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Original Article

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Calcium and Phosphorus in Health and Disease: A Hospital based Study from Kashmir

Tahseen Bilal Rather[1], Gulzar Ahmad Bhat[2], Gowhar Rashid[3], Ishrat Parveiz Bhat[4], Syed Mudassar[5]

Abstract

Background: Calcium (Ca) and phosphorus (P) are indispensable micronutrients to human nutrition for their infinite role in structural and transduction processes. They are the molecules of central attraction due to their role in health and predicting the severity of different diseases.

Aims and objectives: Considering their importance in health and disease, study was aimed to document the normal serum levels of calcium and phosphorus in random population who visited the hospital for consultation during March-May 2021.

Methods: A total of 711 subjects were recruited for the analysis of serum Ca and P. samples were analysed on fully automated analyser (Beckman Coulter, AU5800). Both Ca and P levels were measured by kinetic method.

Results: The median age of the participants was 50 years. The overall median serum Ca and P levels recorded in the study population were as 8.9(Interquartile range [IQR=1.40]) and 3.4(Interquartile range [IQR]=1.18). About 4.5% of the study population was deficient in both Ca and P. Females had higher median calcium levels than males (p-value<0.05). A statistically significant association was observed between Ca and different age groups:-<20y,20-40 (P-value<0.003) and 20-40y,>40y40 (p-value<0.001). Further, a weak negative relationship was observed between Ca and Age (r=-0.118, p-value<0.002). Both Ca and P levels were low in 32(4.5%) patients(p-value<0.004).

Conclusion: Our findings revealed, that our population has calcium and phosphorus levels within the normal reference range, but presence of low Ca and P levels in the small portion of our population may be helpful in efficacious management of serious illnesses including several time and money consuming diseases in future.

Keywords: Serum Calcium; Phosphorous; Observational study; Kashmir.

Introduction

Calcium (Ca) is the third most abundant mineral in the nature and was available to the cells from the very beginning at the start of evolution. The basic regulation mechanisms were present at the very earlier stages in prokaryotes and protists.[1] Besides being the most abundant mineral in our body, it is the vital macronutrient required for normal and smooth functioning of the body[2] and influences a large number of both intracellular and extracellular processes. Growth, development, neural conduction and maintenance of bones and stability of cytoskeleton as well as the regulation of various enzymatic activities depends upon the activity of Ca levels in body.[3] In adults the total content of Ca is 1200g, approximately 2% of the total body weight, 98% of which is stored in bones in the form of hydroxyapatite crystals.[4] Any deviations in the calcium homeostasis can lead to grave conditions like malignant arrhythmia or cardiac arrest.[5] Hypocalcaemia is the signature feature of severely ill patients associated with worst clinical outcomes in various ailments including COVID-19 infections[6,2]

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while as hypercalcemia is observed in acromegaly, sarcoidosis, hypothyroidism, tuberculosis and proliferative disorders.[4] Like Ca, phosphorus (P) is one of the vital intracellular anion present in cell membrane and plays role in cellular metabolism, including synthesis of adenosine triphosphate and 2,3 diphosphoglycerate, enzyme regulation, signal transduction and in maintaining acid base balance.[7] In humans it is present in amounts of 550-770g with maximum (85%) representation found in bones.

Phosphorus haemostasis is disturbed in impaired renal clearance, catabolic reactions, alcohol abuse, diabetic ketoacidosis, acid-base disturbances.[7] Hyperphosphatemia is observed in chronic kidney disease. In one of the studies, it was mentioned that high serum phosphorus levels are associated with increased risk of atherosclerosis in young adults.[8]

Keeping in view the critical nature of above micronutrients in regulating various metabolic activities and scarcity of available literature in study population, particularly limited data available on random screening of serum P and Ca levels in general population, the study was aimed to assess the normal serum levels of Ca and P in general population who visited to the main tertiary care hospital Sheri-Kashmir Institute of Medical Sciences (SKIMS), from different corners of the Kashmir valley.

Materials and Methods

A total of 711 patients were analysed for serum Ca and P during March-May 2021. The analysis was done on fully automated analyser (Beckman Coulter, AU5800). Serum Ca and P levels were measured by kinetic method. The reference ranges of serum Ca and P ranged from 8.5-10.8mg/dL and 2.5-4.5mg/dL respectively. Statistical analysis was done on SPSS v26. The median age of the participants was 50 years (IQR=30). The overall median serum levels of Ca and P in patients were documented as 8.9(IQR=1.40) and 3.4(IQR=1.18) mg/dL respectively.

Results

The median age of the participants was 52 years. The overall median serum levels of Ca and P were recorded as 8.9 and 3.4 mg/dl (table 1). Among 711 patients, 394 (55%) were males and 317 (45%) were females. A statistically significant association was observed between gender and serum Ca (p-value<0.05) (table 2). Similarly, there was also a statistically significant association noted between Ca and different age groups; <20y,20-40 (P-value<0.003) and 20-40y,>40y (p-value<0.001(table 3).

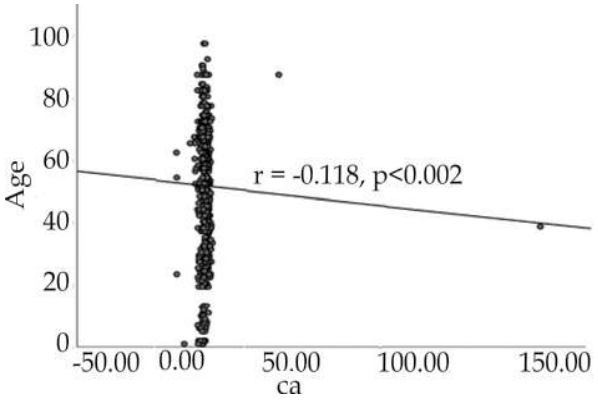

Fig. 1: Representing weak -ve correlation between age and calcium.

Table 1: Median age, Calcium (Ca) and Phosphorus (P) level in our population.

	Age	Ca(mg/dL)	P(mg/dL)
Median	52	8.9	3.4
Interquartile range	30	1.40	1.18

Table 2: Comparison of Ca and P with Gender.

Ca			
Gender	**N**	**Mean rank**	**P- value**
M	394	342.26	<0.05
F	317	373.08	
P			
M	394	344.41	>0.05
F	317	370.40	

Table 3: Comparison of serum calcium and serum phosphorus levels with different age groups in our population.

Ca			
Age	**N**	**Mean rank**	**P- value**
<20	52	85.81	<0.003
20-40	181	125.96	
20-40	181	378.75	<0.001
>40	478	311.54	
<20	52	233.52	>0.05
>40	478	268.86	
P			
<20	52	118.45	>0.05
20-40	181	116.32	
20-40	181	348.98	>0.05
>40	478	322.85	
<20	52	283.12	>0.05
>40	478	268.32	

Table 4: Stratification of serum Ca and P into different groups and there combination.al effect in our population.

		Ca			Chi 2; p-value
		low	**normal**	**high**	
P	low	32(4.5%)	52(7.3%)	2(0.3%)	25.58, <0.001
	normal	146(20.5%)	362(50.9%)	9 (1.3%)	
	high	57(8%)	49(6.9%)	2 (0.3%)	

Further, a weak negative relationship was observed between Ca and Age (r=-0.118, p-value<0.002). On further stratification, we found that both Ca and P levels were low in 32(4.5%) patients, high in 2(0.3%) subjects. Additionally, 362(50.9%) study subjects had both Ca and P in normal range. While as 57(8%) participants were recorded as those subjects who had high serum P levels but low Ca levels. Furthermore, 2(0.3%) subjects were those in which serum Ca levels were documented as high with low serum P levels. (P-value<0.001) (Table 4).

Discussion

Ca and P have regulatory roles in metabolic and signalling pathways.[9,10] There are many studies in our population that assess Ca and P status in relation with various disease.[11,12] The status of Ca and P varies in health and disease.[13] Hypophosphatemia is seen in severe respiratory diseases as observed in various published reports.[14-16] Abnormal Ca levels are also seen in viral induced diseases.[17]

In our study, we found that Sr. Ca and P levels were in normal reference range in most of the study subjects. Low levels of serum Ca and P were also present in a small group subjects. However, we could not find any study, due to very limited data that could validate these results, because our study was aimed at a general assessment of Ca and P in random population that did not included a particular disease as done in previous researches worldwide.[18-20] Low phosphorus levels are associated with mortality in haemodialysis patients as reported elsewhere.[21] A recent study also revealed a low levels of serum Ca and P in severe COVID-19 infection.[9] Our findings also showed a weak inverse relationship of Ca with age. This may be due to malabsorption of calcium in old age due to the deficiency of vitamin D as reported by various studies earlier.[22,23] The limitation of the study was that no proper follow-up was adopted, with no idea about the illness of the subjects.

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Review Article

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Candida Auris: The Emergence of a Multidrug-resistant Fungus

Asha Rani[1], Purnima[2], Anu Saini[3], Vandana Dahiya[4], Nehha Kasturiaa[5], Manoj Kumar Yadav[6], Archana Gupta[7], Anjali Priyadarshini[8], Arpana Vibhuti[9], V. Samuel Raj[10], Ramendra Pati Pandey[11]

Abstract

Candida auris has developed as a multidrug-resistant (MDR) fungal pathogen connected to medical care around the world. Due to its outbreak potential, antibiotic resistance, and high mortality, Candida auris infection has arisen as a significant problem in the care of patients admitted to ICUs in India. Candidemia caused by *C. auris* has been recorded from three continents since the initial report of ear-canal infection by this yeast in Japan in 2009, with a substantial number of cases from India. Some *C. auris* strains have higher minimum inhibitory concentrations (MICs) than amphotericin B and echinocandin compounds, while some *C. auris* strains are resistant to all antifungal medication classes. According to a comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) techniques, *C. auris* isolates exhibit strikingly comparable fluconazole resistance but a broad range of MICs for the other antifungal drug classes. The goal of this research is to learn more about curcumin's antifungal properties. This polyphenolic chemical has been used for medical, culinary, and other uses throughout Asia for centuries. Although curcumin has been shown to have antifungal properties, a current study reveals that curcumin works by disrupting the fungal cell wall.

Keywords: Candida auris; Multidrug resistance; Curcumin.

C. auris risk factors

C. auris infections have the same risk factors as other Candida species. This is usurprising, considering that many Candida species are opportunistic infections that are most commonly seen in severely sick and immunocompromised people. Elderly age, diabetes mellitus, recent surgery, the presence of an indwelling medical device (e.g., central venous catheter), an immunosuppressed state, hemodialysis, a neutropenic state, chronic renal disease, or the use of broad-spectrum antibiotics and/or antifungal drugs are all risk factors for *C. auris* infections.[1] An increase in *C. auris* colonizability was discovered in a study that retrospectively evaluated available patient data. An increase in C. auris colonization or infection was linked to diarrhea and the use of the broad-spectrum antibiotic tetracycline, as well as the second-generation tetracycline derivatives minocycline and tigecycline, according to research that retrospectively reviewed available patient data. These studies show that *C. auris* infections are linked to a wide range of risk factors.[2]

Antimicrobial resistance in C. auris

According to CLSI standards, all *Candida auris* isolates should be tested for antifungal susceptibility. Despite the fact that *C. auris* is

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usually multidrug resistant, antifungal resistance levels might vary greatly amongst isolates. There are presently no documented susceptibility breakpoints for *C. auris*. As a result, breakpoints are determined using data from closely related Candida species as well as expert opinion. At this moment, there is no evidence of a link between microbiologic breakpoints and clinical outcomes. As a result, the following information should be regarded as a broad guide rather than precise resistance breakpoints. Please keep in mind that an increased minimum inhibitory concentration (MIC) for an antifungal agent does not rule out its usage, especially if other antifungal treatments have failed to work for the patient. Many isolates are resistant to numerous kinds of medicine based on these MIC breakpoints. Some *C. auris* isolates from the United States have been reported to be resistant to all three antifungal medication classes.

We've also heard that pan-resistance has been discovered in other nations. In the United States, around 90% of *C. auris* isolates were resistant to fluconazole, about 30% to amphotericin B, and fewer than 5% to echinocandins. Multiple isolates from the same person may be included in these proportions, which may fluctuate as new isolates are examined.[3]

Table 1: MIC breakpoint of antifungal drugs and resistance.

Name of Drugs	MIC Range
FLU	≥32
VRC	N/A
AMB	≥2
AFG	≥ 4
CAS	≥ 2
MFG	≥ 4

FLU, Fluconazole; VRC, Voriconazole; AMB, Amphotericin B; AFG, Anidulafungin; CAS, Caspofungin; MFG, Micafungin (https://www.cdc.gov/fungal/candida-auris/c-auris- antifungal.html).

Ergosterol is the most abundant sterol in fungal membranes and is targeted by azoles (such as fluconazole) and polyenes (e.g., amphotericin B). Fluconazole, a first-line antifungal medicine, suppresses cellular ergosterol biosynthesis by targeting the fungal cytochrome P450-dependent enzyme lanosterol demethylase, which is required for ergosterol formation. In Candida species, ERG11 encodes lanosterol demethylase. Intriguingly, three hotspot mutations in Erg11 (Y132F, K143R, and F126L or VF125AL) have been discovered in fluconazole-resistant *C. auris* strains from various genetic clades. Although fluconazole and amphotericin B-resistant *C. auris* isolates are widespread, echinocandin-resistant isolates (e.g., caspofungin) are uncommon. In Candida species, FKS1 encodes the catalytic subunit of 1,3-beta-D-glucan synthase, which is required for cell wall formation and maintenance. *C. auris* isolates with an S639F mutation in Fks1 were resistant to caspofungin at human therapeutic dosages, but isolates with wild-type Fks1 were sensitive.[4]

- **Role of Melanin in fungus cell wall:** Melanin is a high-molecular-weight pigment that is negatively charged, hydrophobic, and insoluble in aqueous solutions, and it shields fungus from stressors while still allowing them to survive in the host.[5] The fungi produce melanin by two routes from 1,8-dihydroxynaphthalene (DHN) intermediate and from L-3, 4-dihydroxyphenylalanine (L-dopa).[6] Melanin synthesis aids fungal virulence, increases resilience to environmental stresses such as high temperatures, UV radiation, and toxins, and is crucial for invasion and spread. *c. neoformans* melanin, for example, has been related to the spread of yeast cells from the lungs to other organs and is known to alter the host's immunological response.[7]
- **Antifungal properties of curcumin:** Curcumin or diferuloylmethane (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) and other curcuminoids are the major phytochemicals found in the rhizome of Curcuma longa L. (Zingiberaceae family) known as turmeric.[8] Due to a diversity of biological actions, this polyphenolic molecule has piqued the interest of researchers all over the world. Due to the widespread traditional usage of turmeric in food items, several studies have been conducted to examine turmeric and curcumin in the context of preventing fungal spoilage and infections. The active component in turmeric may suppress melanin formation, according to 2012 research published in Phytotherapy Research. Curcumin is a chemical that inhibits the enzyme tyrosinase. This inhibits melanocytes' capacity to produce additional melanin.[9]

- Mannoproteins
- β-(1,3glucan), β-(1,6 glucan)
- Chitin

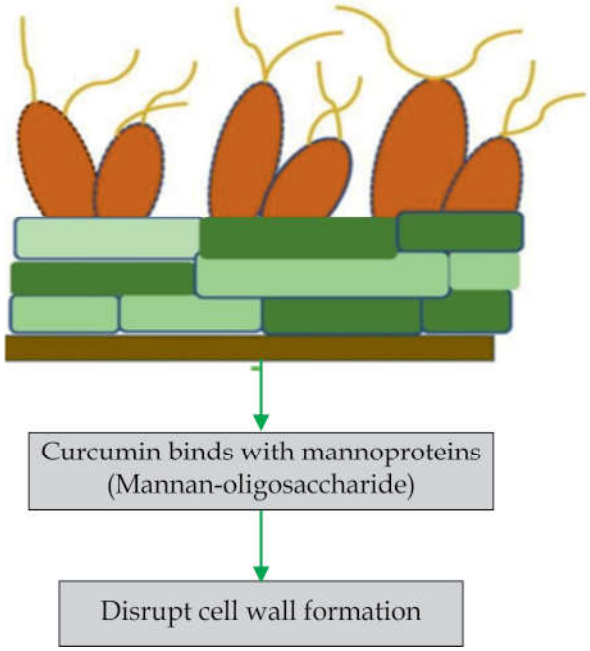

Fig: Schematic representation of candida cell wall and activity of curcumin.

Discussion

C. auris adaptability, genomic structure, virulence factors, and even techniques to treat the fungus in clinics are all being investigated. They've already made significant progress, but there's still a lot to learn about this fascinating organism's general biology, life cycle, other contributors to its genomic flexibility, and how to combat the yeast in patients. *Candida auris* shows some similarities to different species of candida. Some candida species contain melanin. If we treated *Candida auris* with curcumin, there is some possibility that curcumin inhibits the growth of candida auris because in humans, curcumin inhibits the production of melanin.

If this happens, then we can control the *candida auris* infection. Curcumin has been tested for a variety of biological activities, including antifungal activity against a variety of fungal infections, including *Paracoccidioides brasiliensis, Aspergillus niger, Sporothrix schenckii,* and Candida species. Because present antifungal medications have their limits, there has been a surge in interest in identifying new and more effective treatments, particularly those derived from natural sources.

Conclusion

The newly emerged multidrug resistant fungus, *candida auris* is a global health threat and has raised many questions in our minds. What were *C. auris's* initial environmental reservoirs? How did.

C. auris develop multidrug resistance? What allows *C. auris* to survive for lengthy periods of time in clinical settings? Finally, to battle infections caused by *C. auris* and other present and soon-to-be developing fungal diseases, we need to create innovative, safe, and effective antifungals and treatment techniques with a variety of pharmacological targets.

Future Prospective

Melanization might be a promising target for new antimicrobial treatments, so further research into melanin-inhibiting chemicals is needed in the future. Because the genome of *Candida auris* was recently decoded, information from genetic research is anticipated to provide more light on these findings.

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Review Article

RFP Indian Journal of Hospital Infection
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COVID 19 and One Health

Sayan Bhattacharyya[1], Yogendra Mevada[2], Rohit Kumar[3]

Abstract

COVID 19 is caused by the SARS CoV2 virus and is raging across the length and breadth of all the countries of the world at present. A plethora of clinical manifestations are evident in this infection in man, but animals or wildlife and the environment also play a pivotal role in transmission of the infection and persistence and evolution of the virus. A three pronged One Health approach is hence very important for optimum epidemiological surveillance and management of COVID 19. In this article the authors have tried to explain and address these pertinent issues.

Keywords: One Health; COVID 19; Zoonotic; Pandemic.

Introduction

The present pandemic of COVID 19 originated from the wet animal markets in the Hubei province of China. Since then it has spread to every nook and corner of the World. Man, animals and environment, all are important for the disease process and virus survival. Environment may facilitate encounter between animals and man and lead to emergence of many infections like COVID 19. A holistic approach is hence essential for proper understanding of the infection.

Materials and Methods

Thorough and meticulous literature search was done to search for available literature. COVID 19 from human health viewpoint: By now the COVID 19 pandemic is in its third wave. Needless to say, it is very important from human health viewpoint since it spreads effectively from humans to humans via aerosols and rarely fomites or by touching eyes or nose by infected hands.[1] It has led to appreciable morbidity or illness in man and also mortality and has caused tremendous economic losses.[2] Many many mutants and variants of the Novel Coronavirus have emerged by now. Some of these are variants of interest, while some are variants of concern. COVID 19 can affect multiple systems of the body causing protean manifestations like pneumonia, endotheliitis, myocarditis, arrhythmia, deep vein thrombosis, ischemic stroke and pulmonary embolism.[3] It can necessitate some surgical procedures also. It has therefore become imperative that Doctors from all disciplines work in tandem to fight this ongoing pandemic.

COVID 19 from animal health or veterinary perspective: Since the SARS-CoV2 is believed to evolve from Bat Corona viruses and thence Pangolin lineage Corona viruses; a zoonotic perspective assumes some degree of importance.

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Genetic recombination possibly has played a very significant role in the evolutionary history of these viruses.[4] Many types of animals also get the infection from humans occasionally. The virus has infected companion animals, wildlife, zoo and production animals, and also cats, dogs, tigers, lions, gorillas, white-tailed deer, mink, and other animals.[5] Evidence suggests that the emergence of this virus may be a genetic spill over event due to the wet animal markets and others. There are many unsampled virus lineages circulating in horseshoe bats which have zoonotic potential due to the ancestral position of the human-adapted contact residues in the SARS-CoV-2 RBD (Receptor Binding domain).

COVID 19 from environmental angle: Fomites can be a vehicle for transmission of COVID-19 infection. The virus-laden droplets can remain on any surface for an appreciable time, provided the surrounding air is static and does not move.[6] Novel corona virus can survive well on surfaces like plastics and metals. Hence inanimate surfaces and the environment in general can play a decisive role in infection transmission and need to be disinfected regularly. WASH or water sanitation and Hygiene and safe biomedical waste disposal practices can go a long way in prevention of COVID 19 infection and can form a part of a successful One Health approach from environmental perspective.[7]

The all-encompassing One Health viewpoint

The One Health (OH) approach is not new, but it assumes global importance in the context of COVID 19. One Health is an approach which recognizes that the health of humans is closely connected to the health of animals and also the shared environment. One Health is defined by the WHO as "an approach to designing and implementing programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes".[8] One Health is not a new concept, but it has become relatively more important in the last decade.[9]

In the past, many infections like SARS, MERS and Swine flu have caused widespread outbreaks and epidemics. They arose due to coexistence or conflicts between man, wildlife and the environment. The One Health vision is important to study the emergence and natural history of all such infections. In fact, following the outbreak of Severe Acute Respiratory Syndrome (SARS) in 2003, the "One Medicine" concept transformed itself into a much improved all-inclusive approach of "One Health" that incorporated the direct effects of environmental epidemiology on human and animal health.[10] By now it is well established that COVID 19 emerged as Corona viruses evolved through hosts like bats and pangolins. However the reverse is also true.

Animals can also acquire COVID 19 from man. Especially, pet cats and animals belonging to the feline family are notably prone to acquire the infection from man. Mutations and genetic reassortments can occur readily between human and deer Corona viruses and is perhaps the reason for emergence of the recent Omicron variant. It is therefore important for the scientific fraternity to realize at this moment that the SARS CoV2 virus causing COVID 19 has to be eradicated from the infected human host, animals and also the environment. If not possible to eradicate, we all should learn to live with it whilst remembering that man, animals and the environment all have a role to play in its ecology, pathogenesis and persistence. Things like physical distancing, hand washing or hand sanitization and wearing face masks outdoors are also part of the broad One Health approach recognizing the environmental factors in COVID 19 transmission.

There are broadly four areas in which the application of OH has the potential to markedly improve the governance of infectious diseases in general and COVID-19 in particular. Firstly, a good integrated surveillance infrastructure and monitoring of the occurrence of infectious diseases in both humans and animals can facilitate detection of new infectious agents constituting similar genotypes across species and the monitoring of the spatio-temporal spread of those infections. This knowledge can help guide public and animal health officials in instituting appropriate response measures.

Secondly, application of the OH approach can enhance coordination and active collaboration among all the stakeholders who represent seemingly incompatible domains. Thirdly, the OH approach highlights the need of an effective institutional landscape. This can help facilitate adequate regulation of hotspots for transmission of infectious agents among animals and humans, like the live animal markets. Fourthly, integrated human and animal health laboratories can be set up, which will help strengthen the capacity to conduct integrated studies on COVID-19.[11] Finally, OH in practice stresses the need for equitable solutions for infectious disease challenges, indicating that policy response mechanisms and interventions need to be at par with the disproportionate disease

burdens faced by the vulnerable and marginalized populations.[8] The One Health approach can bridge the gap between human medicine, veterinary medicine and environmental science and lead to a broader understanding of the virus and COVID 19 at large. Many courses can also be designed keeping this in mind. For example, a Government institute in India still runs the Master in Veterinary Public Health course for veterinarians to envisage a holistic approach towards public health as well as One Health.

Governments have now realized the importance and relevance of One Health in managing COVID 19, particularly with respect to the zoonotic potential of Corona viruses and the ability of such emerging viruses to jump species barriers. Recently the Department of Biotechnology, Government of India had organized a mega consortium on One Health with respect to COVID 19.[12] Scientists in Russia have started vaccinating animals vulnerable to COVID 19, like rabbits and mink. This will prevent the animals from severe COVID 19, and at the same time will thwart emergence of new mutants and variants that arise by reassortments between human and animal viruses.[13] The FAO or food and agricultural organization is collaborating closely with the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) in a FAO-OIE-WHO tripartite alliance to create and support One Health programmes in order to prevent disruption to food supplies and to manage COVID 19 in a better manner. India is home to a large proportion of the world's livestock farmers, but the lack of a good policy framework that validates the One Health approach in development and health policies is also perceived to be a major obstacle in eliminating poverty and poverty-related diseases.[14]

Discussion

Thus a One Health approach is the need of the hour for better understanding and management of COVID 19. Sir William Osler had promulgated the term "One Medicine", embracing both animal and human medicine way back in the 19th century. Dr Calvin Schwabe rejuvenated the same concept in the realm of veterinary public health .[14] The Wildlife Conservation Society (WCS) coined the term "One World-One Health" in 2007 and suggested 12 recommendations (called the Manhattan Principles) that focussed on adopting a more holistic approach for preventing epidemic disease and maintaining integrity o the ecosystem. The healthcare system was found off guard in managing the pandemic of COVID 19 due to the unpreparedness of the One Health program (15). With time the need to have a broader outlook and realize the importance of One Health for optimum response to COVID 19 and other future pandemics will be felt more. Pandemics like COVID 19 necessitate the proper implementation of the One Health Surveillance system (OHS) in order to focus on multi-sectoral, multidisciplinary, multi-institutional and multispecialty coordination, in all aspects of the response to outbreaks which might involve humans, animals, and their environment .[16]

Medical professionals, veterinarians and environmentalists need to get into the act together to produce a concerted effort to put in place a successful OH approach to manage and monitor COVID 19. If needed, new academic courses and events can be held or added to disseminate this very idea of OH in case of COVID 19, in the scientific community. However, it is also a fact that the most important barriers to such multi-sectoral action are often political, not technical.[14]

Conclusion

A One Health (OH) approach needs to be adopted now by all stakeholders for optimum management of COVID 19. This should not be neglected in the wake of the current pandemic. OH approach is the plausible approach of the future to manage and survey all upcoming pandemics and outbreaks due to such emerging viruses.

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Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

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Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

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