done so far on its contagiousness, communicability, and risk assessments in Islamabad, Pakistan. The present study was aimed to explore the community perceptions, socio-demographic features, healthcare-seeking attitudes, and prevention practices that were contributing to the prevalence and communicability of scabies in a resource-deficient urban community of Islamabad, Pakistan. Data was collected through an orally-administered questionnaire through random sampling. The community responses were entered and analyzed in statistical package SPSS software version 21. The results have shown a high prevalence of scabies (57%) in the target slum community of Islamabad, Pakistan. More than half of the population were found living in congested household settings (61%), deficient in formal /informal education (57%), and were hardly making both ends to meet (59%) with their nominal monthly income (< 6000 Pakistani rupees). It is hence concluded that a low level of community awareness and perception coupled with the socio-demographic features were found to be associated with the high prevalence and communicability of scabies.

Keywords: Scabies,Mite, Vector, Slum, urban

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INTRODUCTION

Scabies is a frequently neglected tropical disease caused by *Sarcoptes scabiei var. hominis* – a microscopic parasitic mite (1). A huge global proportion is infested by this contagious infestation, affecting both genders of all socioeconomic status and races equally (2). Scabietic patients suffer from scratching, intense pruritus, and worse itching due to nocturnal activities of mites which leads to restlessness and insomnia (3, 4). The Incubation period of parasitic mites is variable, itching is noticed within two weeks of exposure (5). Direct and indirect skin contact is the most effective way of transmission such as prolonged exposure (15-20 minutes), hand-holding, and/or sexual contact with an affected individual (6, 7).

Recognized epidemiological risk factors include prolonged exposure with an infected person and immuno-deficiencies developed because of hyperkeratosis (9). A wider range of epidemiological risk factors influence the distribution of scabies in community, including gender, age, ethnicity, unhygienic and overcrowding situations which have been found primarily or secondarily linked with the indicators of poverty and deplorable economic conditions at the community and/or infra-community level (10-15). According to a global prospective study, it is assessed that 300 million scabies cases affect 6% to 27% population per annum, most of which inhabited in resource-deficient areas of the world (16). This significantly high prevalence ratio makes it a significant public health problem in developing world communities, most particularly in overcrowded tropical areas (17-24).

Almost everyday scabies is diagnosed in dermatology clinics of Pakistan (25). An escalating population burden coupled with the surge in urbanization has tremendously enhanced the health-related concerns of the Pakistani community. Data collected from the district health information system of Pakistan reveals that scabies is prevalent in all provinces. It has become a social stigma and spreads quickly among populations living in resource-deficient and congested settings. As the disease is contagious, community education in addition to prevention and control has attained prime importance. The Resurgence of disease holds the potential to cause social and economic losses at the cost of mankind's health, thus, doubling the burden of disease and resulting in a poverty-ridden unhealthy society. Despite ample literature available on the epidemiology of this contagious infestation in impoverished and developing countries relatively infrequent investigations have been made on the fore-mentioned epidemiological risk factors in Islamabad, Pakistan (3, 15, 26). The present study was conducted in slum areas of Islamabad to find out the Knowledge, Attitudes, and Practices (KAP) of communities toward scabies so that a proper community education strategy could be designed for prevention and control against scabies.

MATERIAL AND METHODS

We conducted a cross-sectional qualitative study in an urban slum area of Pakistan's capital-Islamabad. It comprised a total of one thousand households colonized by almost 5000 inhabitants. The climate varied with respective averages of 267mm-309mm for the months of July and August, per annum average rainfall of 1143mm, and relative humidity level up to 55%. The coldest month was January and the hottest month was June with the mean temperatures of 17ºC (max) ± 2.6ºC and 40ºC (max) ± 24ºC, respectively. People were dwelling in generally poor, deprived, and miserably deplorable conditions. Overcrowding was the key feature. A Majority of the participants belonged to the Malakand Agency, Mardan and Khyber Pakhtunkhwa (KPK) characterized by their distinct traditional social beliefs and ancient cultural lifestyles. A Major chunk of the participants were daily wagers and laborers. No proper health care facility except a small clinic equipped with few emergency medicines and unprofessional medical staff was found in the studied area.

We administered a semi-structured oral interview supplemented with a questionnaire-based survey of 397 participants (aged between 18-75 years) inhabiting the impoverished areas of Islamabad. A questionnaire was piloted based on the domains comprising the household information, demographic figures, knowledge, perceptions, and attitudes of participants toward treatment-seeking and prevention practices. All members of the households present at the time of the survey were interviewed. Data were collected and analyzed by using SPSS version-21. The proposal was presented to the Institutional Review Board (IRB) of Health Services Academy for ethical approval. For the sake of confidentiality of data, the responses of respondents were disguised as codes.

RESULTS

Prevalence of Scabies

During the survey, more than fifty percent (57%) of respondents had scabies at the time of the survey. However, almost one-quarter of participants reported the prevalence of disease in at least one of their house members since the last three months of the survey.

Socio-Demographic characteristics

Gender distribution of participants indicates that 53.4% of respondents were males and 46.6% were female. Overall 97% of respondents were married. Fifty-nine percent of the studied population had not received any type of formal education, whereas, a respective 33.2% and 7.6% of respondents had received informal and below the secondary level of education. However, only 3% were reported to receive an intermediate level of education. We found that a meager percentage (1.5%) of respondents had a monthly income ranging from 10,000 to 12,000 Pakistani rupees. However, a respective and

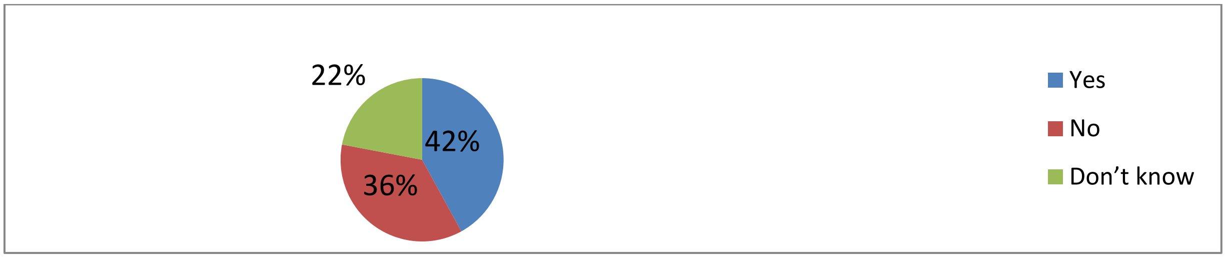

Fig. 1. Participant's Responses on the communicability of Scabies

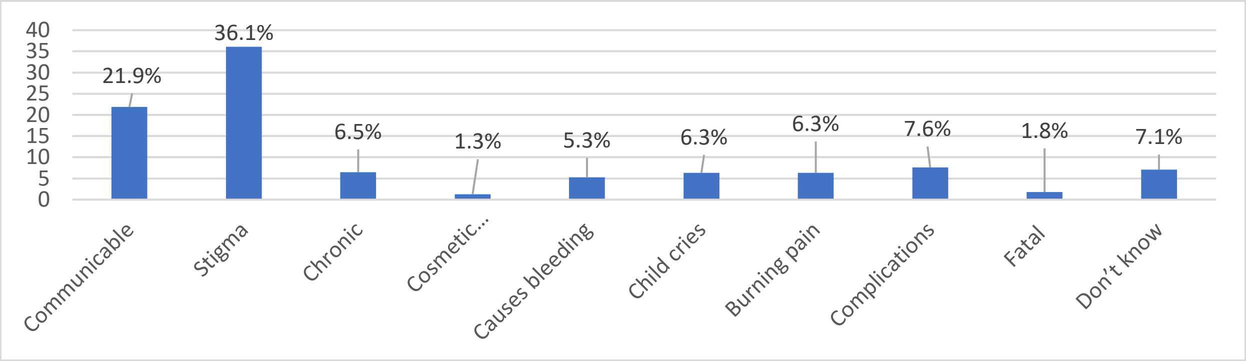

Fig. 2. Basic understanding of complications associated with scabies prevailing amongst the target population

an approximate percentage of 56.9% and 19.4% respondents were found spending their lives at the nominal monthly income of 8000 and <6000 Pakistani rupees. Some 11% of participants indicated no actual and fixed monthly income.

Household Characteristics

The response rate of all households was almost a hundred percent (99%). Information on household characteristics such as type of house, number of rooms per house, electricity, and fuel source was collected. Solar source of electricity was used by three fourth of respondents. Other sources of fuel were wood, gobar, and coal. Sources of water include a hand pump and tube-well. The majority of households were found to contain a separate kitchen. All households used Pit latrine. Almost 70% of households had kept the pet animals inside their houses. Nearly half of respondents (45.6%) reported two to four rooms per house. Almost 41% of the houses comprised 1-2 rooms followed by 11.8% of households having 4 to 6 rooms. The total number of rooms in a household setting is a good forecaster in the transmission of skin ailments. A respective approximate percentage of 48.9% and 12.1% respondents were found sharing a congested setting of 3-6 and 6-9 persons per room in their household settings. Almost 34.5% of household percentage, however, was found in comparatively less-shared settings of 1-3 persons per room (Table 1).

Awareness and perceptions

More than fifty percent of respondents (54%) have shown knowledge about symptomatic conditions like itching in scabies. Information regarding scabies was collected mainly from family members and neighbors (65.5%), followed by the health department (14.6%), newspapers or magazines (13.4%), and TV/Radio (4.2%). Respondents reported sharing the same bed facility (32%) followed by insects (24%), clothes (18%), and close contact (12%) as the main source of spreading the infection. Itching at night was reported as one major symptom of scabies (32%), followed by itchy rashes (8%) and redness (23%). Some 42% of respondents were found aware of the communicability of scabies, whereas 22% were found ignorant of the facts attributed to this infection (Fig. 1, Table 3). This skin infestation was considered a precarious disease because of its association with social-stigma (36.1%), communicability (21.9%), the possibility of complications (7.6%), and cosmetic reasons (1.3%) as shown in Fig. 2 and Table 3. It was found that approximately one-third of respondents (31%) had awareness about the vector of scabies–mite. Human skin was considered as a vector habitat by 27% of interviewed participants, followed by human clothing (19.9%) and animal skin (13.4%).

Control Practices

To prevent scabies, respondents reported maintaining personal hygiene (44.6%) and/or considered it as a neutral practice (25.9%). Others responded in favor of washing hands (54.4%), segregating the patients (14.9%), preventing overcrowding (27%), skin-to-skin contact (32.8%), and by limiting the share of cloths (30%), beds (21.7%) and towel/soap (21.7%) to prevent masses from the exposure to this contagious skin infection (Table 2 and 4). The frequency of taking a bath varied among the respondents from twice a day (11.3%), once a day (39.8%), once a week (37.8%) to once a month (11.8%) (Table 3).

Practices of Respondents

Table 1. Socio-demographic characteristics of the target population

Questions	Status Options	Frequency	Percentage
Gender	Male	212	53.4
	Female	185	46.6
Marital status	Married	386	97.2
	Single	11	2.8
Educational status	Education	234	59
	Informal	132	33.2
	Below intermediate	30	7.6
Income	<6000	226	56.9
	8000	77	19.4
	8001-10000	44	11.1
	10001-12000	6	1.5
	DK*	44	11.1
Number of rooms in the house	1-2	161	41
	2-4	181	45.6
	4-6	47	11.8
	> 6	8	2.0
Number of people living in one room	1-3	137	34.5
	3-6	194	48.9
	6-9	48	12.1
	> 9	18	4.5
Duration of Residence	<6	13	3.3
	Up to 6	26	6.5
	>1	8	2.0
	1-2 >2	95 255	23.9 64.2

*DK = Don't Know

More than half of respondents (52.6%) considered scabies as a treatable disease, 10.1% reported visiting Hakims and /or Homeopaths, followed by 37% who visited doctors for treatment. Nearly half of respondents knew nothing about the treatment of scabies (46.1%), others reported it can be cured by the use of tablets (18.9%), injections (5.5%), and Cream/Lotion (22.4%). To the utmost of the author's surprise, half of the respondents had no idea about the available health facility in their vicinity (54%), whereas, others reported visiting tertiary care hospitals (30%), THQ (30%), and DHQ (8%) (Table 4).

Table 2. Awareness /knowledge regarding Scabies

Attitude of respondents	Options	Frequency	Percentage
Do you maintain cleanliness	Yes	397	100.0
	No	-	
	Don't know	-	
In your opinion is personal hygiene important to prevent Scabies	Yes	177	44.6
	No	117	29.5
	Don't know	103	25.9
How we can prevent the spread of scabies	By isolating the patient	59	14.9
	Personal hygiene	74	18.6
	Environmental Hygiene	124	31.2
	Don't know	81	50
Can washing of hands prevent scabies	Yes	216	54.4
	No	82	20.7
	Don't know	99	24.9
How often you take a bath	Twice a day	45	11.3
	Once a day	155	39.8
	Once week	159	37.8
	One in month	47	11.8
How should scabies patient's clothes be washed	With cold water	46	11.6
	With Hot water	110	27.7
	With soap	121	30.5
	Don't know	109	30.0
What preventive measure you adopt to avoid scabies	Separation of bed	86	21.7
	Separate towel /soap	86	21.7
	Avoid skin to skin contact	130	32.8
	Don't know	94	23.9

Table 3. Respondent's Attitude toward Scabies

Awareness/knowledge about Scabies	**Options**	**Frequency**	**%-age**
Awareness about Scabies or itching at night	Yes	215	54.2
	No	165	41.6
	Don't know	17	4.3
If yes, then the source of information	Health department	58	14.6
	Electronic media	18	4.5
	Print media	8	2.0
	Newspapers/Magazines	260	13.4
	Self/ Neighbors	53	65.5
How do we get infected with scabies	Same bed	82	20.7
	Insects	42	10.6
	Clothes	35	8.8
	Close contact	23	5.8
	Any other	159	56
What are the symptoms of scabies	Intense itch at night	125	31.5
	Pimple-like itchy rash	30	7.6
	Redness	93	23.4
	Any other	87	38
Can Scabies spread from one person to another	Yes	167	42
	No	141	36
	Don't know	89	22
Is scabies dangerous	Yes	220	55.4
	No	177	44.6
If "Yes" why is it dangerous	Communicable	87	21.9
	Stigma	143	36.11
	Chronic	26	6.5
	Cosmetic reasons	5	1.3
	Causes bleeding	21	5.3
	Child cries/cannot sleep	25	6.3
	Burning pain	25	6.3
	Complications	30	7.6
	Can be fatal	7	1.8
	Don't know	1	7.1
Awareness /knowledge about Scabies Vector			
Awareness about scabies mite	Yes	124	31.2
	No	217	54.7
	Don't know	56	14.1
Where does scabies mite live	In human skin	107	27.0
	In human clothes	79	19.9
	In animals skin	53	13.4
	Don't know	39	40

Table 4. Prevention Practices Prevailing among Respondents

Practices of respondents	**Options**	**Frequency**	**%-age**
What are the personal protective measures against scabies	Avoid overcrowding	107	**27.0**
	Avoid sharing clothes	119	30.0
	Any other	118	29.7
	Don't know	52	13.3
Do you think that unhygienic condition is one of the causes of scabies	Yes	250	63.0
	No	147	37.0
Do you think scabies is related to overcrowding at home	Yes	107	27.0
	No	121	31
	Other	37	9
	Don't know	132	42
Do you think that environment is also responsible for the spread of scabies	Yes	127	32
	No	84	21
	Don't know	96	47
Is scabies treatable	Yes	209	52.6
	No	64	16.1
	Don't know	124	31.2
Generally, where would you prefer to go for the treatment of scabies	Doctor	147	37.0
	Hakeem /Homeopaths	40	10.1
	Other	52	13.1
	Don't know	158	39.8
Do you have an idea how scabies is treated?	Tablets	75	18.9
	Injection	22	5.5
	Cream/Lotion	89	22.4
	Any other	183	46.1
	Don't know	4	8
Nearest medical facility for treatment	TCH	120	30.2
	THQ	31	8
	DHQ	33	9
	Other	213	54

DISCUSSION

The high prevalence of scabies (57%) in the impoverished community of Islamabad confirms an endemic status of the disease in Pakistan. It has been found consistent with the finding of a high prevalence of scabies ranging from 18.1-70.2% (35-37). However, the Eastern neighbor of Pakistan, Bangladesh, was reportedly inhabiting a high incidence (98%) of scabies in her madrassahs (38).

The current study was designed to investigate the predominance, beliefs, and understandings of communities based in an urban slum area, which was motivated by the assumption that overcrowding, unhygienic conditions, and poor socioeconomic situations may serve as the breeding ground for the emergence and communicability of this mite-originated widely neglected tropical skin infection. To the author's best knowledge, no reliable data on this contagious skin ailment for this particular slum area has been found at all. Hence, the present study can better be regarded as representative of the other deplorable and resource-deficient communities of Pakistan. As far as the demographic factors are concerned, poor housing, low income, frequent illiteracy, poor hygiene, social and behavioral attitudes or practices facilitate the transmission of a parasitic mite (15, 27-29). The target population was inhabiting in the dismal (nominal monthly earning < 6000 PKR), unhygienic, and congested living conditions which were reported as major contributing factors for the prevalence of disease (15-18). A study conducted in the Internally Displaced Persons (IDPs) camp of Muzaffarabad (Pakistan), agrees well with our findings, that poor hygiene coupled with deplorable social and economic standards were the root cause of scabies (32).

Crowded conditions investigated in this study have been found justifying the high frequency of scabies in this target urban slum community which is in line with the outcomes of the predecessor's studies conducted in Malaysia (8), the UK (39), Brazil (5) and Pakistan (40). Poor and congested living standards in tropical areas have been frequently documented in the literature as the main contributing factors for the proliferation of this contagious skin infestation (17-19). Recent literature has also remarkably confirmed this complex association between unhygienic living conditions, risk behavior, overcrowding, and accelerating ratio of infested persons (11, 28, 37-38). It can best be demonstrated by the fact that high-fold diagnosis of scabies was found in the individuals who lived in the target communities for above six months than those who arrived recently (28).

A considerable level of community awareness was observed for the occurrence and transmission of this contagious skin infestation. Almost half of the participants (42%) were found aware of the transmissible characteristics of the disease, contrary to the rest of respondents who were found ignorant of this widely proven fact, which is consistent with the findings of some predecessors (30). As far as awareness of the disease communicability is concerned, fairly concordant responses of other researchers considering lack of environmental and personal hygiene responsible for the spread of scabies were also reported (33-34).

Community practices like admittedly sharing of household accessories (towels, beds, and clothes) and prolonged physical contact were found as more frequent and persistent means of transmitting the mite infestation from person to person which is consistent with the findings of some other surveys (13, 40). Hence, lack of basic knowledge about personal and environmental hygiene practices has been regarded as a major risk factor, which is consistent with the studies that recognized community unawareness as a major contributing factor, in the high prevalence and communicability of scabies (31).

CONCLUSION

The current study found a high prevalence of scabies in the target population. Most of our findings *i.e.* low socio-economic status, scarce personal and environmental hygiene, overcrowding, personal direct and/or indirect contact with the infected persons, sharing of household accessories, etc. were found as the key contributing factors in the prevalence and communicability of this tropical contagious infestation. It is hereby concluded that the level of community awareness about the prevalence, transmission, and treatment of scabies was fundamentally low which was further compounded by the persisting socio-demographic features (*i.e.* congestion, low income, and lack of hygiene) and thus contributed to the high prevalence of scabies in the impoverished community of Islamabad. This indicates the need for planning comprehensive community education and active intervention strategies to improve public awareness for the prevention and control of scabies.

CONFLICT OF INTEREST

All authors declare **no conflict of interest** in the work.

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ORIGINAL RESEARCH

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Computational and Functional Analysis of Hypodermin C Protein against GWFI

Fatima Tahir[1], Almeera Shoukat[1], Abubakar Ahmed[1], Amna Saleem[1], Hinza Asghar[1], Aqeel Ahmad*[1, 2]

[1*]Department of Biotechnology, Faculty of Life Sciences (FLS), University of Central Punjab, 5400 Lahore Pakistan.
[2] Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan

*CORRESPONDING AUTHOR
Aqeel Ahmad
Address: Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan
aachaudhary142@gmail.com

ABSTRACT

GWFI (Goat Warble Fly Infestation) is the myiasis caused by the larvae dipterous fly *Przhevalskiana silenus* in goats. This fly colonizes under the skin of an animal and increases its size at each larval stage. The larval gut produces the peptide degrading protein called hypodermin C (HyC). This study suggests that how collagen and HyC interact with each other. FASTA sequences of protein were retrieved from NCBI and the aligned sequences showed the highest similarity with serine proteases. The structure of HyC was modeled using I-TASSER online server followed by its validations using Procheck verification tool and physicochemical properties were confirmed by AA-prop. The protein structure of HyC was docked against Collagen by using their PDB IDs, 1HYL and 1BKV respectively. The structure of HyC showed a more determined hydrophobic nature and both structures were also superimposed to find out similarities and differences between them. Glutamic acid, Aspartic acid, Serine, and Lysine are found as interacting residues that are involved in docking with collagen. Generated structure of HyC that was docked against the collagen protein residues as the HyC produced from the gut of the fly binds with the collagen of the animal body and degrades the collagen, the residues which taken part in the binding process could be blocked which ultimately inhibits the binding of the hypodermin C and collagen thus these residues to control the infection. This present study helps in vaccine development against hypodermis, through inhibition of the binding of the collagen with hypodermin C is the strategy towards vaccine production.

Keywords: Hypodermin C, Myiasis, *Przhevalskiana Silenus, Molecular Docking,* Physiochemical

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INTRODUCTION

Myiasis originates from the larvae of hypodermatinae subfamily (Diptera, Oestridae) and the disease is called warble fly infestation (WFI). The members of the Diptera family are specific for their host. *Hypoderma lineatum* and *Hypoderma bovis* are host-specific for cattle and buffalo. *Hypoderma diana* causes disease in roe deer. *Hypoderma actaeon* causes disease in red deer. *Hypoderma tarandi* is host-specific for reindeer. *Hypoderma sinense* causes disease in yaks and *Przhevalskiana silenus* is specific for goats in which it causes the disease called GWFI (goat warble fly infestation) (1). *Pzhevalskiana silenus* fly colonize under the skin, legs, and back of the goat. The fly causes massive economic loss by reducing body weight and growth, changes the quality of hides for tanning purposes, lowers milk and meat production, and causes hypersensitivity reactions in goats (2).

At different larval stages, larvae change their size and color. In the first larval stage L1, the size of the larva is 2-7mm, white which inhabits subcutaneous tissues. At the second larval stage L2, the colors remain the same, but size increases to 8-12mm. At the third larval stage L3, larvae move to the superficial dermis, and color changes from brown to black with an increased size of 13-19mm. The incubation period completes in 3-8 days (3). The parasites produce a large range of serine proteases enzyme that degrades the host body tissue in myiasis (4). HyC is found in the species of hypoderma and many other parasites include Pzhevalskiana silenus. The disease originates by the penetration of larva in the skin of an animal by the activity of collagenase enzymes mainly HyC (5). The hypodermis is caused by the hypodermin C during the incubation period of the larvae. Hypodermin C degrades the subcutaneous tissue of the animal resulting in low performance of the animal by *P.silenus* affects body weight and the immune system of the animal and evokes hypersensitivity reactions in the body (6).

Docking is a computational approach that is used to study the properties and interactions We also studied how Hypodermins bind with collagen which may help devise strategies to develop a vaccine against hypodermosis (7). This study is to model first-time HyC and the generated structure that will be dock against the collagen protein. Molecular docking exhibits the new strategy to make the vaccine for the elimination of disease to overcome the economic loss. As the HyC produces from the gut of the fly binds with the collagen of the animal body and degrades the collagen, the residues which take part in the binding process could be blocked which ultimately inhibits the binding of the HyC and collagen. The inhibition of the binding of the collagen with HyC is the strategy towards the production of the vaccine for the disease.

MATERIALS AND METHODS

The disease-causing protein sequence of the HyC was retrieved from the NCBI (National Centre for Biotechnology Information). The FASTA format of the protein sequence was aligned for the knowledge of sequence similarity with the other proteins using the database BLAST (Basic Local Alignment Search Tool). BLAST showed the highest similarity percentage of HyC with the *Hypoderma lineatum* and serine proteases.

Protein Modeling and Verification:

The protein structure was modeled by I-Tasser and SWISS modeler to obtain the three-dimensional structure. The best three-dimensional structure was selected on the base of the highest C-Score. Hypodermin C and collagenase structure were verified by using Procheck verification tool. For the verification purpose, PDB ID of the collagenase and hypodermin C was retrieved from the RSCB (Protein Database). The PDB ID used was 1BKV and 1HYL for the collagen and hypodermin collagenase respectively.

Physio-chemical properties

The physicochemical properties of a protein are analyzed using the protein physicochemical properties prediction tool AA-Prop. The FASTA sequence of hypodermin C was retrieved from the NCBI. The amino acid sequence was pasted on the protein sequence bar. The results gave the number of total amino acids, molecular weight, atomic composition, kyte, and Doolittle hydrophobicity plot.

Stereochemical Analysis

Stereochemical analysis was performed by Procheck.

Molecular Docking

The protein structure of hypodermin C was docked against the collagen protein to pursue their binding sites. Protein-Protein docking is also a type of molecular docking. The ligand interaction with protein can be modified by changing its affinity is helpful to design the drugs or new molecular probes. One compound or ligand can be interacting with the protein in many different ways. Protein-protein interactions are necessary to study cellular functions. X-ray crystallography and NMR spectroscopy help us to understand the structure of a single protein and complex protein structure.

The main objective of the docking is to understand which residues of the protein are involved in the interaction of hypodermin C and collagen. For the docking purpose, the Cluspro database was used. For the submission of the job, PDB ID of protein was taken from the RSCB (protein database). The 1HYL PDB ID was used for the hypodermin C and 1BKV PDB ID was used for the collagen. (8).

Analysis of Protein Complex Structures:

The resulting models were analyzed by using the software Dimplot and Ligplot. This software gives information on bind residues between proteins. Different chains represent the protein-protein interaction between the residues.

RESULTS

Secondary Structure Prediction:

PDB structure of salmon trypsin, 1HYL was selected to a model of HyC, with maximum query coverage 95%, residue range covered was from 0-230 and 39.39% identity. The predicted 3D structure of HyC was visualized on Pumol and Jmol. The structures of hypodermin C proteins are predominantly composed of 11% helices, 38% beta-sheet as shown in Fig. 1.

Physio-chemical Analysis:

Various physio-chemical properties of HyC are predicted using AA Prop. It calculates many of the parameters (Table 1). Hypodermin has a sequence of 230 aa. The molecular weight of the protein is 28.57 kilodaltons. It also provides several other parameters like, Aliphatic index 88.76%, Aromaticity (Y+W+F): 10.3%, Grand average of hydropathicity (GRAVY) 0.049 which indicates its more interaction with water molecules shown in Table I.

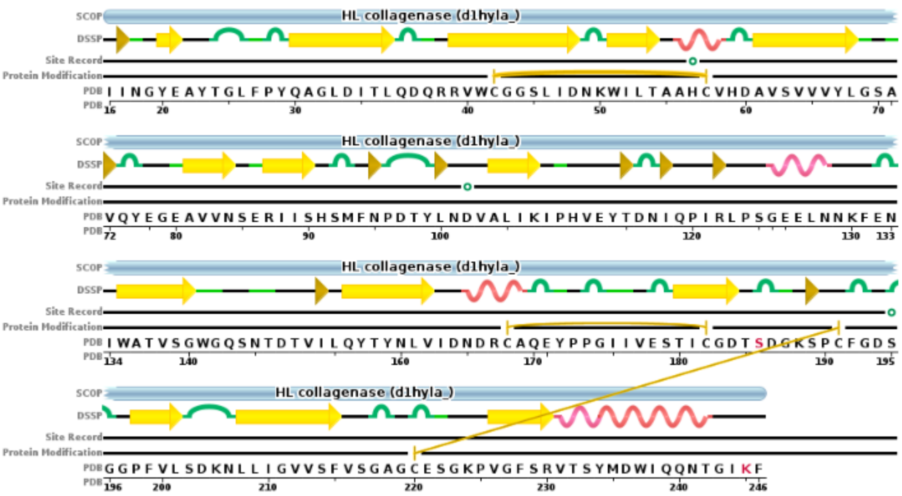


Fig. 1. Secondary structure prediction

Table 1: Composition of residues in Protein

Amino Acid composition	Percentage	Amino Acid composition	Percentage
Ala (A) 19	6.5%	Leu (L) 21	7.2%
Arg (R) 10	3.4%	Lys (K) 7	2.4%
Asn (N) 16	5.5%	Met (M) 6	2.1%
Asp (D) 17	5.8%	Phe (F) 12	4.1%
Cys (C) 7	2.4%	Pro (P) 13	4.5%
Gln (Q) 11	3.8%	Ser (S) 21	7.2%
Glu (E) 16	5.5%	Thr (T) 16	5.5%
Gly (G) 23	7.9%	Trp (W) 5	1.7%
His (H) 7	2.4%	Tyr (Y) 13	4.5%
Ile (I) 24	8.2%	Val (V) 22	7.6%
Leu (L) 21	7.2%	Pyl (O) 2	0.7%

Stereochemical Analysis

The first plot produced by PROCHECK is a Ramachandran plot based on an analysis of 118 structure of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be accepted to have over 90% in the most favored region shown in Fig. 2. The appearance of the plot itself can be modified to some extent by amending the program parameters. Thus, the shading and/or writing of different regions can be switched on or off, then region borders can be drawn in or not drawn in and the individual residues can be labeled as shown in fig.3. Total residues in protein 230 plot statics represented in most favored regions in A, B and L segments 88.5%, additional allowed regions a, b, 1, p 11.5% and residues in generous regions ~a, ~b, ~l, ~p 0.0%.

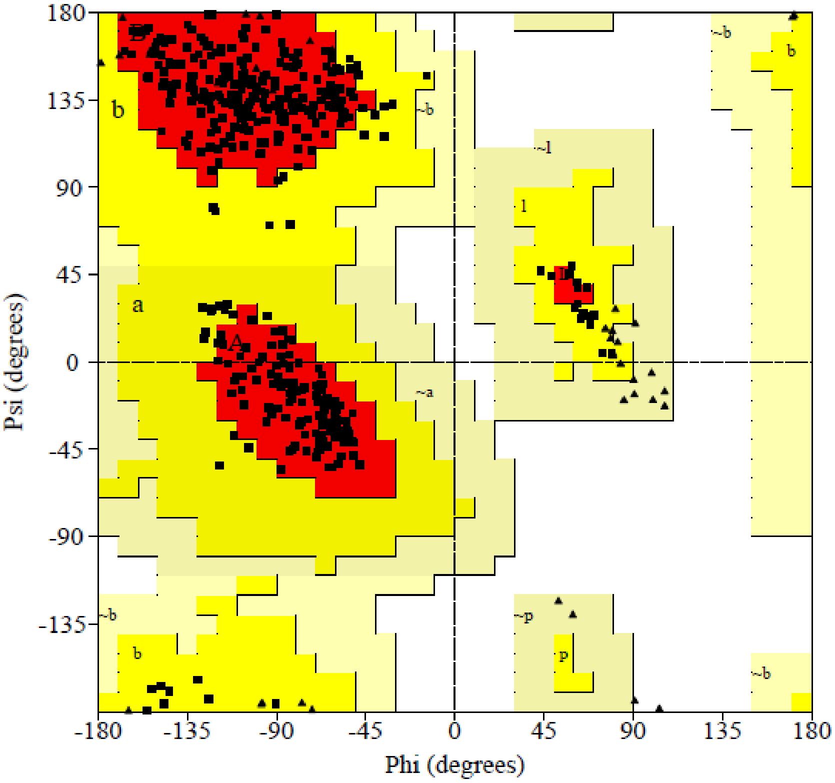


Fig. 2. Ramachandran Plot confirmation of Hypodermin C structure

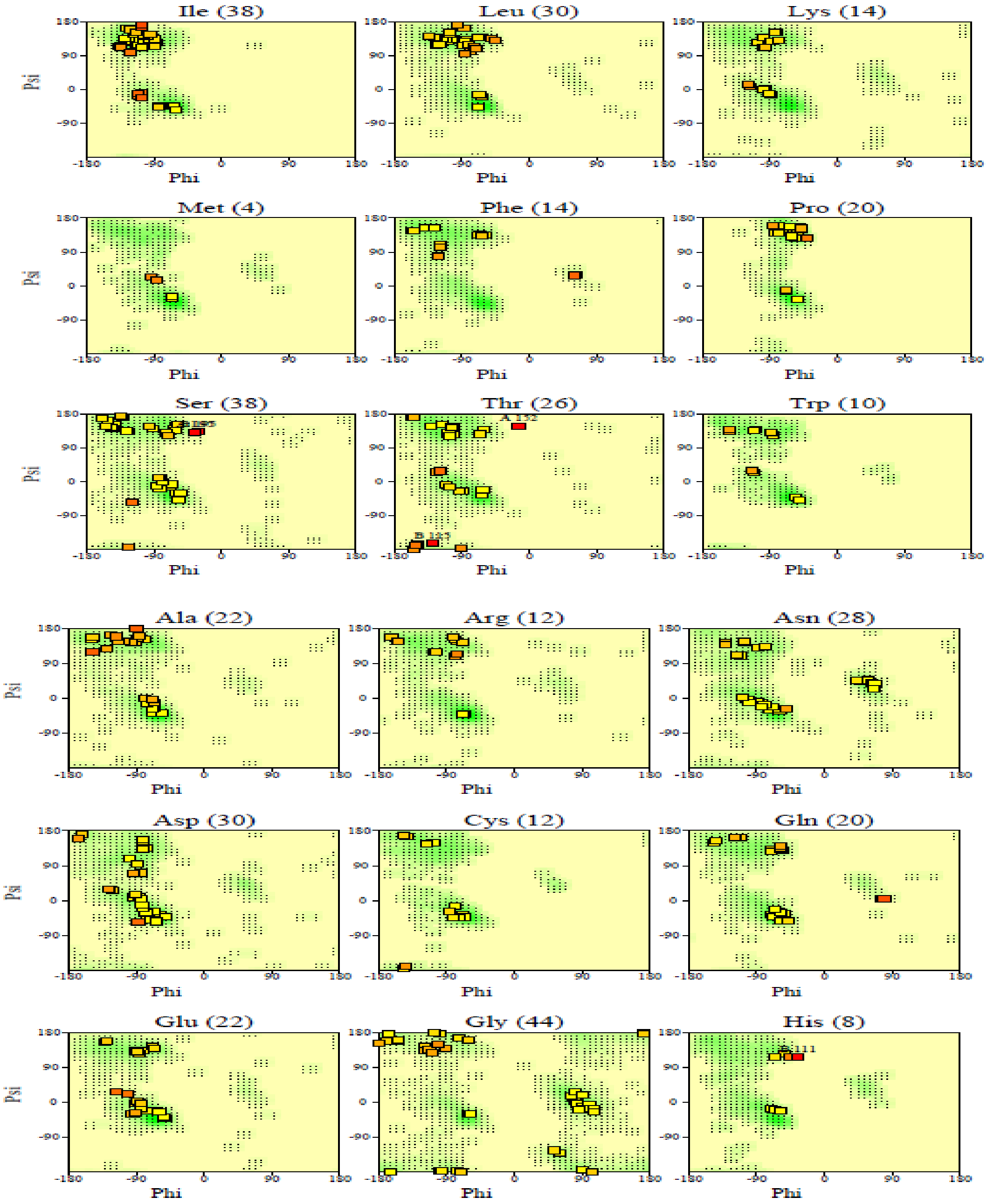


Fig. 3. Ramachandran plot for all residues.

Chi 1 and Chi 2

The numbers of residues are shown in brackets. Those in unfavorable conformations (score < -3.00) are labelled. Shading shows favorable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better shown in Fig. 4. The plots showed a graph of the Chi1 *versus* the Chi2 torsion angles for each residue, where applicable. As each Chi1 and Chi2 can be in one of three preferred configurations *(gauche minus*, *trans* and *gauche plus)*, there are 3 x 3 =9 combinations for the two angles. The nine 'ideal' positions are marked by crosses on the graph.

The graphs showing the first 111 residues in the bar a, b, and c selected from five possible that contains the three shown with absolute deviation of main chain hydrogen bond energy from the ideal value, the beta value of the lemda atom (O, C or S, whichever is used in the definition of the X l torsion angle); the average B value of main-chain atoms; and the average B value of side-chain atoms. The bottom part of the diagram illustrates information given in greater detail in the residue-by-residue listing shown in Fig. 5.

Main and Side-Chain Confirmation

The plot shows the graphs of six main-chain properties of the structure and how these properties compare with well-refined structures at a similar resolution. The dark band in each graph represents the results from the well-refined structures; the central line is a least-squares fit to the mean trend as a function of resolution, while the width of the band on either side of it corresponds to a variation of one standard deviation about the mean. In some cases, the tendency is dependent on the resolution, and in other cases is independent of it. (a) shows the Ramachandran-plot quality assessment, as measured by the percentage of the protein's residues that are in its most favored, or 'core' regions, (b) shows the peptide bond planarity measured by the standard deviation of the torsion angles; (c) shows the measure of bad non bonded interaction, (d) shows the alpha carbon tetrahedral distortion, measured by the standard deviation of the ~ 'torsion' angle, (e) showed the hydrogen bond energies and (f) showed the overall G-factor shown in Fig. 6.

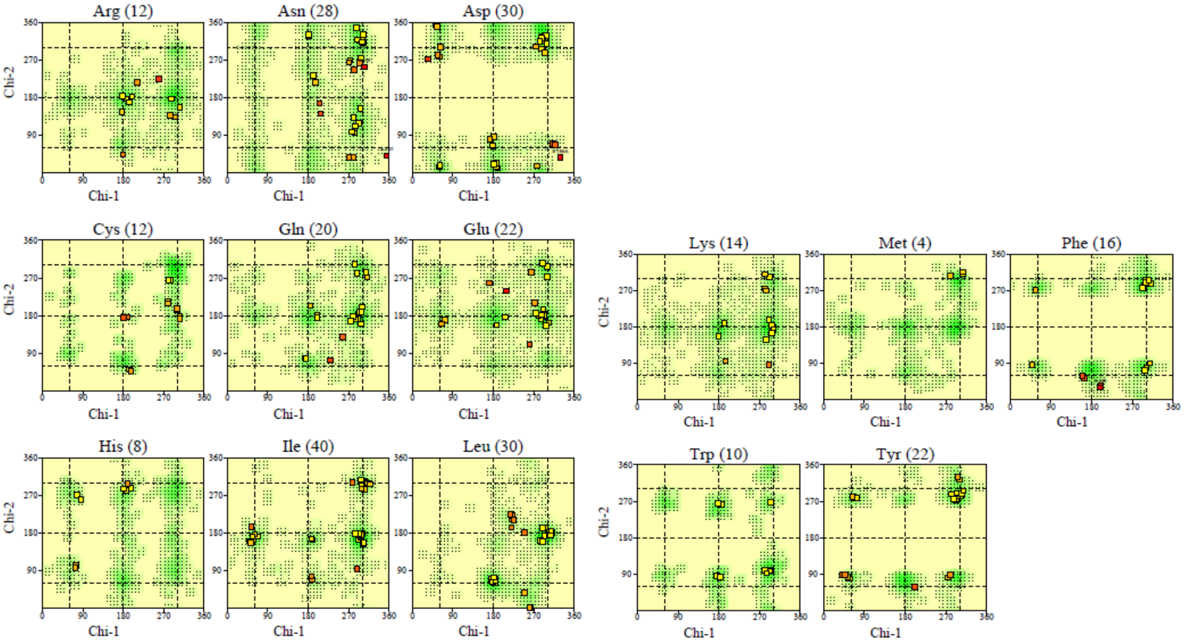
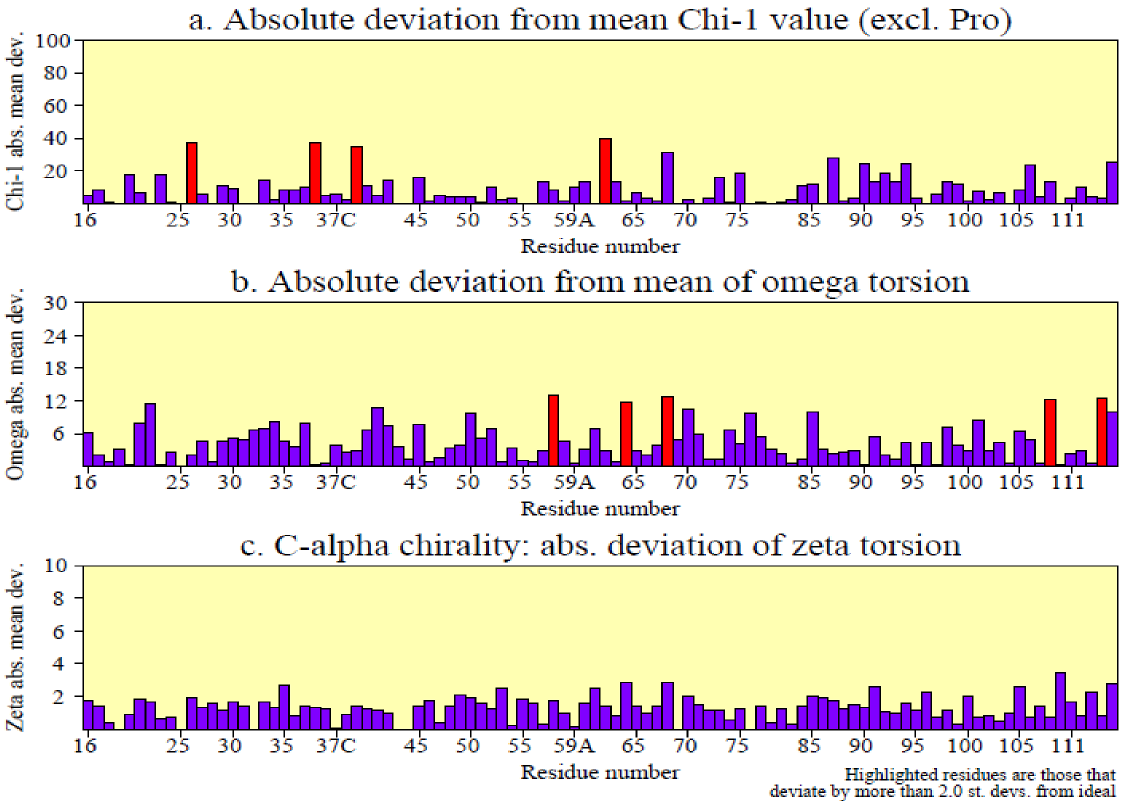


Fig. 4. Ramachandran plot for all residues.



Highlighted residues are those that deviate by more than 2.0 st. devs. from ideal

Fig. 5. Residues properties from the Mean

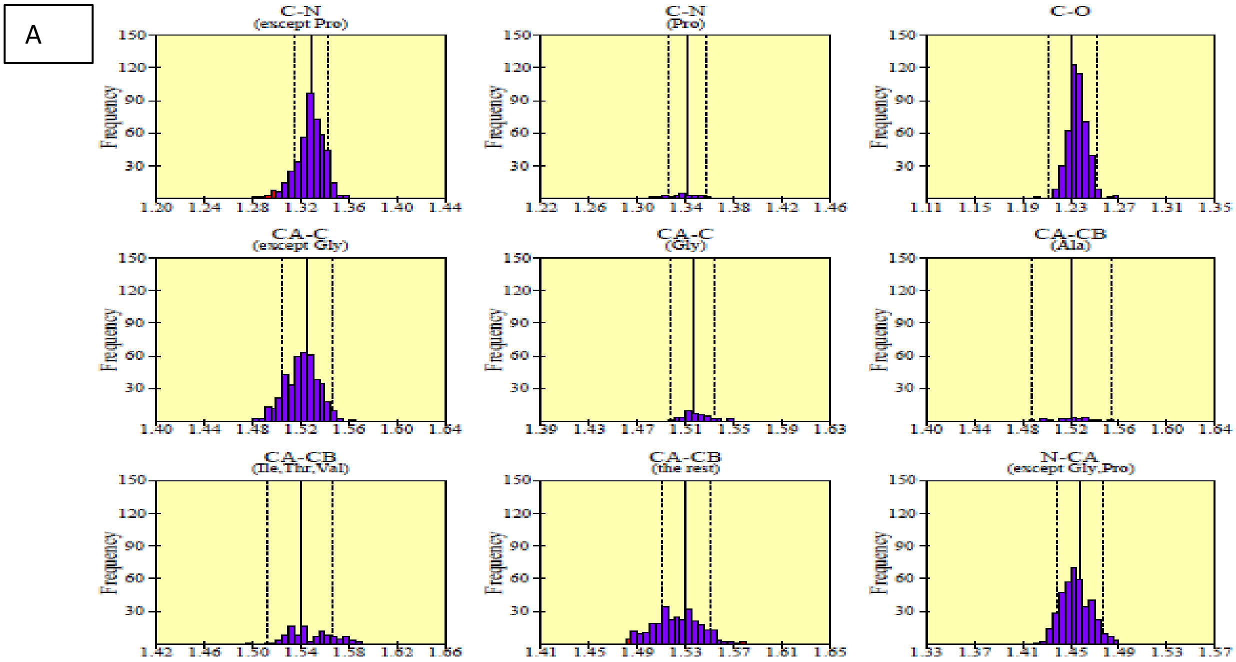


Fig. 6 (a). Bond length side chain different residues (A)

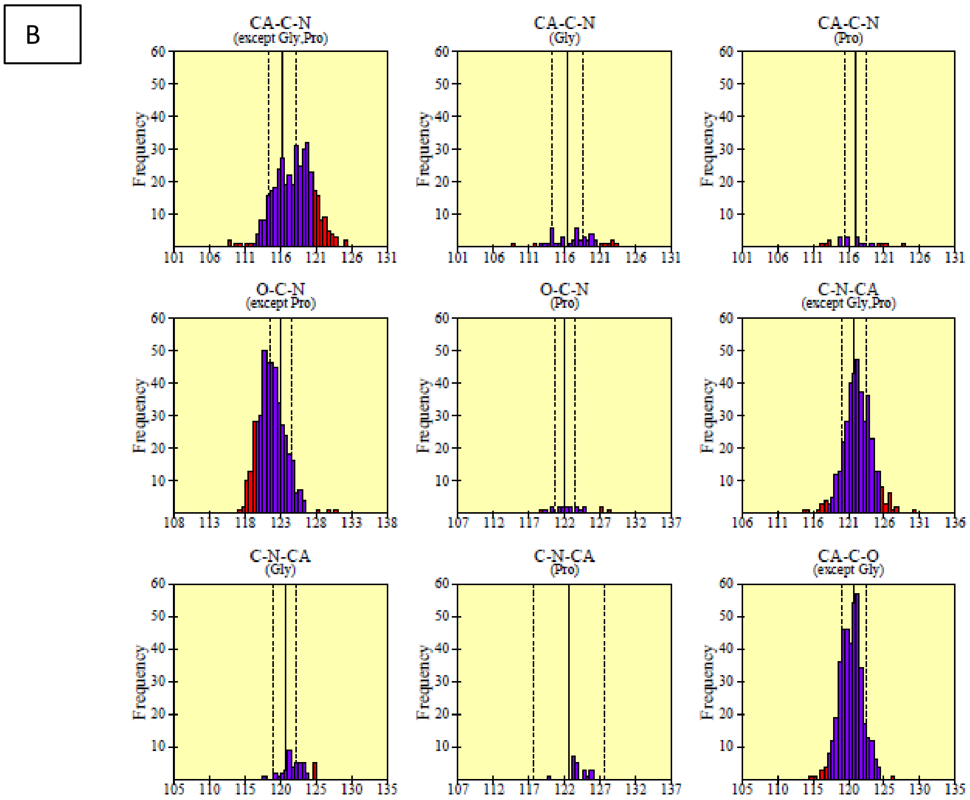


Fig. 6 (b). Bond length Main chain different residues (B)

Sidechain parameters are named chi1, chi2, etc. The chi1 angle is subject to certain restrictions that arise from steric hindrance between the gamma side chain atom(s) and the main chain. The different conformations of the side chain as a function of chi1 are referred to as gauche (+), trans and gauche (-) shown in Fig. 7.

Family and interpretation of domains of Hypodermin C

Interproscan is used for the interpretation of the function of proteins. It classifies the protein into families, subfamilies and gives information about the domains of the protein. Hypodermin C belongs to the family of peptidase S1A, chymotrypsin. The members of this family cause the intracellular digestion of microbes in neutrophils and fibrinolysis. The important catalytic residue is about 230 amino acids. The domains of protein represent the active sites of the protein. In Hypodermin C, this activity is conducted by a charge relay system which includes aspartic acid residues with histidine and histidine is hydrogen-bonded with serine. The biological function of peptidases is proteolysis and the molecular function is the serine-type endopeptidase activity.

Docking Analysis

Interpretation of Protein-protein interaction is the main provocation in the field of proteomics. The docking exhibits the bounded 3D structure of two proteins that interact with each other. The Fourier correlation method covers the translational and rotational spaces between two molecules. The docking results in very few native structures. It incorporates the binding energies of the structures. Other methods are used to purify the interacting regions of the protein (9). The refinement process involves the vanderwaal energy enhances and it improves the surface complementarity between bounded structures.

Cluspro 2.0 selects based on cluster size, given that the lowest energy structures generate the largest clusters. Anyway, the result has been given in the center of the cluster. However, the scores are not a representation of the binding affinity Secondly, even though ClusPro2.0 is CAPRI top-ranked docking software, it does not perform refinement because of the enormous computational power they need for their open-source program (10).

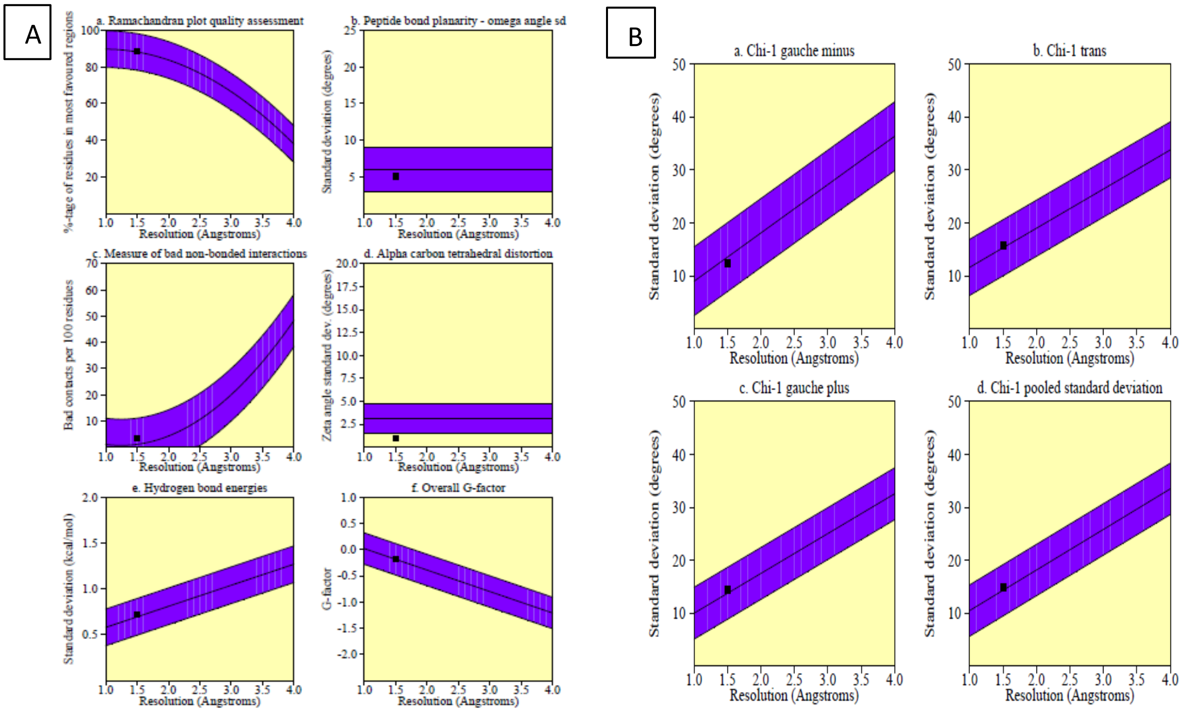


Fig. 7. Main Chain parameters (A)**, Side** Chain parameters (B)

Table 4: Docking Results

Cluster	**Members**	**Representative**	**Weighted Score**
0	141	Center	-858.3
0	141	Lowest Energy	-944.9
1	132	Center	-814
1	132	Lowest Energy	-986.5
2	110	Center	-816.1
2	110	Lowest Energy	-932.4
3	49	Center	-814.3
3	49	Lowest Energy	-877.1
4	46	Center	-863.1

DISCUSSION

Hypodermin C belongs to the family of serine proteases which catalyzes the lysis of protein. This protein comprises 260 amino acids. Hypodermin C causes the lysis of collagen protein. The main goal of this study is to understand the interface interaction between the hypodermin C and collagen. This study explains how the interactive regions of collagen and hypodermin C bind with each other cause the proteolysis in the collagen of bovine animals. The spatial arrangement of aspartate, histidine and serine induce the protein lysis.

Hypodermin C is also called a collagenases enzyme because it belongs to the family of trypsin and chemotrypsin. The members of this family carry out the breakdown of peptide bonds within the amino acids and also called endopeptidases. These enzymes cause intracellular digestion of microbes in neutrophils. The protein sequence was retrieved from the NCBI and sequence similarity was checked through BLAST which showed the highest query coverage with serine proteases. The main purpose of assessment and analysis was how serine is involved in interaction important to the collagenase action of the enzyme as in these proteases' histidine, aspartate, serine and H-bonded with each other. They split positively charged amino acids like arginine and lysine. The breakdown is always done by the residues that are determined by the histidine, aspartate and serine. Serine plays a major role in breaking bonds of the molecule (11). It attacks Serine is necessary for the direct and indirect breakdown of bonds between the molecules. (12). Their active site contains serine, aspartate and histidine, also known as a catalytic triad, but recent studies have discovered (13) that the glutamate and lysine residues are also essential for the endo-proteolysis. The catalytic triad comprising of Asp-Ser-His is present in the active site of the serine protease. This triad plays an important role in the dissolution of the peptide bond of the substrate. The Substrate binds to the surface of the Hypodermin, serine acts as a nucleophile as it has a hydroxyl group (OH). The hydroxyl group of Serine attacks the carbonyl carbon of the scissile peptide bond of the substrate with the assistance of Histidine. The hydrogen of OH of serine is accepted by the nitrogen of Histidine and the pair of electrons move from double bond of carbonyl oxygen of substrate to the oxygen of Serine forming a tetrahedral intermediate. The peptide bond present in the substrate is now broken. The electrons making this bond now move to attack the hydrogen of Histidine. The connection breaks and the previously moved electrons from carbonyl oxygen move back from negative oxygen to recreate the bond. Water formed during the reaction replaces the N terminus of the cleaved substrate and attacks carbonyl carbon. Again, the electrons move to the oxygen and make it negative oxygen. The bond between the oxygen of water and carbon is formed. This reaction is again assisted by Histidine as it takes a proton from the water forming another tetrahedral intermediate. The electrons that make the bond between the serine and carbonyl carbon move to attack this hydrogen that Histidine just acquired from water. Now carbonyl carbon is electron-deficient (3) and it recreates a double bond with the oxygen and the C terminus of the substrate is also ejected.

These flies are one of the causes of morbidity and mortality in the livestock sector and so far, no control strategies exist. For the last decade, researchers are trying to develop vaccines against myiasis. Although there is a dire need for the rapid development in genomic and proteomic analysis, the alternative control strategies are constantly evolving due to the drug resistance that has a strong impact on animal welfare (13). In this study, the structure of HyC was predicted based on homology modeling and its interaction with the collagen protein was studied via molecular docking technique. We are reporting the 3D structure of HyA and HyB for the first time and have shown how the catalytic triad is spatially arranged in the Hypodermin to bring about the proteolytic activity. The models of HyA and HyB were docked against their substrate collagen which showed for the first time how the nucleophilic triad of the HyA and HyB hydrolyze the peptide bond of the host's collagenase, responsible for puncturing the skin of cattle by breaking down the collagen protein which is an integral part of the epidermis to penetrate the body of cattle (14). Our results have illustrated how the characteristic structure of the HyA and HyB of warble fly larvae has been functionally evolved to proteolysis the skin collagen of its host, essential for parasitic activity. These studies also highlight the possibility of developing vaccines against HyA and HyB antigens as their structure is known.

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REVIEW ARTICLE

Life Science Journal of Pakistan
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Meteorological factors and COVID=19 Transmission - A Review

Tabinda Razi[1], Sajida Naseem[1], Asma Naeem[2], Ishrat Perveen[2*], Tallat Anwar Faridi[3], Saba Sabahat[4] ,Hafiza Najma Naeem[5]

[1]Department of Zoology, University of Education, Lower Mall Campus, Lahore, Pakistan.

[2]Department of Zoology, Faculty of Science, University of Central Punjab, Lahore, Pakistan.

[3]Institue of Public Health, Department of Allied Health Sciences, University of Lahore, Pakistan

[4]Rashid Latif College of Pharmacy, Lahore, Pakistan.

[5]Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

***Corresponding author**

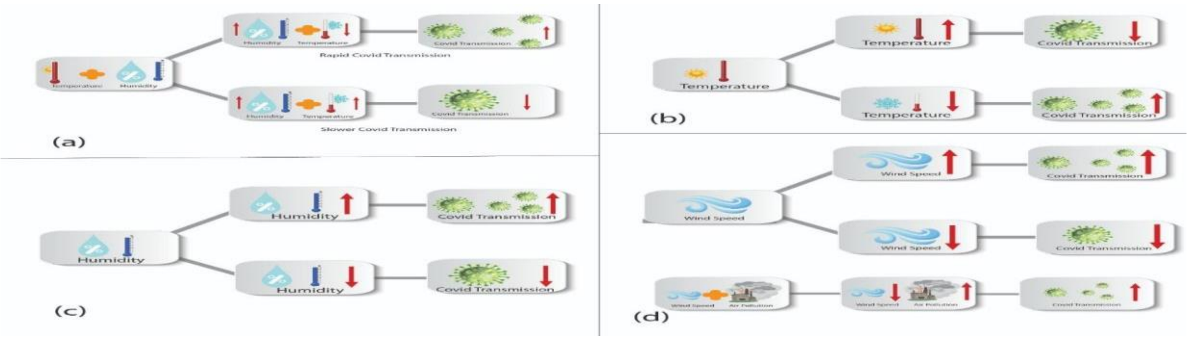
Ishrat Perveen

Address: Department of Zoology, Faculty of Science, University of Central Punjab, Lahore, Pakistan.

Email Address: ishrat.parveen@ucp.edu.pk

GRAPHICAL ABSTRACT

Effect of Environmental and/or Climatic Patterns (a) Temperature + Humidity (9, 12, 14, 16, 17, 31), (b) Temperature (10, 11) (c) Humidity (11), and (d) Wind Speed + Air Pollution (16, 18) on COVID-19 transmission



ABSTRACT

The coronavirus (SARS-CoV-2), emerged and identified by the end of the year 2019, has been found responsible for a series of lockdowns and a global medical emergency. Since then, several epidemiological studies have been conducted to identify and resolve the mysteries associated with the life-threatening viral strain (SARS-COV-2). The disease caused by SARS-CoV-2 is widely known as COVID-19. Researchers have well-established both the direct and indirect relationships between various climatic patterns, environmental and meteorological factors (*i.e.* air quality index (AQI), humidity, temperature, wind speed, etc.), and a surge in emergence, stability, and transmission of the COVID-19. The current review aims to dispense the relative variation in COVID-19 emergence, survival, stability, and transmission ratios due to the variant environmental, meteorological, and/or climatic factors.

Keywords: Contagiousness, Coronavirus, transmission, Air Quality Index, Pandemic

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INTRODUCTION

Corona-Virus Disease 2019 (COVID-19), has been proven widely as a fatal pulmonary pandemic, primarily emerged from Wuhan city (Hubei, Province), China. The gradual surge in the SARS-COV-2 viral infection was soon morphed into a global pandemic situation (1). The SARS-COV-2 virus exhibits distinct characteristic features like crown-spikes, positive-sense RNA, unsegmented and enveloped structural appearances, etc. (3-8).

There are different genomic structures of Coronaviruses which divides them into seven sub-groups *i.e.* alpha, beta, gamma, delta, MERS-COV, SARS-COV (beta), SARS-COV-2 (Novel coronavirus). Alpha and beta sub-groups of Coronaviruses principally affect human beings, whereas, others may primarily affect animals. The latter types of viral sub-groups may further transmit their infections from animals-to-humans *i.e.* SARS-COV (beta), MERS-COV, and SARS-COV-2 (Novel Coronavirus or COVID-19) (44).

Globally collected data on COVID-19, under WHO auspices, , entails more than 2,265,354 mortalities and 5, 2021, 104,165,066 cases of COVID-19 infection, worldwide (2). Since the pandemic hit the countries hard, across the globe, an average of four thousand cases of SARS-COV-2 infection emerged on a daily basis in Pakistan. There is no perfect statistical tool to compare the variation in COVID-19 emergence, stability, and human-to-human dispersal cases among countries around the globe. A Look at the peak percentages provides an insight into the SARS-COV-2 toll of each country, which compares it in its region and the world overall. In the Asia region, Bangladesh is reported with a high peak percentage (73%) followed by Pakistan (64%), Iran (55%), and India (51%), respectively, as given in fig.1. (45).

The current article illuminates the interconnectedness between the COVID-19 emergence, variability, dispersion in the atmosphere, human-to-human transmission, and any aid or abate in its contagiousness level for various environmental indicators, abiotic factors, and meteorological parameters. The influences of abiotic and/or environmental factors such as temperature, humidity, climate patterns, weather conditions, wind speed (WS), air pollution level, and/or air quality index (AQI).

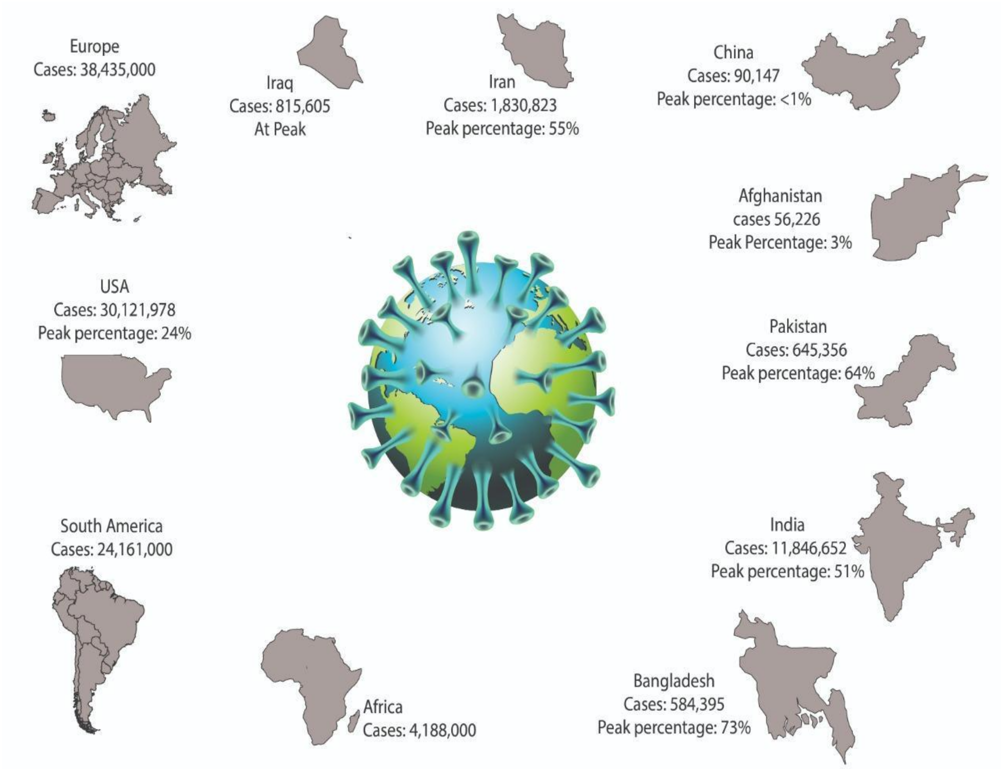

Fig. 1. A comparative Insight of The Peak Percentages (%) and COVID-19 Infection Cases around the Globe (45)

Table- 1. Impact of various Environmental, meteorological, and climatic patterns on the COVID-19 emergence, stability, and transmission rates

Sr. No.	Environmental/ Meteorological and climatic factors	Countries/ Regions	Key Findings	Reference/s
1.	Weather and/or Climatic patterns (particularly Temperature)	Spain, Switzerland, the US, Turkey, Germany, UK, France, Italy, Iran, Chile, Saudi Arabia, Brazil, Malaysia, Philippines, South Africa, Pakistan, Thailand, Indonesia, India	● Low temperature promotes the COVID-19 transmission. ● High temperature reduces transmission.	(10)
2.	Meteorological factors like Temperature	China	● A negative correlation has been found between temperature and COVID-19 transmission. ● The surge in COVID-19 transmission becomes unavoidable and uncontrollable in high populous and cold regions.	(9)
3.	Relative humidity (RH) and Temperature (Temp.)	China (Mainland), Hong Kong, and Singapore	● High RH and low temperature promote COVID-19 transmission. ● High RH and high temperature reduce transmission.	(11)

4.	Average relative humidity (ARH) & Average temperature (AT)	China (mainland)	● Average level of temperature (AT) and relative humidity (ARH) are negatively associated with COVID-19. ● The associations are not consistent throughout Mainland China.	(31)
5.	Relative humidity (RH) and Temperature (Temp.)	India (Delhi)	● A positive correlation of temperature with COVID-19 cases. ● A negative correlation of RH with COVID-19 cases.	(18)
6.	Humidity and Temperature	United States (New Jersey)	● A negative correlation b/w temperature and emerging COVID-19 cases. ● A positive and direct association b/w humidity and emerging COVID-19 cases.	(13)
7.	Relative humidity (RH) and Temperature (Temp.)	Italy, USA, France, UK, Spain, Germany, China, Brazil, Turkey, Canada, Russia, Belgium, Iran, Netherlands, Portugal, India, Ecuador, Peru, Switzerland, and Saudi Arabia	● High temperature and high RH reduce the COVID-19 transmission. ● Social distancing and precautionary measures have been proved vital and effective in containing the surge in SARS-COV-2.	(16)
8.	Ambient temperature (AT), air quality, and Relative Humidity (RH)	Malaysia (Kuala Lumpur)	● Emerging COVID-19 cases has confirmed a weak positive association with relative humidity (RH). ● A weak negative correlation exists between ambient temperature (AT) and new COVID-19 cases.	(12)
9.	Mean temperature and relative humidity (Spatio-temporal analysis)	Africa	● An inverse relationship b/w temperature, RH & surge in COVID-19.	(14)
10.	Humidity & Temperature	Bangladesh	● High humidity with high temperature may help in reducing the COVID-19 outbreak. ● Social distancing in addition to other precautionary measures may aid in abating the global pandemic.	(15)
11.	Climatic Patterns	Italy, France, Spain, Germany, Canada, USA, the UK, Russia, Scandinavian countries, Africa (sub-Saharan), Latin America.	● Cold and temperate warm climatic patterns were found favorable home grounds for the rapid COVID-19 spread. ● Arid and tropical environments are less favorable for SARS-COV-2 transmission and/or COVID-19 emergence/ outbreak..	(21)
12.	Weather and/or Climatic patterns	China	● The unaccompanied weather parameters cannot contain the spread of COVID-19.	(5)
13.	Climate and/or weather patterns	Spain, Switzerland, the US, Turkey, Germany, UK, France, Italy, Iran, Chile, Saudi Arabia, Brazil, Malaysia, Philippines, South Africa, Pakistan, Thailand, Indonesia, India	● Warm and humid states/ countries experienced fewer COVID-19 incidences as compared to the cold and dry countries.	(10)

14.	Air pollution, climatic patterns, and economic conditions	Latin America and the Caribbean region	● Warm and humid countries experience fewer COVID-19 incidences as compared to cold and dry countries. ● Data varies with region.	(17)
15.	Air Quality Index (AQI), Relative Humidity (RH), and Temperature	China	● A significant association has been developed b/w AQI and COVID-19 emergence/ transmission. ● The delayed effect of the Air Quality Index (AQI) on confirmed cases is noteworthy on days 1-3. ● Low relative humidity increases the effect of AQI on the surge of COVID-19 emergence and/or transmission.	(24)
16.	Air pollutants, Wind Speed (WS)	Italy	● High levels of air contamination/ pollution and low (WS) wind speed are responsible for the surge in COVID-19.	(26)
17.	Climatic patterns, air pollutants, Particulate Matters (PM) & economic conditions	LAC cities, Mexico City (Mexico), Santo Domingo, San Juan, Lima (Peru), Bogota (Colombia), Manaus (Brazil), Guayaquil (Ecuador), Santiago (Chile), Sao Paulo (Brazil), Buenos-Aires (Argentina)	● A significant association has been found b/w COVID-19 surge and Prevalence of Particulate matter (PM_{10}, $PM_{2.5}$) and NO_2.	(17)
18.	Air pollutants & Ozone (O_3)	India (Delhi)	● Weak association of air pollutants with daily COVID-19 cases. ● Significant positive association of O_3 with daily COVID-19 cases.	(18)
19.	Particulate Matters (PM)	The United States (New Jersey)	● Delayed effect (0-2 days) of particulate matter ($PM_{2.5}$) and air quality index on SARS-CoV-2 surge and dispersal.	(13)
20.	Air Quality Index (AQI)	China	● A decrease in coronavirus infections due to the mobility restrictions imposed at global and national level/s during the lockdown, aided in improving air quality.	(27)
21.	Wind speed	India (Delhi)	● A significant positive correlation between wind speed and COVID-19 cases.	(18)
22.	Wind Speed	The USA, Italy, Spain, France, Germany, The UK, Turkey, Iran, Russia, China, Canada, Brazil, Belgium, India, Netherlands, Switzerland, Portugal, Ecuador, Peru and Saudi Arabia	● Wind speed facilitates the emergence, survival and dispersal rates of COVID-19.	(16)
23.	Atmospheric stability, wind speed and air pollutants	Italy	● Low wind speed in polluted regions accelerated the transmission dynamics of COVID-19.	(26)
24.	Wind speed	Africa	● An inverse relation has been found b/w wind speed and COVID-19 surge and dispersal ratio.	(14)

25.	Climatic Patterns, Air pollutants, wind speed and economic conditions	Caribbean region and Latin America	● An inverse correlation has been confirmed b/w wind speed and rapidly surging COVID-19 cases.	(17)

RESULTS

Since the pandemic outbreak, the global researcher's community raced to identify and reveal the untold mysteries regarding the life-threatening viral strain (SARS-COV-2), which may aid in containing the emergence, viability, and worldwide dispersion of COVID-19. The scientific community has confirmed the high stability, sensitivity, and viability of SARS-COV-2 toward heat. With an increase in temperature up to 70°c, the viral inactivation span was reduced up to merely five minutes. Even at 4°c, the reduction in viral infectious titre was observed on the fourteenth day. SARS-COV-2 was found extremely stable at wide pH ranges, 3-to-10 (30).

Researchers have established the fact that diminished viability of SARS-COV-2 in high-temperature ranges is responsible for the low emergence, stability, viability, transmission, and/or dispersal rates of COVID-19 in hot climatic zones. A negative correlation between the COVID-19 transmission and temperature, however, was found by the scientists indicating that regions with high-temperature ranges were less prone to viral transmission (10, 11, 29, 31). According to the recent research, it has been verified that propagation of SARS-COV-2 was relatively more difficult to contain the viral transmission in cold and populous zones as given in Table 1 (9, 28).

The scientific community has confirmed a correlation between the humidity, temperature, and COVID-19 outbreaks in Bangladesh. It was found that high humidity along with high-temperature temperature ranges may help in reducing the COVID-19 outbreaks (15). Similarly, an inverse relationship between the relative humidity, temperature, and COVID-19 surge in Africa has been found through the Spatio-temporal analytic method (14). A cumulative effect of relative humidity and temperature indicated an increase in COVID-19 dispersal and/or transmission rate in the regions with high humidity and low-temperature levels. However, high temperature along with high humidity levels has been found to decrease the transmission rate. A negative effect of temperature coupled with the direct influence of humidity on the SARS-CoV-2 transmission has been detected with a similar correlation in the South-east Asian region, Africa, Bangladesh, Latin America, and Caribbean (LAC) cities and global hotspots of viral load as shown in Table-1 (11- 17). On the contrary, a plausible positive correlation between the temperature and the rapid surge in the emergence of COVID- 19 cases in India (Delhi) has been recorded (18). Human respiratory defenses against the infection are decreased in winters mainly due to the temperature dependency of SARS-COV-2 and cold nasal passageways during the cold seasons (19). However, a positive and direct correlation between humidity and a surge in COVID-19 emergence, survival, transmission, and dispersal rates, has been explained on the basis that high humidity accelerates the viral deposition on moist surfaces (20). Conversely, Average humidity (relative) and temperature (AT) ranges have also been observed to be indirectly related to the COVID-19 dispersion amongst human beings. However, this relationship was not found consistent all over China (28).

Weather conditions and climatic patterns have been found amongst the important indicators for the emergence, viability, stability, dispersion, and/or transmission rates of SARS-COV-2. A group of researchers found out, through a survey conducted in America, China, and some European, African, and Scandinavian countries, that cold and temperate-warm climate states were found as favorable breeding grounds for the rapid surge in transmission, emergence, and viability of SARS-COV-2 (21). On the contrary, arid and tropical climatic patterns having countries and/or states were found abating the feasibility and contagiousness of COVID-19 (10, 5, and 21). A Malaysian group of scientists surveyed to develop a possible association between the emerging COVID-19 cases and air quality parameters. The emerging cases of COVID-19 in Kaula Lumpur were found in weak respective negative and positive associations with the humidity (RH) and temperature (AT) parameters (12).

Studies have identified and developed a strong and positive correlation between the emergence, viability, transmission, and dispersal rate of COVID-19 with the varying abiotic factors like wind speed, atmospheric temperature, relative humidity, dew/frost, and precipitation rates. Researchers have demonstrated from the evidence collected from COVID-19 outbreak/cases of twenty countries that the reduction in viability, emergence, stability, survival, and dispersion rates of COVID-19 is caused by high temperature and highly humid atmospheric conditions (16).

It was reported that high temperature mitigates the COVID-19 dispersal and/or transmission ratio. On the contrary, cold weather (Temp. ↓) conditions promote the SARS-COV-2 survival and/or dispersal ratio (16).

Wind speed intensity coupled with the surface pressure aids the fast-track dispersal and accelerates COVID-19 transmission to far-flung destinations (32, 33). COVID-19 transmission cycle powered by the high intensity of wind speed along-with surface pressure can be explained without the human-to-human exposure (16, 32, 33). Wind speed (WS) directly aids and/or promotes the COVID-19 incidences on a global scale (16, 18). On the Contrary, Wind speed was found inversely and/or indirectly correlated to SARS-CoV-2 infections in Africa and Latin America, and Caribbean (LAC) cities (14, 17).

Global scientists raced together to unveil the possible impacts of air pollution and air quality parameters on the COVID-19 survival, emergence, viability, transmission, and/or dispersal rate of COVID-19, but still they are knowledge deficient in this regard. In general, good air quality facilitates healthy living and prevents respiratory disorders in people of all ages (26, 34-40). Human exposure to various air pollutants/ contaminants *i.e.* ozone ($O_{3)}$, Particulate matters (i.e. $PM_{10,}$ $PM_{2.5}$), PAH (Poly-aromatic Hydrocarbons) and Carbon Monoxide (CO) makes them susceptible to various respiratory and pulmonary distresses (17, 1, 8, 22, 23, 29).

In addition to this, human exposure to air pollutants induces and enhances oxidative stress and reduces their immunity and ability to resist viral infection (41-43). In a nutshell, the above-mentioned plausible impacts of air pollutants may directly impede the healthy quality of life and curtail the human ability to fight against the challenges imposed by SARS-COV-2 against human survival (43). The emerging COVID-19 cases were overall reduced, according to research evidence from China, due to the reduced human activity during the lockdown period (27).

Compromised air quality index (AQI) has a significantly delayed effect on COVID-19 emergence, survival, and viability ratios which are heavily affected by the temperature and humidity levels. Low temperature (10-20°C) and humidity (RH) ranges were found to increase the influence of AQI on transmission and/or dispersion ratios of COVID-19 cases (24, 25).

CONCLUSION

The global scientific community raced together to reveal the interconnectedness between the various climatic factors, ecological parameters, and meteorological indicators which either aid or abate the COVID-19 emergence, viability, stability, dispersal, and/or transmission rates, which in turn puts a direct or indirect impact on the contagiousness of SARS-COV-2. The current review depicts that the majority of studies have put forward a plausible direct correlation of wind speed, humidity, and air pollution with COVID-19 viability, stability, and transmission rates. Climatic patterns of a country/state were also established by numerous studies as significant indicators for the COVID-19 dispersion. Cold and temperate regions were found as heavens on the earth for the viability and stability of COVID-19 and vice versa. Several studies have confirmed high casualties in the countries with the bad air quality index (AQI) parameters along with the low wind speed. Putting in a nutshell, irrespective of the emergence of the COVID-19 (how did it come into being? How did it first transfer to human beings?), the deteriorated climatic factors *i.e.* temperature, air pollution, and air quality index (AQI) speak volumes about the undue anthropogenic interruptions in the environment. Hence, to control the current life-threatening viral pandemic, it has become mandatory to improve the quality of life by reversing the necessary climatic and meteorological parameters as they were originally, before the industrial revolution and globalization.

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