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# Journal of Monno Medical College

## Advisory Board



## Editorial Board

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**Journal of Monno Medical College**
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***Editorial***

# Monkeypox outbreak in non-endemic countries: Is there a pandemic threat like COVID-19?

Stating from 7 May 2022, new cases of Monkeypox have been reported from several countries/states including/across UK, USA, Europe, Australia and UAE in which the disease is not endemic.[1-3] The recent outbreaks in non-endemic countries are creating concerns, often a fear, may be due to widespread use of social media by all levels of population. But understanding the facts related to history, epidemiology and management of the disease, it appears that it might not be creating a pandemic threat like that of COVID-19, because- (i) it is not very rapidly transmitting across the communities with a high potential of a pandemic threat, (ii) it is not frequently causing serious life-threatening complications, (iii) it is a self-limiting illness and most people recovered within few weeks, (iii) even antiviral drugs as well as vaccine and good quality of diagnostics are available.

Let's look back to the history of Monkeypox. In 1958, Monkeypox virus was first detected in captive monkeys of a research laboratory. The first human Monkeypox cases were reported in 1970 in the Democratic Republic of Congo and later in 2003 introduced in USA.[4,5]

The etiological agent, the Monkeypox virus, is related to Smallpox virus and causes a rare illness found mainly in Central and West African countries near the tropical rainforest.[6,7] There are two main clades of the virus - West African and Central African (Congo basin) clades- the Congo basin clade has been found to cause more severe disease and thought to be more transmissible.[7]

Despite its name, Monkeypox is a misnomer since it most commonly infects and spreads between small African mammals and rodents. It can transmit to human when someone has a close relationship with the infected animals like as monkeys, rats and squirrels or person-to-person through virus-contaminated objects such as bedding and clothing. The virus can enter the body through broken skin, respiratory tract, body fluids or eyes, nose or mouth. It has not been previously described as a sexually transmitted infection, but it can be passed through direct contact during sex. In fact, it can spread if someone is in close contact with an infected person for a long time.[6-8] Some of those who were infected in 2022 are gay and bisexual men.[1]

Early symptoms of Monkeypox include fever, headache, bloating, back pain, lymph node swelling, chills and fatigue. It causes rashes, often starting at the mouth, then spreading to other parts of the body, usually the palms of the hands and the soles of the feet. The rash changes and eventually it forms a wound and which later falls off. The infection usually lasts 14 to 21 days and gets better on its own. The potential complications of Monkeypox include secondary infections, pneumonia, sepsis, encephalitis, keratitis with vision loss etc. This infection could be dangerous in young children, pregnant women and people with weak immune systems.[7,9]

Amid of this bizarre global situation created by COVID-19 pandemic and Ukraine war, the good news is that this infection with Monkeypox is mild in nature in most of the cases, and clears up on its own within a few weeks.[1,7,9] Though it can sometimes be more serious, this can be a leading cause of death (10%) due to infection with West African stains.[2,7] Interestingly, there was no death in 2003 outbreak reported from USA[4] which may suggest that with better management the mortality rate could be lower. Though this is a rare kind of viral infection, fortunately vaccine, antivirals and diagnostics are available for this. The US FDA has approved a vaccine, i.e. MVA-BN which basically developed for Smallpox (third generation smallpox vaccine), gives 85% protection against Monkeypox. This vaccine is known as "Imvanex" in EU; "Imvamune" in Canada and "Jynneos" in USA, requires to be administered in 2 doses, given 28 days apart.[7,10,11] The incubation period for this Monkeypox infection is long and usually varies between 5 and 21 days.[7,9] This provides high-risk contacts such as healthcare workers and laboratory workers, a window for obtaining post-exposure vaccines that can prevent infection. Vaccination against this virus is most likely aimed only at the immunity of those who have come into contact with infected people, known as the ring strategy.[12] An oral antiviral- "Tecovirimat" which inhibits the function of a major envelope protein required for the production of extracellular virus is available and approved by FDA USA for treatment in 2018.[13] Monkeypox can be diagnosed using real-time PCR and cell culture.[7,14] It is an urgent need that in such anxious, fearful and pessimistic situation, these information need to be disseminated quickly, so people can feel at ease, physicians can work confidently and policy makers can plan for future Monkeypox fights. Finally, it appears that the Monkeypox would not be able to turn into a pandemic threat like that of the COVID-19.

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*Original Article*

# Drug Prescription Patterns for Bronchial Asthma in a Tertiary Level Hospital in Bangladesh

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**Abstract**

***Background:*** Asthma is a chronic inflammatory disorder of the airways. The prevalence of asthma increased steadily over the latter part of last century. ***Objectives:*** To evaluate drug prescription pattern for bronchial asthma in a Tertiary Level Hospital. ***Methodology:*** This was a cross-sectional, observational study conducted in the department of Pharmacology, Mymensingh Medical College, in collaboration with the departments of Respiratory Medicine and Medicine out-patient departments in Mymensingh Medical College and Hospital, Mymensingh. ***Results:*** A total of 160 patients were selected non-randomly for the study. Age distribution indicates that majority (76, 47.5%) of patients were in the 28-37 years age group, followed by 18-27 years (44, 27.5%). Out of the 160 patients, 139 (86.88%) were treated with combination therapy and 21 (13.12%) were treated with monotherapy. Most of the patients (140, 87.5 %) used Fixed Dose Combination (FDC) therapy and the mostly used combination (131, 93.57%) was Salmeterol and Fluticasone. Combination of Salbutamol and Ipratropium bromide was used in only 9 (6.43%) cases as FDC therapy. Routes of administration of the anti-asthmatic drugs were inhalation and oral- of which the major route was inhalation (245/468 doses, 52.35%) and the other was 223 (47.64%). ***Conclusion:*** Majority of patients were treated with combination therapy. Mostly used FDC therapy was combination of Salmeterol and Fluticasone.

**Keywords:** Bronchial Asthma, Drug Prescription papers, Fixed Dose combination Therapy.

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## Introduction

Bronchial asthma associated with airflow restriction due to airway smooth muscle contraction often leading to difficulty in breathing and hypoxia and bronchial hyper-reactivity, being a chronic inflammatory condition of the respiratory tract.[1] The various triggers of asthma are infection, dust mites, molds and mildew, insects, pollens, pets, irritants, stress, smoke, food etc. Main characteristics of asthma is coughing, wheezing, shortness of breath, chest tightness.[2] Worldwide, asthma cases are increasing at a rate of 50 per cent every decade, and asthma would become the 3rd leading cause of death as per World Health Organization by the year 2020.[3]

To address the cases of asthma, different terms like allergic or asthmatic bronchitis, wheezy bronchitis, intrinsic and extrinsic asthma are used frequently by the physicians.[4] Factors affecting this disease include urbanization, air pollution, passive smoking, and also allergens.[5]

Over time and pulmonary function test diagnosis of the disease is usually made on the pattern of symptoms and/or response to therapy.[6] Following international consensus on asthma management, it is reasonable to hope that prescribing in the community should be in line with recognized guidelines to optimize asthma treatment.[7] Daily inhaled corticosteroid therapy as monotherapy or in combination with adjunctive therapy is the preferred treatment for all patients with persistent asthma.[8-10]

There are about 500,000 annual hospitalizations (individual aged 18 years or younger which is 34.6%) due to asthma and inhaled glucocorticoids are the most effective controller medications currently available.[11] Irrational use of medicines is currently a serious problem worldwide- WHO estimates that more than 50% of all medicines are used irrationally. Considering these facts, WHO is promoting rational use of

medicine and defines rational use of medicines as "that patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements, for an adequate period of time, and at lowest cost to them and their community".[12]

To evaluate drug prescription pattern for bronchial asthma in a Tertiary Hospital in Bangladesh that can be utilized in the development of treatment regimen this study was conducted.

**Methodology**

This study carried out over one year (July 2017 to June 2018) in the department of Pharmacology with collaboration of the department of Respiratory Medicine and Medicine in Mymensingh Medical College and Hospital, Mymensingh. This was a cross-sectional type of observational study.

Data were collected from outdoor prescriptions from the patients by questionnaire. Data related to type of drugs used, monotherapy, combination therapy, route of administration, drug schedule, type of bronchial asthma and various drug delivery devices were collected. Data related to knowledge of using meter-dose-inhalers and nebulization were also collected.

Collected data were checked and edited first and processed with the help of software Statistical package for social sciences (SPSS) version 21 and analysed. In view with the objectives of the study, an analysis plan was developed. Statistical analyses were done using appropriate statistical tool. Data expressed in categorical variables were presented as frequency and mean with standard deviations for continuous variables. Statistical significance was assessed at the 0.05 level for all analyses.

**Results**

In this study, of the 160 recruited patients, most common age group suffering from Bronchial Asthma were among 28-37 years (76, 47.5%) and second most common age group was 18-27 years (44, 27.5%). (Table 1) Considering sex of the enrolled patients suffering from Bronchial Asthma, it was found that majority of them were females (118, 73.75%). (Table 2) As reported occupation of the participants, it was found that majority were home makers (110, 68.8%), followed by service holders (25, 15.6%) and business person (14, 8.8%). (Table 3)

Table 1: Age distribution of patients

| Age groups in years | Number of patients | Percent | Mean+ SD |
|---|---|---|---|
| 18-27 | 44 | 27.50 | |
| 28-37 | 76 | 47.50 | |
| 38-47 | 30 | 18.75 | 33.5+10.35 |
| 48-57 | 4 | 2.50 | |
| 58-67 | 2 | 1.25 | |
| 68-77 | 4 | 2.50 | |
| **Total** | **160** | **100.00** | |

Table 2: Sex distribution of patients

| Sex | Number of patients | Percentage (%) |
|---|---|---|
| Male | 42 | 26.25 |
| Female | 118 | 73.75 |
| **Total** | **160** | **100.00** |

Table 3: Reported occupation of the enrolled patients

| Sl no | Occupation | Number of patients | Percent |
|---|---|---|---|
| 1. | Home maker | 110 | 68.75 |
| 2. | Service holder | 25 | 15.63 |
| 3. | Business person | 14 | 8.75 |
| 4. | Daily worker | 8 | 5.00 |
| 5. | Others | 3 | 1.87 |
| | **Total** | **160** | **100.0** |

Out of 160 patients, 140 (87.5%) were treated with fixed dose combination (FDC) therapy and remaining 20 (12.5%) by monotherapy. (Figure 1) Mostly used (131, 93.57%) FDC therapy was combination of Salmeterol and Fluticasone, whereas, combination of Ipratropium Bromide and Salbutamol was used in only in 9 (6.43%) of cases as FDC therapy. (Table 4)

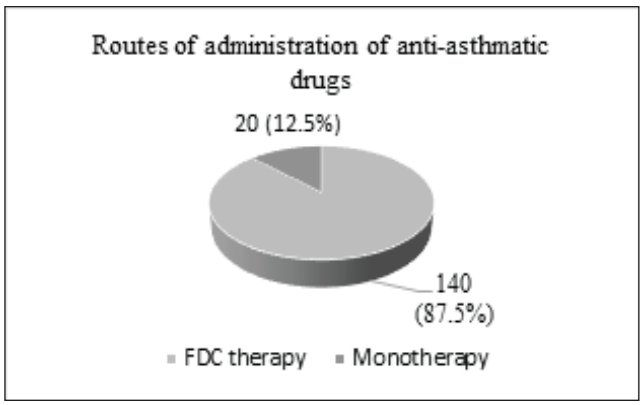


Figure 1: Routes of administration of anti-asthmatic drugs (n=160)

Table 4: Status of the patients with fixed dose combinations

| Name of drugs in FDC* | Number of patients | Percent |
|---|---|---|
| Salmeterol + Fluticasone | **131** | 93.57 |
| Ipratropium bromide + Salbutamol | **9** | 6.43 |
| **Total** | **140** | **100.00** |

*FDC- Fixed dose combination

Two different routes of administration of the anti-asthmatic drugs were recorded- oral and inhalation, which included a total of 468 doses of medications. Of them, majority 245 (52.35%) of the total anti-asthmatic drugs-doses prescribed were with inhalation dosage forms and remaining 223 (47.65%) by oral route. (Table 5)

Table 5: Routes of administration of anti-asthmatic drugs

| Sl no | Routes of administration | Frequency of doses | Percentage (%) |
|---|---|---|---|
| 1. | Inhalation | 245 | 52.35 |
| 2. | Oral | 223 | 47.64 |
| | **Total** | **468** | **100.0** |

## Discussion

In this study, majority (47.5%) patients were in 28-37 years age group, which is almost similar with the study by Prasad et al,[13] who reported mostly affected group of patients by bronchial asthma were 30-40 years. However, the present study is different with that one by Karki et al[3], where young patients were affected more in the age group of 16-30 years. Considering occupation, majority (68.75%) of the patients were homemakers in this study.

The result of the present study is also supported by Karki et al[3] reported that 92.6 % asthmatic patients were on combination drug therapy and only 7.4 % patients were on single drug therapy. Further, in the study by Shimpi et al,[14] investigators found that the percentage of single drug therapy was higher than our study, where it was 24% and the rest (76%) were treated with multiple drug therapy.

The mostly used Fixed Dose Combination (FDC) was Salmeterol +Fluticasone (93.57%), which is different from the study conducted by Rajathilagam et al, where most commonly used FDC was Montelukast + Levocetirizine (36.8%).[11] The result is similar to the study done by Prasad et al in (2015 in eastern India).[13]

Regarding route of administration of the drugs in this study, almost all (93.57%) were by inhalation route. The inhalation route is the most favourite, because it delivers more drugs locally in the respiratory tract with fewer side effects. Whereas, other studies reported somewhat different findings.[1,3,13] Rafeeq and Murad1 found 34.8% drugs prescribed by oral route and 61.3% drugs via inhalational route. Study conducted by Karki et al[3] shows contrasting results, which reported that 66% anti-asthmatic drugs were taken orally, and remaining 34% by inhalation route. The study conducted by Prasad et al[13] also reported that 60% drugs were prescribed via inhalation route and 38% by oral route.

Limitation of the study include primarily the study period-although this study was for a calendar year from July to June, Bronchial asthma patients are usually widely available during the winters of December to February each year in Bangladesh. Moreover, we could not include child patients-because they were not available in the study sites of outpatients' department of Respiratory Medicine.

## Conclusion

Majority of patients were treated with fixed dose combination (FDC) therapy and the mostly used FDC was combination of long-acting ß-2 agonist (Salmeterol and steroid (Fluticasone). Therefore, the current practice of using FDC in the treatment of Bronchial asthma is rationally practiced following recommended regimen.

**Conflict of interest:** None declared

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*Original Article*

# Psychosocial Stressors among Generalized Anxiety Disorder Patients in a Tertiary Level Hospital of Bangladesh

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**Abstract**

***Background:*** Psychosocial stressors are the most common perpetuating factors of the Generalized Anxiety Disorder (GAD) patients in Psychiatry outpatient departments. ***Objectives:*** The objective is to identify the psychosocial stressors among GAD patients visiting a tertiary level hospital. ***Methods:*** A cross-sectional study was conducted from June 2019 to December 2020, involving 227 individuals aged above 18 years and residing in Manikganj. Patients visiting Psychiatry outdoor department of the tertiary level hospital (Monno Medical College and Hospital, Gilondo, Manikganj) were taken as study subjects. Psychiatric diagnosis was confirmed by consultant Psychiatrist following standard definition. A semi-structured questionnaire was used to collect socio-demographic information and psychosocial stressors. Ethical issues were maintained throughout the study. ***Results:*** Our analysis shows a strong association between psychosocial factors and GAD as majority of the patients had at least one of the psychosocial stressors involved. During considering psychosocial factors, it was seen that higher number of the respondents mentioned to have financial crisis (154, 67.8%) and familial disharmony (109, 48%). Other factors (75, 33.0%) such as Covid-19 infection, effect of lockdown, home isolation had also contributed as psychosocial factors. Family member in the abroad were found among 67 (29.5%) and disease related anxiety among 57 (25.1%) of the respondents. Contraceptive use (45, 19.8%) was found as another contributing factor during the study period. ***Conclusions:*** Most of the patients with GAD have association with psychosocial stressors in this particular demographic region. Hormonal contraceptive use in female patients was an atypical finding- hence this study shows need of the larger research in this area and it points the association of psychosocial stressors among GAD patients.

***Keywords:*** Psychological stressors, Tertiary level Hospital, Generalized Anxiety Disorder.

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## Introduction

Anxiety is a discomforting feeling which includes worry or fear that ranges mild to severe in intensity. It is common for everyone to experience a feeling of anxiety at some point in his life. Anxiety disorder is an emotion, characterized by feelings of worried thoughts, tension, and physical changes such as increased blood pressure.[1] There are several forms of anxiety disorder including generalized anxiety disorder (GAD), social anxiety disorder, panic disorder, and specific phobias.[2]

The GAD can cause an excessive tension and worry in the daily calamities and problems on most days- it lasts for at least 24 weeks, where the person experiences difficulty in performing day-to-day works.[3] The emotional problem of GAD is characterized by autonomic hyperactivity, increased motor tension, and increased vigilance and scanning with lacking of panic attacks.[3] Anxiety disorders had the highest 12-month prevalence (8.1%) of all psychiatric disorders. In Bangladesh, the prevalence of anxiety disorders was found 4.5%, which is second common mental disorders among the adult population of Bangladesh.[4] It was reported that the spread of GAD ranged from 1.5% to 3% among adults.[5]

The treatment of anxiety disorders may be complicated by the potential presence of co-morbid psychiatric disorders, including other anxiety disorders, mood disorders, substance use disorders and personality disorders. In addition to co-morbidity, psychosocial stressors and behavior such as self-harm, may require clinical attention as they have the potential to affect course, prognosis or treatment of the disorder.[5] Previously, GAD had found to be associated with a wide range of adverse psychosocial contexts of childhood, including parental psychopathology, disruption of attachment

between the child and parents, acute life events, abuse, overprotective parenting, loss experiences, parental avoidance and modelling of anxious behavior.[6]

The objective of this study was to identify the psychosocial stressors among GAD patients attending a tertiary level hospital in Bangladesh.

**Methodology**

This was a cross-sectional study, conducted at a tertiary level multidisciplinary teaching hospital, Monno Medical College and Hospital, Gilondo, Manikgonj from June 2019 to December 2020. Majority of the enrolled patients came from different Upazilas of Manikganj and some others from Nagorpur Upazila of Tangail. Among the patients attending Outpatient Department (OPD), who were 18 years and above, were evaluated by the consultant Psychiatrist according to standard diagnostic criteria[3] and those who were diagnosed as generalized anxiety disorder (GAD) were included in the study. Patients from both sexes were included. A semi-structured questionnaire was used to collect socio-demographic information including age, gender, marital status, educational attainment, occupation, and residence. Psychosocial stressors reported during consultation were recorded by the researchers. The patients were analyzed based on important socio-demographic variables, and psychosocial stressors. Informed written consents were taken from the study participants after explaining them the nature and purpose of the study, their right of not taking part in the study, their anonymity in the study, etc. The data was analyzed by using SPSS-17.

**Results**

Among the 227 enrolled patients, majority (92, 40.5%) were of 31-45 years age groups, followed by (62, 27.3%) of 18-30 years. The mean age ± was 40.68 years. Majority of the respondents (151, 66.5%) were female, from rural areas (191, 84%) and married (181, 79.7%). Based on education, the highest number of respondents (89, 39.2%), Based on education, completed in primary level and one third (72, 31.7%) did not attend any school education. Monthly income less than 15,000 taka (133, 58.6%) comprised of the major percentages of the respondents, followed by 15,001- 30,000 taka (82, 36.1%). (Table-1)

Among the respondents, the most prevalent co-morbid disorder was hypertension (23, #10.1%), followed by irritable bowel syndrome (20, 8.8%) and peptic ulcer disease (16, 7.1%). (Table-2)

During considering psychosocial factors, it was seen that higher number of the respondents had financial crisis (154, 67.8%) and familial disharmony (109, #48%). Other factors such as Covid-19 infection, lockdown effect, home isolation had considerable contribution (75, 33%,). Family member staying abroad were found among 67 (29.5%) and disease related anxiety were present among 57 (25.1%) of the respondents. Hormonal contraceptive use (45, 19.8%) was found another factor during study period. (Table -3).

Table -1: Socio demographic profile of the respondents (n-227)

| **Age groups** | **Frequency** | **Percentages** |
|---|---|---|
| 18-30 | 62 | 27.3 |
| 31-45 | 92 | 40.5 |
| 46-60 | 56 | 24.7 |
| 61 and above | 17 | 7.5 |
| **Total** | **227** | **100.0** |
| Mean age in years± | 40.68 ±3.66 | |
| **Sex** | | |
| Male | 76 | 33.5 |
| Female | 151 | 66.5 |
| **Total** | **227** | **100** |
| **Marital status** | | |
| Married | 181 | 79.8 |
| Unmarried | 18 | 7.9 |
| Divorced | 6 | 2.6 |
| Separated | 4 | 1.8 |
| Widow | 18 | 7.9 |
| **Total** | **227** | **100.0** |
| **Residence** | | |
| Rural | 191 | 84.1 |
| Urban | 36 | 15.9 |
| **Total** | **227** | **100.0** |
| **Education** | | |
| Not schooling | 72 | 31.4 |
| Up to primary | 89 | 39.2 |
| Up to SSC | 40 | 17.6 |
| Up to HSC | 12 | 5.3 |
| Graduation and above | 14 | 6.2 |
| **Total** | **227** | **100.0** |
| **Occupation** | | |
| Farmer | 29 | 12.8 |
| House wife | 117 | 51.5 |
| Business | 11 | 4.8 |
| Service | 22 | 9.7 |
| Student | 10 | 4.4 |
| Un-employed | 38 | 16.7 |
| **Total** | **227** | **100.0** |
| **Monthly income** | | |
| Less than 15,000 taka | 133 | 58.6 |
| 15,000±30,000 taka | 82 | 36.1 |
| 30,001±45,000 taka | 12 | 5.3 |
| **Total** | **227** | **100.0** |

Table-2: Co-morbidity status of the respondents (n-227)

| **Co-morbid disorder** | **Frequency** | **Percentages** |
|---|---|---|
| HTN | 23 | 10.1 |
| DM | 11 | 4.9 |
| HTN & DM | 13 | 5.7 |
| IBS | 20 | 8.8 |
| PUD | 16 | 7.1 |
| Others | 10 | 4.4 |
| No Co-morbidity | | 59.0 |
| **Total** | **227** | **100.0** |

Notes: HTN- Hypertension, DM-diabetes mellitus, IBS- Irritable bowel syndrome, PUD- Peptic Ulcer diseases

Table-3: Psychosocial factors among responded (n=227)

| Psychosocial factors | Number (percent) of the patients | |
|---|---|---|
| | Present | Absent |
| Family member staying abroad | 67 (29.5) | 160 (70.5) |
| Familial disharmony | 109 (48.0) | 118 (52.0) |
| Financial crisis | 154 (67.8) | 73 (32.2) |
| Diseases related anxiety | 57 (25.1) | 170 (74.9) |
| Hormonal Contraceptive use | 45 (19.8) | 106 (70.2) |
| Other contributing factors | 75 (33.0) | 152 (67.0) |

## Discussion

Age distribution among the participants of current study showed that more than two-fifths of the respondents (92, 40.5 %) were within 31 to 45 years of age. This finding is similar to study done in South Africa and Malaysia.[7,8] In this study, most of the patients were female (151, 66.5%) and this was consistent with many other studies.[7-10] Most of the respondents were married (181, 79.7%) and the similar finding was observed in a Malaysian study.[8] But one South African study found only 35% cases married, of which about 25% were divorced, separated and widowed.[7] In this present study, majority of the respondents came from rural area (191, 84.1%) and monthly incomes were mostly less than 15,000 BDT (133, 58.6%). Majority of the lower income patients usually attended this institute as it is situated in rural area and treatment cost is cheaper.

The highest number of the co-morbid disorders of this study was hypertension (23, 10.1%)- similar findings were found previously.[8] In addition, irritable bowel syndrome (20, 8.8%) and peptic ulcer disease (16, 7.0%) were found in significant numbers in this study which differs with the study mentioned above. Another study showed that in irritable bowel syndrome, up to 95% of the patients had generalized anxiety disorder (GAD) or panic disorder.[11] In another study, panic disorder and GAD were in sufferers with peptic ulcer disease.[12]

Among psychosocial issues, this study discovered that about 59% affected persons had financial crisis and 48.0% patients had familial disharmony- these findings are close to the comparable studies in abroad.[7,8] However, their extraordinary stressors discovered housing problems, problems at work (40%) in Malaysia,[8] as well as 25% patients had abuse and neglected history records in South Africa.[7]

In this study, disease related anxiety was found among 25.1% of the GAD respondents. Nurun Nahar et al in a study in BSMMU found that 59.6% of the patients had co-morbid GAD and chronic medical conditions,[15] which is distantly related with this observation. About 33% of different factors like COVID-19, impact of lockdown, and domestic isolation were the newly rising psychosocial factors in this pandemic, that is found similar with an internet survey in Bangladesh.[16] In this study, 19.1% of the patients gave the records of using hormonal contraceptives, but no comparisons had been suggestive on the time. However, two observations in abroad showed exclusive findings. One in New work by Keely Cheslack-Postava et al found that hormonal contraceptive use reduced the sub thresholds anxiety disorders.[17] And the other study by Zettermark et al in Sweden confirmed that hormonal contraception increases the chance of psychotropic drug use in adolescent ladies. [18]

The methodological choices were constrained as there were few limitations including small sample size, public anxiety on visiting Hospital due to Covid-19 pandemic, short duration. One of the reasons of small sample size is supposed to be the increasing cases of COVID-19 during data collection for this study. Future studies are recommended overcoming the shortcomings with larger sample size and extended duration of study period.

## Conclusion

Based on the analysis of those who responded, it can be concluded that family member in abroad, familial disharmony, financial crisis, diseases related anxiety, oral contraceptive use and others like COVID-19 infection, lockdown effect, and home isolation are the factors causing Generalized Anxiety Disorder (GAD) in this particular demographic area. The results suggest that financial crisis and familial disharmony were the major psychosocial stressors among the patients with GAD in this area. The incidence of GAD would be less and the prognosis would be better if these factors could be managed carefully. To better understand the implications of these results further studies and community level approach could address the importance of stressors removal in treatment of GAD.

**Conflict of interest:** None declared

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*Original Article*

# Morphometric Study of Acromion Process of Left Scapula among Bangladeshi Population

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**Abstract**

***Background:*** The acromion is a process on the scapula of pectoral girdle and articulates the clavicle. The morphometry of the acromion process, it's relation to coracoid process and supraglenoid tubercle is important to determine dimensions of the subacromial space. Variations of acromion process and subacromial space can encourage clinical conditions of subacromial impingement syndrome, rotator cuff diseases and degenerative changes. ***Objective:*** Aim of this study was to determine the morphometric values of acromion process and thereby help managing the related clinical conditions as well as for the anthropological studies. ***Methodology:*** The study was completed on 140 dry adult human left scapulae derived from 89 males and 51 females. Morphometry of the acromion process of the scapulae was measured with the help of digital Sliding Calipers and metallic wire and scale. ***Results:*** The average maximum length of acromion process was 53.22 (±6.17) mm and 46.20 (±4.99) mm, maximum (average) width of acromion process was 29.83 (±3.05) mm and 26.25 (±3.03) mm, acromio-coracoid distance was 36.72 (±5.86) mm and 32.95 (±4.09) mm, acromio-glenoid distance was 29.98 (±3.97) mm and 27.17 (±3.38) mm among males and females respectively. ***Conclusion:*** Morphometric knowledge of the study might be useful for the Orthopaedic Surgeons and the Radiologists for determination of gender and for the Anthropologists when studying the evolution of bipedal gait.

***Keywords:*** Acromion process, Morphometry, Acromio-coracoid distance, Acromio-glenoid distance.

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## Introduction

The scapula (shoulder blade) connects the humerus with the clavicle, is a flat triangular bone of pectoral girdle. There are three processes on the scapula- the spine, the acromion and the coracoid processes. The acromion projects forward from lateral end of spine. The medial border of acromion is short and close to its anterior end is marked by a small oval facet for acromio-clavicular joint.[1]

The inferior aspect of acromion, together with coraco-acromial ligament and coracoid process, form a protective arch over the shoulder joint. The rotator cuff tendons, subacromial bursa and biceps tendon all pass beneath this arch. Any process acquired or congenital, that narrows the space may cause mechanical impingement.[2]

The slope and length of acromion and the height of the arch are closely associated with degenerative changes.[2] The knowledge regarding the shape and various distances of acromion process provides benefit to the orthopedic surgeons during surgical repair around the shoulder joint. It is also helpful to anthropologists during their study on evolution of acromion. This study may also be helpful to the forensic experts for determination of gender.[3]

The morphometric study of acromion process of scapula is very important in the field of forensic anthropology and in clinical sciences. The scapula plays important roles in impingement syndrome of shoulder joint and rotator cuff diseases.[4] This painful process is caused mainly by the friction of the inferior surface of the anterior part of acromion process.

From the above information, this study was planned to determine the morphometric values of acromion process of scapulae.

## Methodology

It was a cross-sectional, analytical type of study. The study was carried out in the department of Anatomy, Sir Salimullah

Medical College (SSMC), Dhaka, Bangladesh from July, 2013 to June, 2014. Eighty-nine male and fifty-one female dry adult human left scapulae were collected from the department of Anatomy, SSMC, Dhaka National Medical College and Delta Medical College, Dhaka. International Congress of Prehistoric Anthropometry and Archeology, Geneva gives an agreement on 'paired bilateral structures' measurements of left sided are recommended.[5] Hence, for this study dry adult human left scapula was the inclusion criteria.

The variables related to acromion process were measured as follows: (i) Maximum length of acromion process (MLA) - as the distance between most superior point and most inferior point on acromion process. (Figure 1) (ii) Maximum width of acromion process (MWA) - as the distance between lateral and medial borders at midpoint of acromion process. (Figure 2) (iii) Acromio-coracoid distance (Acd) - as the distance between tip of acromion process and tip of coracoid process. (Figure 3) (iv) Acromio-glenoid distance (Agd) - as the distance between tip of acromion process and supraglenoid tubercle. (Figure 4)


Figure 1: Maximum length of acromion process (MLA) measurement by metallic scale

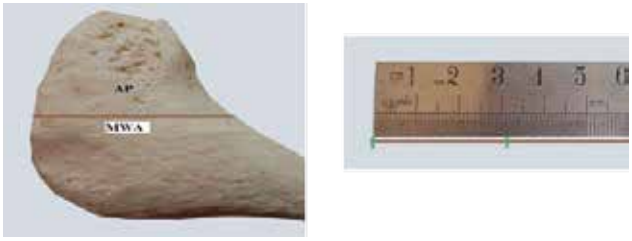


Figure 2: Maximum width of acromion process (MWA) measurement by metallic scale

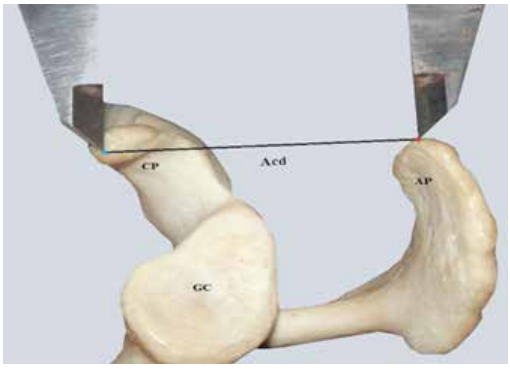


Figure 3: Acromio-coracoid distance (Acd) measurement by Slide Calipers

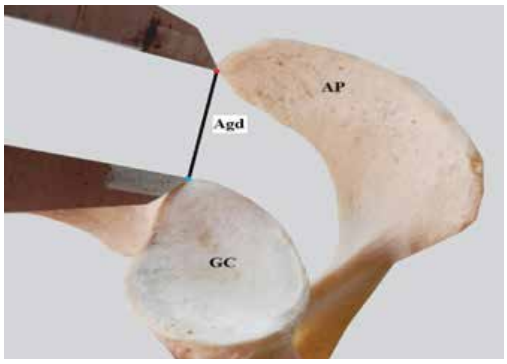


Figure 4: Acromio-glenoid distance (Agd) measurement by Slide Calipers

The variables were measured with the help of Vernier Calipers, a metallic wire and a metallic scale. The mean value of three measurements was recorded for each variable to exclude observer's error.

An unpaired Student's t-test was used to perform statistical analysis, p-value <0.01 was considered statistically significant. The study was approved by the Institutional Ethics Committee (IEC), SSMC, Dhaka.

## Results

The mean ± SD of maximum length of acromion process among males and females was 53.22 (±6.17) mm and 46.20 (±4.99) mm, the maximum width of acromion process was 29.83 (±3.05) mm and 26.25 (±3.03) mm, the acromio-coracoid distance was 36.72 (±5.86) mm and 32.95 (±4.09) mm, the acromio-glenoid distance was 29.98 (±3.97) mm and 27.17 (±3.38) mm in male and female respectively. The variables were greater in male than in female and were statistically significant. The mean and standard deviation values of various variables were shown in Table 1 (Maximum length and width of acromion process of scapula) and Table 2 (Acromio-coracoid and acromio-glenoid distance).

Table 1: Maximum length and width of acromion process of scapulae in males and females

| Sex | Maximum length of acromion process (Mean ± SD in mm) | Maximum width of acromion process (Mean ± SD in mm) |
|---|---|---|
| Male | 53.22 ± 6.17 | 29.83 ± 3.05 |
| (n=89) | (37.77-70.50) | (24.16-36.64) |
| Female | 46.20 ± 4.99 | 26.25 ± 3.03 |
| (n=51) | (29.25-56.15) | (20.59-35.05) |
| P value | 0.000** | 0.000** |

Comparison between sex was done by unpaired Student's 't' test
**= P value < 0.01, significant at 1% level of significance (two tailed)

Table 2: Acromio-coracoid distance and acromio-glenoid distance of scapulae in males and females

| Sex | Acromio-coracoid distance Mean ± SD in mm | Acromio-glenoid distance Mean ± SD in mm |
|---|---|---|
| Male (n=89) | 36.72 ± 5.86 (24.18-55.45) | 29.98 ± 3.97 (19.86-37.75) |
| Female (n=51) | 32.95 ± 4.09 (23.63-41.00) | 27.17 ± 3.38 (18.25-33.00) |
| P value | 0.000** | 0.000** |

Comparison between sex was done by unpaired Student's 't' test
**= P value < 0.01, significant at 1% level of significance (two tailed)

## Discussion

In this study, maximum length of acromion process, maximum width of acromion process, acromio-coracoid distance and acromio-glenoid distance were found higher in the males than that of females and were found statistically significant ($p<0.01$). The result clearly indicates the difference in sex.

The maximum length and maximum width of acromion processes in the present study were found higher than those of the studies carried out by Pushpa,[4] Paraskevas et al,[6] and Singroha et al,[7] who carried out their studies on Indian, Greek and North Indian populations respectively. In this study, for more accurate measurements, we placed metallic wire on the acromion process and then collected measurements from the wire. This was done to capture curvatures of the acromion process. This is why values of our reported measurements were higher than the others.

The acromio-coracoid and acromio-glenoid distances in the present study were found higher than those of the studies carried out by Paraskevas et al,[6] and Pushpa,[4] who carried out their studies among Greek and Indian populations respectively; but lower than that of the study carried out by Singroha et al[7] among North Indian population. The variations might be due to different race and ethnicity. Mansur[8] in 2012 and Burke[9] in 2008 did not differentiate sex, rather worked on the whole population. Hence, the values of their studies have not been brought under comparison spectrum.

Limitations of the present study include small sample size, which was small because of the scarcity of the bones. The samples were collected from the Anatomy departments of different medical colleges in Dhaka. The sources of these bones were not definite and authentic.

## Conclusion

The morphometry of acromion process in the present study can help the Orthopaedic Surgeons during surgical repair around the shoulder joint. It may also help the Radiologists to successfully carry out the interpretation of images of shoulder joint. The Anthropologists may also get interest in the morphometry of scapula when studying about the evolution of the bipedal gait. This study might also be useful to forensic experts in determination of gender.

**Conflict of interest:** None declared

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*Original Article*

# Knowledge and Awareness regarding Dengue Fever among the Adult Population in a Sub-urban area near Dhaka City

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**Abstract**

***Background:*** Dengue Fever (DF) is a preventable vector-borne viral disease transmitted by Aedes mosquito. It is a major public health problem in Bangladesh, especially in the urban and suburban regions. Since the first-detected epidemic of dengue in Bangladesh in 2000, around 49,000 people suffered from the disease. Most of the patients were from Dhaka or its neighboring districts. ***Objective:*** The study was carried out with the objective to assess the level of knowledge and awareness regarding DF among adult people of a selected sub-urban community. ***Methodology:*** The study was cross-sectional descriptive in nature. It was carried out at Keraniganj Upazilla, Dhaka. Data were collected by face-to-face interview with a semi-structured questionnaire. A total 540 adult persons were included in the study. ***Results:*** Total 9 questions were asked to assess the knowledge of DF. It was found that 425 (78.7%) of the respondents did not know about the causative agent, but 428 (79.3%) of the respondents knew that mosquito or Aedes mosquito is the vector of the disease. Some 287 (53.1%) of the respondents did not know about the biting time of the mosquito, but 318 (58.9%) of the respondents correctly knew about the breeding places of Aedes mosquito. Again, some 288 (53.3%) of the respondents did not know about the clinical features of dengue and 314 (58.1%) of them knew about preventive measures of DF. It was revealed that 186 (34.4%) respondents had poor knowledge about dengue who answered 0 to 3 questions correctly, 262 (48.6%) respondents had some knowledge answering 4 to 6 questions correctly and only 92 (17%) respondents had good knowledge about dengue and answered 7 to 9 questions correctly out of total 9 questions. ***Conclusion:*** The findings of the study indicate that knowledge regarding DF is very poor among the community people. Therefore, awareness building strategy directed towards bringing up a significant change in the knowledge among the people is essential.

***Keywords:*** Acromion process, Morphometry, Acromio-coracoid distance, Acromio-glenoid distance.

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## Introduction

Bangladesh is situated in the tropical and sub-tropical regions of the Southeast Asia and like other countries of the region has become a suitable habitat for the dengue vector and increased transmission of the disease. Only sporadic cases of the disease were reported from Dhaka and other parts of the country before 2000.[1] A sudden outbreak of Dengue occurred in 2000, causing a serious public health concern when around 5,551 cases and 93 deaths were reported in the country.[2] During the outbreaks of the disease from 2000–2017, both types of the Aedes mosquito vectors (A. aegypti and A. albopictus) were identified in Bangladesh.[3] The re-emergence of dengue and the recent emergence of Chikungunya due to chikungunya virus, both spread by the Aedes mosquitoes, are very worrying and have created a huge burden of morbidity and mortality with insufficient allocation of resources.[4]

It is important to investigate the knowledge about a preventable disease like dengue as prevention measures are the key point to control such a disease and knowledge level of general people are directly linked to the effectiveness of a control programs. Knowledge of disease agent, vector, breeding places of the vector, clinical features, preventive measures and treatment methods is fundamental information about dengue fever (DF) which is necessary to collect and analyze.

Therefore, the objective of this study was to assess the level

of knowledge about DF among the sub-urban people in Bangladesh which shall in turn facilitate understanding the situation and conducting any preventive measure against the disease.

**Methodology**

This cross-sectional descriptive study was conducted from December 2018 to March 2019 in the sub-urban region of Keraniganj upazila, under Dhaka district. Convenience sampling technique was followed to select the 540 adult respondents from Ainta and Konda villages of Keraniganj Upazila. After taking verbal consent from the respondents following introducing and informing the study purpose and objectives, data were collected by face-to-face interview.

A semi-structured questionnaire, based on socio-economic characteristics and dengue fever related knowledge measuring questions was used for this purpose. Statistical analyses were performed using the 21st version of SPSS® software. Before collection of data, verbal permission was taken from the respondents by informing them the purpose, procedure, expected duration, nature, and anticipated physical and psychological risks and benefits of participation. Confidentiality of data and privacy of the respondents were maintained strictly.

**Results**

Among the respondents, 377 (69.8%) were female and 163 (30.2%) were male. Mean age of the respondents was 37.83 (SD ±15.4) years ranging from 18 to 87 years, among whom majority (151, 28.1%) were between 18 and 25 years. Regarding religion of the respondents, almost all (536, 99.3%) were Muslims, 210 (38.9%) had 1 to 8 years of schooling, 348 (64.4%) were housewives, 464 (85.9%) were married and 345 (63.9%) respondents were from nuclear families. Average monthly family income of the respondents was Tk. 20,998 (SD ±14,369). It was also revealed that, 373 (69.1%) respondents had aedes mosquito breeding places around their households. (Table 1)

Total 9 questions were asked to assess the knowledge level about dengue fever (DF). It was found that 526 (97.4%) of the respondents heard the name of dengue, 425 (78.7%) did not know about the causative agent and 428 (79.3%) of them knew that mosquito or aedes mosquito is the vector of the disease. Some 287 (53.1%) of the respondents did not know about the biting time of aedes mosquito, and 318 (58.9%) correctly knew about the breeding places of aedes mosquito. A total of 288 (53.3%) of the respondents did not know the signs and symptoms of dengue, whereas, 468 (86.7%) of the respondents did not know the complications of DF. Majority (461, 85.4%) of the respondents gave incorrect answers about the persons who may suffer more severe complications of dengue and 314 (58.1%) of the respondents knew about prevention measures of DF.

Regarding overall knowledge level assessment, it was found that 186 (34.4%) respondents had poor knowledge about dengue, who answered 0 to 3 questions correctly, 262 (48.6%) had some knowledge answering 4 to 6 questions correctly and only 92 (17%) had good knowledge about dengue who answered 7 to 9 questions correctly out of 9 knowledge-assessing questions.

Table 1: Socio-demographic characteristics of the respondents (n = 540)

| Name of variables | Frequency | Percentage | Mean ±SD |
|---|---|---|---|
| **Sex** | | | |
| Female | 377 | 69.8 | |
| Male | 163 | 30.2 | |
| **Age in years** | | | |
| 18-25 | 151 | 28.0 | |
| 26-35 | 143 | 26.5 | 37.8 (±15.4) |
| 36-45 | 101 | 18.7 | |
| 46-55 | 63 | 11.6 | |
| 55+ | 82 | 15.2 | |
| **Religion** | | | |
| Muslim | 536 | 99.3 | |
| Hindu | 4 | 0.7 | |
| **Education** | | | |
| <1 yr schooling | 119 | 22.0 | |
| 1 to 8 yrs schooling | 210 | 38.9 | |
| 9 yrs to SSC | 188 | 34.8 | |
| Bachelors | 21 | 3.9 | |
| Masters | 2 | 0.4 | |
| **Occupation** | | | |
| Unemployed | 28 | 5.2 | |
| Housewives | 348 | 64.4 | |
| Job holders | 39 | 7.2 | |
| Businessmen | 82 | 15.2 | |
| Day laborers | 19 | 3.5 | |
| Students | 22 | 4.1 | |
| Doctor/Engineer/Teacher | 2 | 0.4 | |
| **Marital status** | | | |
| Single | 47 | 8.7 | |
| Married | 464 | 85.9 | |
| Divorced | 2 | 0.4 | |
| Widow/widower | 23 | 4.3 | |
| Separated | 4 | 0.7 | |
| **Family type** | | | |
| Nuclear | 345 | 63.9 | |
| Joint | 195 | 35.1 | |
| **Monthly family income (Taka)** | | | |
| 0-10,000 | 126 | 23.3 | 20,998.1 |
| 10,001-25,000 | 288 | 53.3 | (±14,369.3) |
| 25,001-35,000 | 78 | 14.4 | |
| >35,000 | 48 | 8.9 | |
| **Aedes mosquito breeding places around housing** | | | |
| Present | 373 | 69.1 | |
| Absent | 167 | 30.9 | |

Table 2: Overall knowledge level

| Category | Frequency | Percent |
|---|---|---|
| Poor knowledge (0—3 correct answers) | 186 | 34.4 |
| Some knowledge (4—6 correct answers) | 262 | 48.6 |
| Good knowledge (7—9 correct answers) | 92 | 17.0 |
| **Total** | **540** | **100** |
| Average number of correct answers | 4.3 (SD ±2) | |

## Discussion

Considering sociodemographic criteria of the respondents, it was found in the present study that majority (377, 69.81%) were male and the highest number of them were in the 18-25 years age group (151, 28.1%), followed by 26-35 years (143, 26.5%). A similar study conducted in India by Singh et al5 found that 318 (59%) of the respondents were male and majority of them were young adults (20-40 years) and literate.

In our study, a total of 9 questions were asked to assess the knowledge of dengue fever (DF). It was found in this study that majority (425, 78.7%) of the respondents did not know about the causative agent; but surprisingly 428 (79.3%) of the respondents knew that mosquito or aedes mosquito is the vector of the disease. Majority (287, 53.1%) of the respondents did not know about the biting time of aedes mosquito; but 318 (58.9%) of the respondents correctly knew about the breeding places of aedes mosquito. Some 288 (53.3%) of the respondents did not know about the clinical features of dengue and 314 (58.1%) of the respondents knew about preventive measures of DF.

A study conducted by Sharmin et al[6] found that participants had high levels of knowledge regarding the transmission of dengue, Aedes breeding, and biting prevention methods. Another study conducted by Sharmin et al[7] found that majority of the respondents had possible breeding site for Aedes mosquitoes in their compound and practiced good habit in preventing the Aedes mosquitoes from breed. A study conducted by Karim et al[8] found interviewing 195 individuals that some 7% were illiterate and 18% had a college degree. Some 91% individuals knew mosquito as the vector, 32% identified clear stagnant water as the breeding place, while 22% knew about bleeding manifestations. Another 71% felt dengue as a severe disease and 84% had a positive attitude towards consulting a doctor for the illness. Sharmin et al[9] found in their study that out of 223 individuals interviewed, 93% identified fever as a cardinal symptom of DF. The knowledge about other signe symptoms of DF was low among participants. Only 17.5% knew that DF is transmitted by Aedes mosquitoes. The correct timing of biting was known by only 14% respondents. Despite low knowledge, the participants had good attitude and most of them reported good preventive practices against dengue prevention and control. Banu et al[10] found in their study that almost 68% of the individuals knew mosquito causes vector-borne diseases irrespective of their educational status and majority of them were daily wagers. Amongst them more than 70% of them were using protective measures. Majority (38%) of them responded the probable breeding sites were plastic pots, muddy pots and vessels.

It was revealed in this study that 186 (34.4%) respondents had poor knowledge about dengue who answered 0 to 3 questions correctly, 262 (48.6%) respondents had some knowledge who answered 4 to 6 questions correctly and only 92 (17%) respondents had good knowledge about dengue who answered 7 to 9 questions correctly out of total 9 questions. A similar study conducted in India by Villanes et al[11] found more than 50% of the participants to have poor knowledge regarding DF. A study by Raheel et al[12] found that 53.2% of the respondents had good knowledge about dengue and it was found that the main source of information was from mass media (76.6%). However, only 43.4% were found to have good attitude towards dengue. Multiple Logistic Regression analysis showed there was no association between socio-demographic characteristics with the level of knowledge and attitude towards dengue. There was also found no association between knowledge of dengue and the attitude of the respondents towards dengue. Faisal et al[13] found in their study that more than half of the parents (54%) had good knowledge about signs, symptoms, and modes of transmission of dengue. Approximately 47% considered dengue as a serious but preventable disease in which they are vulnerable. Nevertheless, a majority (77%) did not use effective dengue preventive methods such as screening of homes and 51% did not use bed nets. Educational attainment (OR, 2.98; CI, 1.23–7.23) was positively associated with knowledge of dengue. There was no correlation between knowledge about dengue and preventive practices (p=0.34).

## Conclusion

The findings of the study indicate that knowledge regarding dengue fever is not satisfactory among the sub-urban community people. Therefore, awareness building strategy directed towards bringing up a significant change in the knowledge among the people regarding the causative agent, vector, mode of transmission, prevention & treatment are essential. In this regard, policy makers, community leaders, volunteers and environmental activists should plan & implement coordinated awareness building programs involving the general community people to combat the4 situations.

**Conflict of interest:** None declared

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*Review Article*

# Updates on Laboratory Diagnosis of COVID-19 infections

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**Abstract**

***Background:*** COVID-19, the disease caused by SARS-CoV-2, beginning in the late 2019 as an outbreak in Wuhan, China- now, became the world's most feared pandemic among communities around the globe. While we are investing almost all our efforts to save us from the infection, the diagnosis, still at the end of about 2-years of dreadful pandemic, remains difficult for common people. Although, this is a notorious, but enveloped virus, and therefore, is easily destroyable with heat (so, cannot easily survive in the environment) and detergents (so, can be easily killed by applying soap and water), is now returning consecutively in waves of variants with increasing virulence and transmissibility. Because of new variants of the virus, diagnostic approaches to identify the virion as a whole (by cell culture) or in parts (by detecting antigens) or viral products (by detecting antibodies against significant antigens) becomes difficult. The currently available laboratory test methods are ranging from rapid tests at point-of-care (detecting viral antigen(s)) to the genome sequencing. In between, majority of the tests like electron microscopy and cell culture are not routinely practiced, because of their high-end costly equipment and set up. All of these tests have extensively different results due to unpredictably variable presence of the virus (and its products) in clinical specimens as well as costs among the brands. And these limitations are now putting the healthcare professionals along with their patients in discomforts of unsatisfactory management. Yet, the rapid tests are widely practiced for screening purposes, followed by confirmation by real-time reverse transcriptase polymerase chain reaction (rtRT-PCR) tests. This is a simple and comprehensive review of the diagnostic approaches considering all relevant issues of the virus (SARS-CoV-2) and the disease (COVID-19) immunobiology with the publications available up to February, 2022.

***Keywords:*** Acromion process, COVID-19, SARS-CoV-2, Laboratery Diagnosis Update

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## Introduction

The real horror name COVID-19 warrants back to the dateline of 31 December 2019, when Wuhan Municipal Health Commission in China reported to the WHO country office that a series of Pneumonia cases emerged in Wuhan (under the province Hubei of China) with clinical presentations resembling viral pneumonia. The cases of the primary outbreaks were mostly found having epidemiological link with the large sea-food market in Wuhan.[1-3] Specimens from the hospitalized patients (majority of them were sellers of the seafood market in Wuhan) were sent to Wuhan Institute of Virology, scientists analysed one of the specimens by metagenomics analysis and found 79.6% sequence identity to SARS-CoV BJ01 (GenBank accession number AY 278488.2). Scientists also found a short region of RNA-dependent RNA polymerase from a bat coronavirus and conducted a full-length sequencing that shows a 96.2% sequence homology.[4,5] Inoculation of respiratory secretions from infected individuals into Vero E6 and Huf7 cell lines and human airway epithelial cells brought to the isolation of a novel virus, whose genome sequence showed belonging to the Coronaviridae family. Soon the virus was characterized as a novel beta coronavirus and named as the '2019-nCoV'.[1,6] The viral infection was found to spread to the surrounding countries very soon potentiating a pandemic threat and then throughout the globe establishing the world's dreadful pandemic.[7] On 30 January, WHO was very scare to declare a Public Health Emergency of International Concern (PHEIC).[7,8] The virus was later renamed by the international Committee on Taxonomy of Viruses (ICTV) as SARS-CoV-2.[8-10]

Although in the last twenty years, mankind has faced three different coronavirus outbreaks (SARS-C0V-1 in 2003,[11] MERS-CoV in 2012,[12] and SARS-CoV-2 pandemic in 2019), the last one appeared as the most devastating in all considerations.[13]

Now, at the end of about two years of dreadful massacres of the world's economy and all-stage livelihoods of the citizens

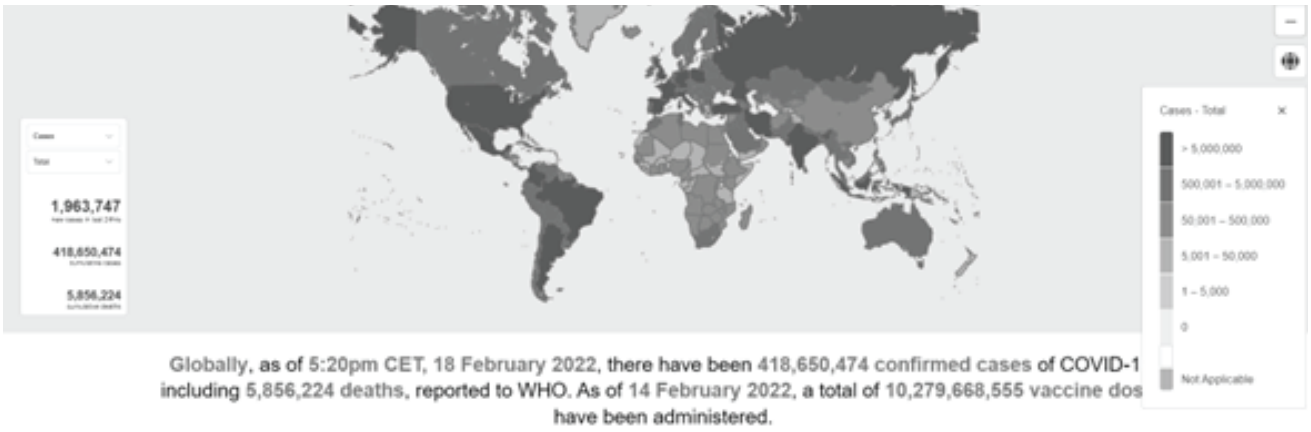


Figure 1: COVID-19 situation as on 18 February, 2022 (source: WHO Coronavirus Dashboard, available at: https://covid19.who.int/ , accessed 20.02.2022)

and availability of many promising vaccines, the transmissions of the virus and consequent morbidity and mortality could not be yet made under good control.[14] (Figure 1).

For laboratory diagnosis of a viral infection, including the SARS-CoV-2 infections, usually multiple evidences are required starting from clinical presentations by the infected person to laboratory data exploring from the clinical specimens. Clinical presentations by the patients are due to the induced pathology upon cells or tissues of the host, culminating into tissue injury and the resultant clinical features experienced. Whereas, laboratory data mostly related to direct (the microorganism itself as observed by microscopic examination, or structural component of the organism as exemplified by detection of antigens or Nucleic acids by molecular diagnostics like Nucleic Acid Amplification Test (NAAT)) or indirect (by detection of antibodies produced in response to the antigens of the organism) evidences in favour of the suspect pathogen.

**Brief immunobiology of SARS-CoV-2**

While considering laboratory diagnosis of COVID-19, it directs to the identification of SARS-CoV-2, the causative virus, as a whole virion or its structural components like antigens or whole-genome nucleic acid or specific gene segments available in clinical specimens. Therefore, detailed knowledge on structural components and immunobiology of the virus including pathogenesis and evolution of the variants in essential to select right specimen along with the most appropriate test and laboratory preparedness.

As it is known that the SARS-CoV-2 was first documented by the CDC country office China as a novel coronavirus, and is an RNA virus containing approximately 27-32 kb of positive sense single stranded RNA.[9,10,15] This betacoronavirus is an enveloped virus, containing a large nucleoprotein (N) having three trans-membrane protein antigens (S) incorporated into the lipid envelope and two smaller proteins- membrane protein (M) and envelope protein (E).[16-18] When observed under an electron microscope, the virus appears as spherical particle with variable diameters around 100nm without spikes.[19,20] The genome of the SARS-CoV-2 has at least six open reading frames (ORFs) and accessory genes, comprising

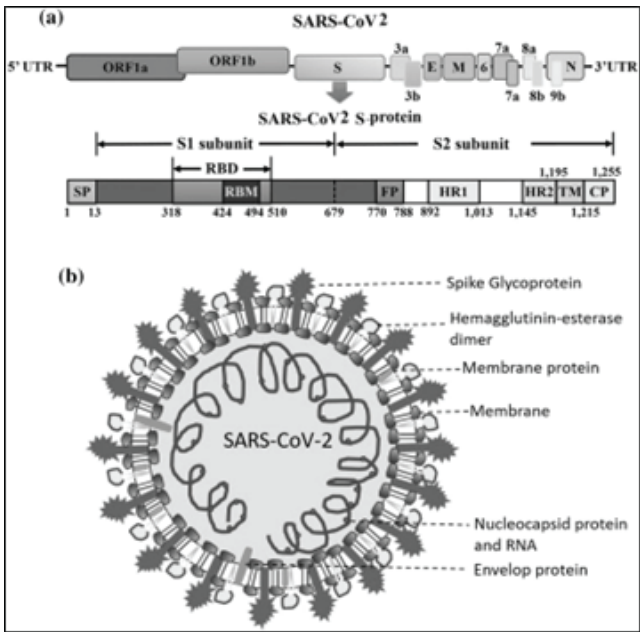


Figure 2: Schematic representation of (a) the genome and (b) virion structure of SARS-CoV-2.
[Reproduced with permission from original source available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196923/ ]

of 11 coding regions that encode 12 potential gene products. At 5′ terminal, two-thirds of the genome consists of two ORFs (e.g., ORF1 and ORF2), which encode two polyproteins namely pp1a and pp1ab which are further cleaved into 11 and 16 non-structural proteins, respectively. The 16 proteins are responsible for genome and viral replication. Whereas, at the 3′ terminal, genes for the structural proteins (e.g., S, E, M & N) are located. Other gene products include spike (S), ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF.[10,15,16,21] Four of these structural proteins are important for coronavirus infectivity, namely S, E, M and N.[13,15,16,21-23] The S protein is responsible for host specificity, viral attachment to the receptor and fusion with cell membrane.[13,22] The N protein interacts with viral RNA to form the ribonucleoprotein and protects viral RNA genome.[23,24] The E protein helps in virion assembly and ion channel actions.[14-16,18,19] The M protein is the key for assembly of viral particles by interacting with all other structural proteins.[13,22-26]

Investigators made it clear that like SARS-CoV-1, SARS-CoV-2 also infects humans through the angiotensin-converting enzyme (ACE-2) which is highly expressed in organs of the humans including respiratory and gastrointestinal tracts, blood vessels, bone marrow, spleen, thymus, lymph node, liver, kidney and brain. This receptor regulates the interspecies and human-to-human transmission through interactions with S protein of the virus.

Soon after the first wave of COVID-19 pandemic during early 2020, most of the areas of the globe face the second and subsequent waves of infection. Scientists could identify the strains of SARS-CoV-2 in the second wave by whole genome sequencing. The new strain in Houston, TX, USA were found to have a Gly614 amino acid replacement in the spike protein- these mutated variants have been found linked to increased transmission and infectivity. Patients infected with the Gly614 variant strains had significantly higher virus loads in the nasopharynx on initial diagnosis.[27]

While the world communities were facing repeated waves of infections and the life-threatening complications due to COVID-19, the globe is now under newer challenges of emerging variants of the SARS-CoV-2. The World Health Organisation (WHO) readily announced the simple, easy-to-say labels for SARS-CoV-2 variants of concern and variants of interest using letters of the Greek alphabet.[28] The variant lineages were also named using computational tool for Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLin/ PANGO Lineage).[29] Among the variants those having clear evidence indicative of a significant impact on transmissibility and severe morbidity have been identified as Variants of Concern (VOCs) and those having evidences that could imply a significant impact on transmissibility and severity have been identified as Variants of interest (VOIs). Some other variants with genetic changes and having no evidence of phenotypic or epidemiologic impact are suspected of posing a future threat are designated as 'Variants under monitoring (VUM)'.[30-32] Yet another group that have been reclassified on at least one of the following criteria: (i) the variant is no longer circulating, (ii) the variant has been circulating for a long time without any impact on epidemiological situation, (iii) scientific evidence demonstrates that the variant is not associated with any concerning properties, have been designated as 'Formerly Monitored/ De-escalated Variants'.[30,31]

**Approach to diagnose COVID-19**

**Testing strategies for SARS-CoV-2**

Selection of tests for SARS-CoV-2 in symptomatic or asymptomatic individuals depends upon the objective of screening, diagnosis or public health surveillance.[33]

**Screening tests:** are intended to identify people with SARS-CoV-2 infection who are asymptomatic and do not have known or suspected exposure to COVID-19 patients for the purpose of employment/work, travel and study. Screening test helps to identify the unknown cases, so that preventive measures can be taken for further transmission during the individual's stay or movement.[33]

**Diagnostic tests:** are intended to identify current infection and should be performed on any one who have signs and symptoms consistent with SARS-CoV-2 infection and/or following recent or suspected exposure to COVID-19 patients, irrespective of vaccination status of the individual.[33]

**Public health surveillance tests:** are intended as a part of the ongoing, systematic collection, analysis and interpretation of health-related data for planning, implementation and evaluation of the intervention measures to control and contain COVID-19 in a community.

**Specimen collection**

Before planning for the diagnostic approach for COVID-19 infection, appropriate selection of the specimen that will be analysed for the evidence is very important. Because, right selection as well as rightly collection of the appropriate specimen may yield the highest sensitivity of the diagnostic approach. At the same time, adequate safety measures (personal protective equipment (PPE) and packaging for transport) are very crucial for infection prevention and control perspective to break the chain of infection.[34,35]

Upper respiratory tract specimens, including nasopharyngeal swab (NPS) are usually collected. When collection of NPS is not possible, other upper respiratory specimens like oropharyngeal swab, nasal mid-turbinate swab, nasal swab from anterior nares, and nasopharyngeal wash/ aspirate can be collected.[36] In some instances, when infection spreads downwards to involve the lung parenchyma, the virus can be missed- in these cases, lower respiratory tract specimens like sputum or bronchoalveolar lavage fluid (BALF) may be the alternative choice.[37] For initial diagnostic testing, the Centers for Disease Control and Prevention (CDC), USA recommends collection and testing of upper respiratory

specimen.[38] In a study by Yang et al analysing more than 3.5k clinical specimens found that during the first 14 days of symptoms onset (dso), sputum possessed the highest positive yield (73.4%-87.5%), followed by nasal swabs (53.1%-85.3%) for both severe and mild cases of COVID-19.[39] The investigators could identify viral RNA from BALF collected from severe cases within 14 dso and lasted up to 45 days- notably, no viral RNA was identified in BALF from the mild cases. In another study, investigators compared throat washings, nasopharyngeal and oropharyngeal swabs among hospitalized and confirmed COVID-19 patients between 0-15 dso and found good sensitivities of 85%, 85% and 79% respectively.[40] Whereas, another study by Jeong et al demonstrated viable SARS-CoV-2 in saliva, urine and stool specimens of COVID-19 patients up to 11-15 dso. They also demonstrated that viral shedding in saliva, urine and stool specimens were almost equal to or higher than those in nasal/oropharyngeal swabs.[41] Viable viruses have also been isolated from urine and stool specimens from COVID-19 patients up to 11-15 days of clinical course.[41,42] Stool specimens were found excreting the viruses among patients who did not have diarrhoea.[43] In a rapid review, Zhou and O'Leary identified that assessing against a composite standard, anterior nares swabs are less sensitive (42-94%) than nasophayngeal swabs (79-100%).[44] Another systematic review and meta-analysis found sensitivity of saliva (88%) superior to nasal (82%) and oropharyngeal (84%) swabs.[45] And some investigators found saliva comparable to nasopharyngeal swab.[46]

After collection, all specimens for antigen detection should be placed in a tube containing viral transport medium (VTM) and transported to the laboratory as early as possible.[38]

For serological tests, blood is the specimen for detection of antibodies against the virus, including the vaccine effectiveness evaluation- although, some leading authorities including US FDA (Food and Drug Administration) do not recommend antibody testing to assess immune status after vaccination.[47] Antibody detecting tests are especially important among the asymptomatic individuals.

Specimens should be stored at 2-8$^{O}$C for up to 72 hours after collection- for further delays in shipment or testing, the specimens should be stored at -70$^{O}$C or below. For transport, specimens should be packed following triple package system for transport of infectious biological specimens.[34,38, 50]

**Infection prevention and control (IPC) measures:**

Although IPC measures are very essential for all aspects of COVID-19 patient management, including the safety of the patients and healthy people in the community as well as the healthcare workers and the environment, the recommended

Table 1: Specimens for diagnosis of COVID-19 infection

| Sl no | Specimen* | Indication | Test(s) name | Sensitivity | Highest Sensitivity on dso | Reference (s) |
|---|---|---|---|---|---|---|
| 1. | Nasopharyngeal swab | Screening, Diagnosis & Surveillance (suspected case of COVID-19 at OPD collected by a trained HCP) | NAAT, Ag-detecting RDT | 53.1-85.3% | 5-7 | 38,39 |
| 2. | Oropharyngeal swab | Diagnosis (suspected case of COVID-19 at OPD collected by a trained HCP) | NAAT | 45.7-72.7% | 5-7 | 38,39 |
| 3. | Nasal swab from anterior nares/ mid-turbinate swab | Screening & Diagnosis (suspected case of COVID-19 at home or OPD) | Ag-detecting RDT, NAAT | 42-94% | 5-7 | 38,43 |
| 4. | Nasal/ pharyngeal wash | Diagnosis (suspected case of COVID-19 at home or OPD) | NAAT | 85% | 5-7 | 38,39 |
| 5. | Saliva | Diagnosis (suspected case of COVID-19 at home or OPD) | NAAT | 88% | 5-7 | 38,44 |
| 6. | Sputum | Diagnosis (hospitalized cases with respiratory distress) | NAAT | 73.4-84.5% | 7-14 | 38,39 |
| 7. | Bronchoalveolar lavage fluid | Diagnosis (hospitalized cases with respiratory distress) | NAAT | 100% | 10-15 | 38,39 |
| 8. | Anal swab/ Faeces | Screening & Surveillance (convalescence period) | NAAT | 36.7% | 11-15 | 39, 41-43 |
| 9. | Urine | Screening & Surveillance (convalescence period) | NAAT | 0%-NA | 11-15 | 39, 41 |
| 10. | Blood | Screening & Surveillance (asymptomatic suspects) | Serology (ELISA) | 84.3% | 14-30 | 38, 48, 49 |

Dso- day after symptom onset, NA-not available, *Multiple specimens are recommended for greater sensitivity.

practices of IPC are equally important during laboratory handling of the patient for specimen collection, specimen preparations for testing and disposal. There are very specific recommendations for PPE use and disposal, specimen collection, handling, transport, testing and disposal by the global leading authorities of healthcare system.[34,35,38]

The SARS-CoV-2 is considered as a biosafety level-3 organism. All laboratories handling clinical specimens should perform risk assessments and follow standard precautions, including hand hygiene and use of specific PPE such as laboratory coat or gown, gloves, eye protection or a disposable mask and face shield to protect skin and mucous membrane of the eyes, nose and mouth.[34]

Work surfaces and equipment should be decontaminated using recommended disinfectants like hypochlorite solution, 70-90% Ethanol, povidone-iodine etc.[34,35]

Initial processing of specimens (before inactivation of viruses) should be performed in a properly validated biological safety cabinet (BSC) or an equivalent containment device. Non-propagative laboratory works like NAATs should be conducted in an environment equivalent to biosafety level-2 (BSL-2), whereas, propagative works like virus culture requires a containment laboratory with inward directional airflow equivalent to BSL-3. Point-of-care assays and antigen-detecting rapid diagnostic tests (RDTs) can be performed on a laboratory bench, wearing proper PPE and using appropriate disposal systems in place.[35]

**Selection of laboratory test method**

For the diagnostic approaches of COVID-19 infection considering sensitivity, specificity and current practices, four common panels of laboratory tests are considered: (i) molecular diagnostics- using the gene sequences of the virus that expresses different proteins of the virion (Nucleic Acid Amplification Tests (NAATs)), or the total genome sequencing for characterization to be useful for developing diagnostic approaches as well the vaccines; (ii) serological tests- the antigens and antibodies of the virus can be identified using corresponding antibody and antigen containing reagents that also includes the rapid tests at point-of-care (POC); (iii) microscopy- the virion (SARS-CoV-2) morphology can be identified by an electron microscope; and (iv) culture- the virus can be cultured in different cell lines including simian and human cells.

In the context of SARS-CoV-2, the newer concepts of laboratory diagnosis that include better antibody reagents and more sensitive assays for direct analysis of specimens, molecular genetics techniques and genomic sequencing for direct identification of the virus should be adopted primarily.

*(i) Molecular diagnostics:*

Molecular diagnostics in this section, actually refers to nucleic acid-based tests. Currently, nucleic acid amplification tests (NAATs) are the mainstay of confirmatory diagnosis of COVID-19. The NAATs detect nucleic acid (RNA) of SARS-CoV-2, usually from upper and lower respiratory tract and include but not limited to: reverse transcriptase polymerase chain reaction (rtRT-PCR) and isothermal amplification which includes nicking endonuclease amplification reaction (NEAR), transcription mediated amplification (TMA), loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HAD), clustered regularly interspaced short palindromic repeats (CRISPR) and strand displacement amplification (SDA).[47] The NAATs has been authorized for different settings, for examples- rtRT-PCR for laboratory setting with trained personnel or some others (isothermal rapid tests) can be performed at POC or even can be self-administered at home or at other non-healthcare locations.[51]

In addition, a cartridge-based nucleic acid amplification test (CBNAAT) GeneXpert, following documented high-level success and wide acceptability in diagnosis of tuberculosis, Cepheid Xpert Xpress SARS-CoV-2 have been authorized for emergency use by the US FDA,[52,53] as well by the WHO.[54] The Xpert Xpress is a 50-minutes RT-PCR-based assay detects the pan-sarbecovirus E gene and the N2 region of the N gene as its SARS-CoV-2-specific target.[52,55,56]

**Real time reverse transcriptase polymerase chain reaction (rtRT-PCR):**

The rtRT-PCR assay is the gold standard for diagnosis of COVID-19 and is one of the best and accurate laboratory methods for detecting, tracking and studying the SARS-CoV-2 from respiratory specimens, including saliva. This method amplifies a small segment of viral RNA genome, which is converted to cDNA first and then is amplified subsequently. The DNA amplification is monitored in real time using a fluorescent dye or a combination of a quencher molecule and a sequence-specific DNA probe labelled with a fluorescent molecule.[57,58] The most important aspect of this test is that the amplification and analysis are carried out in a closed system, minimising the chances of false positive reactions.[36] A variety of RNA gene targets are used by the manufacturers for one or more of the Helicase (*Hel*), envelope (env), nucleocapsid (*N*), spike (*S*), transmembrane (*M*), RNA-dependent RNA polymerase (*RdRp*) and open reading frame (*ORF1a* and *ORF1b*) genes.[58] In this assay, the viral RNA is measured by cycle threshold (ct)- the number of cycles required for the fluorescent signal to become detectable. A 'ct' value of less than 40 is reported as PCR-positive.[36] Meantime, the WHO published the 'Technical Specifications for Selection of Essential In Vitro Diagnostics for SARS-CoV-2 which includes the series of specifications for SARS-CoV-2 Nucleic Acid Test including intended use (detection target, test purpose, specimen type, testing population etc), performance characteristics (clinical sensitivity->95%, specificity->99%, limit of detection etc), technical and operational characteristics (principle of the assay, specimen stability, turnaround time-4-5 hours, test limitations, etc).[59]

Investigators developed and evaluated a novel, one step nested quantitative real-time PCR (OSN qRT-PCR) for highly sensitive detection of SARS-CoV-2 targeting *ORF1ab* and *N* genes.[60] The sensitivity of OSN qRT-PCR assay was 1 copy/reaction being 10-times higher than that of the commercial qRT-PCR kit (requiring 10 copies/ reaction) and some qRT-PCR negative specimens were detected by OSN qRT-PCR showing higher specificity. Other investigators analysed and validated the OSN qRT-PCR finding it superior showing great potential for detection of SARS-CoV-2, especially in patients with low viral load.[61]

**Loop-mediated isothermal amplification (LAMP)**

This is a novel method of nucleic acid amplification that can amplify few copies of DNA to $10^9$ in less than one hour under isothermal conditions and with higher specificities.[62] Scientists reported in 2020 that a reverse transcription loop-mediated isothermal amplification (RT-LAMP) have been developed for specific detection of SARS-CoV-2 designing the primer set to target the nucleocapsid gene of the virus RNA with detection limit of $10^2$ copies of RNA/reaction, which is close to that of qRT-PCR.[63] This test can specifically detect viral RNA of SARS-CoV-2 with no cross-reactivity with currently circulating other coronaviruses, MERS-CoV and other respiratory viruses including influenza viruses. This assay exhibited a rapid detection span of 30-minutes combined with colorimetric visualization and thus the isothermal amplification conjugated with a single tube colorimetric detection may contribute to a simple-to-perform, time-efficient, less expensive yielding high sensitivity and specificity for public health laboratories with limited capacities.

Another group of scientists also developed the RT-LAMP assay for SARS-CoV-2 using a mismatch-tolerant amplification technique and similarly, based on predominantly detection of the N gene.[64] For this purpose, they aligned the SARS-CoV-2 genomic sequence with those of the six other human coronaviruses and several sets of SARS-CoV-2-specific LAMP primers, targeting *N*, *S*, and RdRp genes, were developed. Comparing with a qRT-PCR assay, they found that the SARS-CoV-2-RT-LAMP assay has a high sensitivity and specificity with robust reproducibility and the results can be monitored using a real-time PCR machine or visualized by colorimetric change from red to yellow. The completed reaction time was within 30-minutes for a real-time fluorescence monitoring and 40-minutes for visual detection.

**GeneXpert Diagnostics**

In line of continuous demand of rapid, easy-to-use at POC, the GeneXpert concept of diagnosis, having previous excellent experiences with tuberculosis, have been considering by a few manufacturers.[52] The first of such test device with a rapid, real-time RT-PCR test for qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasopharyngeal, oropharyngeal, nasal or mid-turbinate swab or nasal swab/aspirate) was approved for emergency use (Emergency Use Authorization- EUA) by the US Food and Drug Authority on 21 March, 2020 in the name of 'Cepheid Xpert® Xpress SARS-CoV-2' test as well as by the WHO.[53,54]

The Cepheid Xpert® Xpress was then using throughout the globe encouraging many multi-center studies. In one of the multicenter studies in Wuhan, China, investigators reported 96.1% positive percent agreement (sensitivity) and 96.2% negative percent agreement (specificity) with Chinese National Medical Products Administration (NMPA)-approved RT-PCR.[65] Another group of researchers in USA also evaluated the Xpert® Xpress in their multiple centers and found positive agreement of 99.5% and negative agreement of 95.8% with standard-of-care NAATs with a short-time results in approximately 45 minutes.[66] The investigators recommended this technology for the acute-care hospitals in high-prevalent areas, where rapid triage decisions are required for better management of COVID-19 patients. Others also found almost similar results (among them, the UK group found a better agreement) and made similar recommendations.[67,68]

*(ii) Serological tests:*

**Antigen detecting rapid tests**

Upon the widespread expansion of COVID-19 infection, a wider proportion of infected community members and a rapidly increasing threat of infecting family inhabitants, there was a strong urge of extending the COVID-19 diagnostic test capacity for a cheaper, faster and easier-to-use at point-of-care (POC). Eventually, rapid diagnostic tests were designed by manufacturers throughout the globe and considering SARS-CoV-2 antigens (spike protein or nucleocapsid) detection by coating corresponding immobilized antibodies on the device.[69] Meantime, the WHO developed a set of technical specifications for selection of SARS-CoV-2 antigen-detecting rapid diagnostic tests (Ag-RDTs), including the detection target (nucleocapsid protein), specimen type (upper respiratory, nasopharyngeal or nasal swabs), test population, intended users, clinical sensitivity and specificity (minimum 80% and 97% respectively).[59]

There could be several different types of Ag-RDT kits like chemiluminescence immunoassay (CLIA), fluorescent immunoassay (FIA), lateral flow immunoassay (LFIA) or lateral flow fluorescent immunoassays- the lateral flow assays being commonly known as immunochromatographic tests (ICTs). The Ag-RDT results can be interpreted without any instrument and available within 10-30 minutes.[59,70,71]

As the Ag-RDTs perform best in individuals with high viral load, WHO recommends that the Ag-RDTs are indicated for the following specific populations and settings: (i) for primary case detection in symptomatic individuals suspected to be infected and asymptomatic individuals at high risk of

COVID-19; (ii) for contact tracing; (iii) during outbreak investigations and (iv) to monitor trends of disease incidence in communities as well as to use the Ag-RDTs that meet minimum performance requirements of >89% sensitivity and >97% specificity.[59]

But the Ag-RDTs are not always promising. In a study in University Hospital Tor Vergata, Rome, Italy during May-September 2020 with 50 nasopharyngeal swabs (collected from emergency department or infectious diseases ward) tested by COVID-19 Ag Respi-Strip (Coris Bioconcept, Belgium), sensitivity of the rapid antigen test was found 30.77%.[72] Whereas, investigators in another study in Thailand during March-May 2020, rapid antigen detecting test prerformed by StarndardTM Q COVID-19 Ag kit (SD Biosensor®, Republic of Korea) from respiratory specimens found a very high sensitivity and specificity of 98.33% and 98.73% respectively. They compared the antigen tests with the real-time RT-PCR test (AllplexTM2019 n-CoV assay (Seegene Korea).[73] The Results of these two studies were also evaluated for diagnostic accuracy by the Cochrane database systematic review. They included forty-eight studies reporting fifty-eight evaluations for antigen tests. They found that estimates of sensitivities were varying considerably among the studies and there were differences between symptomatic and asymptomatic patients (72.0% and 58.1% respectively). Average sensitivity was higher in the first week (78.3%) after symptom onset than in the second week (51.0%) of symptoms. The authors of the Cochrane review also found that the sensitivity was higher in those with PCR cycle threshold (ct) values <25, compared to those with ct values >25 (94.5% vs 40.7%).[74] Nevertheless, an ultra-rapid (within 3 minutes) antigen detection test was found very promising (93.3% sensitivity and 100% specificity) for qualitatively detecting nucleocapsid protein of the virus from nasopharyngeal swabs.[71]

There could be also false positive results with the Ag-RDTs, because of the cross-reacting antibodies embedded with other coronaviruses circulating in a community. These unfortunate false positive results are mostly associated with tests that target nucleoprotein (NP) antigens- whereas, tests that target a highly conserved subunit (S1) of the spike protein of the virus are less likely to yield false-positive reaction. However, mutations in the spike protein occurs frequently among variants of the virus leading to invalidate these test potentialities. Therefore, laboratories and the COVID-19 management team should be careful about the possible false-negative and false-positive results with the Ag-RDTs.[75]

Ag-RDTs for COVID-19 are mostly positive when viral loads are the highest and patients are most infectious- typically 1-3 days prior to and during the first 5-7 days after onset of symptoms.[76]

**Antibody detecting assays**

Serological tests to detect antibodies (IgA, IgM and IgG) to SARS-CoV-2 have been using in people with active infection and in convalescent cases. Because seroconversion occurs with a median range of 18-21 days after exposure to the virus, the antibody-detecting assays are not suitable for diagnosis of the early stage COVID-19 infections.[77] But the antibody-detecting tests are found very promising especially in low resource countries.[77,78] In a Cochrane database systematic review on antibody tests for identification of current and past infection with SARS-CoV-2 found substantial heterogenicity in sensitivities of IgA, IgM and IgG antibodies- which showed low sensitivities during the first week of symptoms onset, rising in the second week and reaching their highest values in the third week.[79] The combination of IgM/IgG showed pooled sensitivities of 30.1% during 1st week, 72.2% during the 2nd week and 91.4% during the 3rd week. During the next weeks (21 to 35 days), sensitivities for IgM/IgG were 96.0%. Similarly, in an evaluation of performances of two rapid IgM-IgG combined antibody tests, comparing with RT-PCR results, showed 100% specificity and varying sensitivities from 35.7% (0-5 days) to 100% (in patients >15 days of symptoms onset).[80]

Two kinds of antibody-detecting tests are currently available: (i) quantitative antibody detecting enzyme linked immunosorbent assay (ELISA), and (ii) point-of-care qualitative lateral flow chromatographic immunoassays (RDTs).

The ELISA kits are usually based on recombinant nucleocapsid (rN) or spike (receptor-binding domain) (rS) proteins, where ELISA plates are coated with monoclonal mouse anti-human IgG/IgM and subsequent steps including addition of sera specimens, incubation, washing, addition of enzyme (horseradish peroxidase, HRP)-conjugated rN/rS proteins, washing and addition of substrate (tetramethylbenzidine, TMB), incubation and finally reading are similarly employed as for other sandwich ELISAs.[48,81]

In the study by Pan et al, the investigators found similar increasing sensitivities with the days of symptoms onset (11.1%, 92.9% and 96.8% among blood specimens collected during the 1st week, 2nd week and after 2nd week respectively) with colloidal gold-based immuno chromatographic strips targeting SARS-CoV-2 IgM or IgG or both, considering RT-PCR as gold standard with nasopharyngeal swabs from the patients.[78] The rates of IgG detection were higher at all three stages of infection and combined IgM-IgG showed the highest positivity during the intermediate stage (2nd week of symptoms onset).

However, as mentioned earlier, the US Food and Drug Authority (FDA) recommended that the currently authorized SARS-CoV-2 antibody tests should not be used to evaluate a person's level of immunity or protection from COVID-19 at any time, especially after COVID-19 vaccination.[47]

*(iii) Microscopy:*

The first electron micrograph of a virus (Poxvirus) was published in 1938 and since then, the electron microscope (EM) was one of the first methods to diagnose viral diseases

during the 1940s, and this has been a reliable tool for classification of viruses following their ultrastructure.[82,83] The EM can be applied to many biological specimens and can also hasten routine cell culture diagnosis by observing the growing viruses.[84]

The EM was later associated to virus isolation by cell culture[85,86] and serological methods.[2,87,88] However, after development of the molecular methods of diagnosis, namely real-time quantitative PCR methods or direct nucleic acid extraction associated to next generation sequencing from clinical specimens replaced almost all microscopic tests.

Microscopical identification of SARS-CoV-2 requires either Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM).[89] However, SEM was proved to be very rapid and efficient tool compared to classical TEM providing a detailed and complete infectious cycle.[90,91] Although, Indian scientists identified the 70-80 nm round virus particles with surface structures on the envelope as morpho-diagnostic features of coronavirus-like particles in the real-time RT PCR-confirmed clinical specimens by TEM, they recommended that imaging thin sections of infected cells by conventional and cryo-ultramicrotomy methods could provide more detailed information that they could not resolve of some interesting features in their images.[92] However, some other scientists in USA earlier used the cryo-electron Microscope to identify the spike glycoprotein trimers of the virus to facilitate medical countermeasure development.[93] The scientists could identify the predominant state of the spike glycoprotein molecule having one of the three receptor-binding domains and they also provided the biophysical and structural evidence that 2019-nCoV (later renamed SARS-CoV-2) spike protein binds angiotensin-converting enzyme (ACE)-2 with higher affinity than SARS-CoV does.

Figure 3 Scanning Transmission Electron Microscope (STEM). Available online at: https://uwaterloo.ca/metrology/tem-stem

In spite of many advantages provided by the electron microscopy, the test procedure has some default limitations for routine usage: (a) requires high costly set up, (b) extensive experience of analysis and interpretation required to rightly identify the virus particles from other cytoplasmic structures in an infected cell.[94,95]

*(iv) Culture:*

As viruses are obligate intracellular parasites, their propagation requires living cells and viral culture has long been considered as 'gold standard' for diagnosis of viral diseases because it secures an isolate for further analysis.[96]

The value of viral isolation is exemplified by its most significant role in providing epidemiological data, in the diagnosis of new or unknown infections and yielding infectious virions for further study.[20, 97,98] Likewise, the emergence of COVID-19 disease by SARS-CoV-2 was rapidly identified by isolation of the virus by co-culture into VERO cells (kidney epithelial cells of African green monkey) as well as into human airway epithelial cells.[6,98] These isolations rapidly encouraged the testing for antiviral agents' susceptibility and repurposing of newer agents.[98] Further cell lines were also explored and found 6 Simian and one more human cell line (Caco-2) to support growth of the SARS-CoV-2. The cytopathic effects (CPE) were found variable- the lysis of cell monolayer observed within 48-72 hours in the Simian cell lines and no CPE was found in Caco-2 in spite of intense multiplication.[98]

Scientists recently developed a biosafety level-2 cell culture system for production of transcription and replication competent SARS-CoV-2 virus-like particle (trVLP) using the Caco-2 cells. They developed a 96-well format high throughput screening for antivirals discovery and identified some potent antivirals (salinomycin, tubeimoside I, monensin sodium, lycorine chloride and nigericin sodium) against SARS-CoV-2.[99]

**Detection of Variants**

Several variants of the circulating SARS-CoV-2 are now of great concern (Variants of Concern-VOCs) and monitoring of theses variants are the essential events of management of the pandemic.[30,31,100] Although nucleic acid amplification tests (NAATs), based on reverse transcriptase (rtRT-PCR), are generally considered as a gold standard for detection of SARS-CoV-2 and some of these tests can use one or multiple target genes for amplification, and some of the SARS-CoV-2 VOCs (e.g., Alpha [B.1.1.7] and Omicron [B.1.1.529]) generate a negative (S-gene target failure [SGTF] or significantly weaker positive S-gene result in the RT-PCR assays, some assays that include an S-gene target may fail

detection of the VOCs.[101-106]

## Conclusion

Rapid and accurate identification of SARS-CoV-2 is the mainstay of COVID-19 patient management. Sooner the appropriate diagnosis, earlier the healthcare professionals would be able to manage cases of COVID-19 to contain the infection along with its life-threatening complications. Newer methods are currently introducing into every nation increasing capacities of the healthcare workers. It is highly expected that very soon, the pandemic will come to an end with the coordinated management efforts all over the globe.

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viii. ***Tables-*** Prepare tables according to standard requirements. Include tables after 'Reference' section to supplement and not to duplicate the text in 'Methodology' or 'Results' sections. Number tables consecutively in the order of their first citation in text and supply a title for each. Title of the table should be short but self-explanatory, containing information that allows readers to understand the table's content without going back to the text. Be sure that each table is cited in the text. Give each column a short or an abbreviated heading. Place explanatory matter in footnotes, not in the heading. Explain all non-standard abbreviations in footnotes and use symbols to explain information, if needed. For using data from another published or unpublished source, obtain permission and acknowledge that source fully.

ix. ***Illustrations (Figures)***- Should be either professionally drawn and photographed or submitted as photographic quality digital prints. For Radiological and other clinical/ diagnostic images as well as pictures of Pathology specimens or photomicrographs, send high-resolution photographic image files. Figures should be made as self-explanatory as possible. Ensure titles and detailed explanations belong in the legends- not on the illustrations (figures) themselves. Number figures consecutively in the order as they been cited in the text. If a figure has been published previously, acknowledge the original source and submit written permission from the copyright holder to reproduce it. In the manuscript, legends for illustrations should be on a separate page, with Arabic numerals corresponding to illustrations.

x. ***Units of measurement-*** Measurements of length, height, weight and volume should be in metric units (meter, kilogram or liter) or their decimal multiples. Temperatures should be in Celsius. Blood pressures should be in millimeters of Mercury.

xi. ***Abbreviations and symbols-*** Use only standard abbreviations. Avoid abbreviations in the 'Title' of the manuscript. The spelled-out abbreviation followed by the abbreviation in parenthesis should be used on first mention, unless the abbreviation is a standard unit of measurement.

**B.2.2. For Case Reports-** Limit within 2,000 words excluding up to 30 references and up to 4 tables and figures- corresponding a maximum of 3 printed pages of the JMoMC. Divide text into an abstract, an introduction, the case presentation, discussion and conclusion. For using identifiable pictures of patients, provide patient's informed consent for this publication which includes his/her awareness of possible consequences after publication.

**B.2.3. For Reviews-** Limit within 4,000 words excluding up to 110 references and up to 6 tables and figures. Divide text into an abstract, an introduction that outlines the main themes, brief subheadings and/or an outline of important unresolved questions.

**B.2.4. For Letters to Editor-** Limit within 500 words excluding up to 5 references and up to 2 tables and figures- that corresponds to 1 printed page of the JMoMC.

**C. Manuscripts management for JMoMC**

**C.1. Manuscript receive and management:** Manuscripts are received throughout the year and a submitted manuscript is usually published and posted to the author within a highest of 9-months of submission. However, this timeline may be prolonged in cases of: (a) bad submission time (3-months before publication datelines June and December each year), unless requested); (b) bad preparation (not followed appropriately the JMoMC requirements), (c) bad responses (failing to respond within set timeline and response is inadequate).

**C.2. Stages and timelines of Management**

**C.2.1. Stage 1:** Editorial Scanning (usually completed in 1st month of submission)

a. Received papers are entered into receive register giving an ID and acknowledged;
b. Editorial scanning- checked for appropriateness, integrity and plagiarism;
c. Primary author response- sent to corresponding author for primary response.

**C.2.2. Stage 2:** Peer Review (usually completed in 2nd month of submission)

a. Processed for Peer reviews (select Peer(s), sent to reviewers with timeline);
b. Sent to corresponding author for responses with a timeline;
c. Cross-check by Editorial staff for accommodation of the review comments.

**C.2.3. Stage 3:** Decision of Acceptance/ Rejection (usually completed within 4th month of submission)

a. Information of 'Acceptance'/ 'Rejection' communicated with the corresponding author;
b. Accepted papers are processed for Pre-Press version and submitted to Printing Press;
c. Decisions of rejections of the submissions are made in cases of serious violation of publication ethics including plagiarism, allegations of misconduct pre- and post-publication, authorship dispute, undisclosed conflict(s) of interest, research misconduct (fabricated study and data falsification), unethical practice during research, and duplicate publication.

**(2) Stage 4: Publication (usually within 9th month of submission)**

a. Printed hard copies are distributed soon after publication (in no cost currently) to the faculty members of MoMC, Libraries of the BMDC-registered Medical/ Dental institutions in Bangladesh and the authors of the publications;
b. Usually 3-copies of the published issue are sent for the authors of a publication to the address of communication of the corresponding author.

# Journal of Monno Medical College

## REVIEWERS OF THE ISSUE (June, 2022, Volume 8, Number 1)

1. Professor SM Akram Hossain, MBBS, M.Phil. (Anatomy), MHPEd (Australia)
2. Professor Saif Ullah Munshi, MBBS, M.Phil. (Virology), PhD
3. Professor ATM Farid Uddin, MBBS, M.Phil (Pharmacology)

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