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## Diseases

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Highlights

Acute Lymphoblastic Leukemia

Childhood Asthma in Upper Egypt

Discovering Thoughts, Inventing Future

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DISEASES  
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## Parasitic Infection as a Risk Factor for Childhood Asthma in Upper Egypt

By Alameldin M. Abdallah MD, Randa E.Abd-Elkader MD  
& Doaa A.Yones MD

*Assiut University*

**Abstract- Background:** Asthma and allergic diseases are serious public health problems in many middle and low-income countries. We examined the relationship between parasitic infection and the development and severity of asthma among children living in Assiut Governorate, Upper Egypt.

**Methods:** A cross sectional study was conducted on 140 children suffering from bronchial asthma (78 males and 62 females) aged from 5 to 14 years attending Assiut University Children Hospital. As well as 70 apparently healthy children with matched age and sexas controls. Beside meticulous history taking and clinical examination all patients and controls undergone; pulmonary function test, stool analysis, antibodies to *Toxocara canis*, antibodies to *Ascaris lumbricoides*, IL-5 level and Leukotriene E4.

**Results:** *Ascaris lumbricoides* and *Toxocara canis* infections were detected in sera of 26 (18.6%), 26 (18.6%) patients respectively, whereas *Giardia* infection was detected in stool of 28 (20%) of patients. Among patients infected with *Ascaris* 15,9, and 2 patients had severe, moderate and mild asthma respectively.

**GJMR-F Classification:** NLMC Code: WC 695



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# Parasitic Infection as a Risk Factor for Childhood Asthma in Upper Egypt

Alameldin M. Abdallah MD <sup>α</sup>, Randa E. Abd-Elkader MD <sup>σ</sup> & Doaa A. Yones MD <sup>ρ</sup>

**Abstract- Background:** Asthma and allergic diseases are serious public health problems in many middle and low-income countries. We examined the relationship between parasitic infection and the development and severity of asthma among children living in Assiut Governorate, Upper Egypt.

**Methods:** A cross sectional study was conducted on 140 children suffering from bronchial asthma (78 males and 62 females) aged from 5 to 14 years attending Assiut University Children Hospital. As well as 70 apparently healthy children with matched age and sex as controls. Beside meticulous history taking and clinical examination all patients and controls undergone; pulmonary function test, stool analysis, antibodies to *Toxocara canis*, antibodies to *Ascaris lumbricoides*, IL-5 level and Leukotriene E4.

**Results:** *Ascaris lumbricoides* and *Toxocara canis* infections were detected in sera of 26 (18.6%), 26 (18.6%) patients respectively, whereas *Giardia* infection was detected in stool of 28 (20%) of patients. Among patients infected with *Ascaris* 15, 9, and 2 patients had severe, moderate and mild asthma respectively. While among patients infected with *Toxocara* 13, 10, and 3 patients had severe, moderate and mild asthma respectively. As regard patients infected with *Giardia* 15, 12 and 1 patients had severe, moderate and mild asthma respectively. Among controls *Giardia* infection was detected in stool of 4 children (2.8%). Among controls *Giardia* infection was detected in stool of 4 children (2.8%).

**Conclusion:** Infection with *Ascaris*, *Toxocara* and *Giardia* is more common among asthmatic children so infection with these parasites may be a risk factor for bronchial asthma among Upper Egyptian children.

## Briefpoints

**What is known:** The multidimensional relationship between parasitic infections and asthma. and atopy.

The immunomodulatory effects of some parasites and their protective effects upon asthma.

*A. lumbricoides* eggs were associated with an increased prevalence of asthma.

**What is to add:** Infection with *Ascaris*, *Toxocara* and *Giardia* is more common among asthmatic children than healthy children.

Infection with these parasites may be a risk factor for development of bronchial asthma among Upper Egyptian children.

Infection with these parasites may be a risk factor for increased asthma severity among these asthmatic children.

## 1. BACKGROUND

Asthma as one of the most common allergic diseases causes major public health problem in many developed and developing countries. Asthma is characterized by chronic inflammation of the airways and it is one of the most common diseases among children worldwide. Asthma affects 300 million people worldwide<sup>1</sup>.

*What is known* The multidimensional relationship between parasitic infections and asthma. and atopy has been previously reported in many studies<sup>2</sup>. However, the association between parasitic infection and childhood asthma and atopy remains controversial<sup>3</sup>.

The immunomodulatory effects of some parasites and their protective effects upon asthma had been addressed in many studies. On the other hand *A. lumbricoides* eggs were associated with an increased prevalence of asthma and anti-*Ascaris* IgE had been reported to be associated with an increased risk of asthma symptoms<sup>4</sup>.

Human toxocariasis is a cosmopolite helminthic zoonosis caused by *Toxocara canis* and *Toxocara cati*, which are common roundworms of dogs and cats, respectively<sup>5</sup>. It has been reported that an increased risk of wheeze in some populations may be associated *Toxocara* infections and that may be caused by the host response to the parasite or by parasite-enhanced Th2 responses to aeroallergens<sup>6</sup>.

Activation of Th2-type immune response which takes place in giardiasis and proved by enhanced IgE production pointed to and confirmed its association with allergy. Also IgE production is larger and more severe in allergy-complicated giardiasis than that of uncomplicated cases<sup>7</sup>.

The aim of this study was to assess the relationship between certain parasitic infection and the development and severity of asthma among children living in Assiut Governorate, Upper Egypt.

**What is to add** Infection with *Ascaris*, *Toxocara* and *Giardia* is more common among asthmatic children so

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infection with these parasites may be a risk factor for bronchial asthma among Upper Egyptian children.

## II. MATERIALS AND METHODS

A cross-sectional descriptive study was performed which included 140 children with persistent bronchial asthma (78 males and 62 females) recruited at Assiut University Children Hospital, during the period from January, 2015 to January, 2016. Their ages were ranging from 5 to 14 years. As well as 70 apparently healthy children with matched age and sex were participated as controls.

### Inclusion criteria

Agreement to participate; recurrent episodes of coughing, wheezing and breathlessness, especially if aggravated or triggered by exposure to inhaled allergens, viral infection or exercise and relieved by the use of bronchodilators, corticosteroids or subcutaneous epinephrine. Children should not take anti-parasitic medication in the previous 6 months and provided three samples for parasite tests on alternate days.

### Exclusion criteria

Not meeting all inclusion criteria, other causes of wheezy chest such as: tuberculosis, foreign body inhalation, bronchiectasis, bronchopneumonia or any other anatomic or congenital malformations

*All cases and controls included in the study were subjected to:*

- i. Meticulous history taking including
- ii. Thorough clinical examination
- iii. *Laboratory investigations:* pulmonary function tests (PEFR and FEV<sub>1</sub>), stool examination, absolute eosinophilic count, IgE antibodies to *Ascaris lumbricoides* by serology, IgG antibodies to *Toxocara canis* by ELISA, serum IL-5 level and urinary Leukotriene E4 in urine.

As regard the severity of asthma, we classified patients into 3 groups according to the Global Initiative for Asthma 2002<sup>8</sup>.

*Group I:* 20 patients had mild persistent asthma (12 males and 8 females).

*Group II:* 60 patients had moderate persistent asthma (34 males and 28 females).

*Group III:* 60 patients had severe persistent asthma (32 males and 26 females).

### a) Stool Examination

We collected stool samples from all participants in sterile clean stool plastic disposable cups with lids labeled with the patient's serial number, name, age, and sex, group of BA and date of collection. Within half an hour all collected samples were examined parasitologically. We used iodine and lactophenol cotton blue for direct wet smear. Then, fomol-ether

sedimentation was done to the stool samples and examined.

### b) Urinary Leukotriene E4

Urinary LTE4 levels were assessed using the commercially available enzyme immunoassay (Cayman Chemical; Ann Arbor, MI, USA).

### c) Blood Samples

We collected blood samples from the participants by venipuncture. Cellular assay (AEC) was performed (Eosinophilia corresponded to levels above 400/mm<sup>3</sup>), then the serum samples collected were stored at -70°C until the serological analysis.

### d) Total IgE levels

We used ELISA to measure total IgE levels where levels above 200 IU/mL were considered high. All samples were measured in duplicate.

### e) Human IL-5 Level Assay

Human enzyme-linked immunosorbent assay kitare used to measure IL-5 levels (Biosource International, Inc., Camarillo, California, USA), according to the manufacturer's instructions. The lowest level of detection of IL-5 was 2 pg/mL. The intra-assay coefficient of variation was 7.4%, and the inter-assay coefficient of variation was 10%.

### f) Detection of *Ascaris lumbricoides* Infection in serology

We measured specific IgE levels against *Ascaris* by the CAP-FEIA fluoro enzyme immunoassay method (Phadia AB, Uppsala, Sweden).

### g) Detection of *Toxocara canis* Infection in serology

Were prepared excretory/secretory antigens from laboratory cultivated second stage larvae of *T. canis* according to the method of Sugan et al.<sup>9</sup>. The antigen was stored at -70°C until used as a crude antigen. We used ELISA technique to detect IgG against *T. canis* according to Van Kanpen<sup>10</sup>. ELISA plates (Flow Lab. Cat. No., 76-321-05) were coated by the prepared antigen.

### h) Statistical analysis

We used SPSS statistics version 22 (IBM Corporation, NY, USA) to analyze our data. Values were expressed as means and standard deviation (SD). Qualitative variables were presented as number (n) and percentage (%). We used Chi-square test to compare qualitative variables between groups. Unpaired *t*-test and Mann-Whitney "U" tests were used to compare quantitative variables. Anti-*Ascaris* IgE was classified into quartiles based on the distribution of the study participants.

## III. RESULTS

Regarding pulmonary functions, all groups of patients showed significantly lower PEFR% and FEV<sub>1</sub>%

than controls but only FEV<sub>1</sub>% was insignificantly lower in mild group than controls. Regarding AEC, all patients showed significantly higher values than controls. IL-5 was significantly higher in different groups of patients

than controls. Furthermore, asthmatic patients whatever collectively or subgroups showed significantly higher urinary LTE<sub>4</sub> levels than controls (Table 1).

**Table (1):** Pulmonary functions, A.E.C., serum IL-5 and urinary LTE4 of studied patients versus controls

	I Patients (n: 140)	II Mild patients (n:20)	III Moderate patients (n:60)	IV Severe patients (n: 60)	V Controls (n: 70)	P.value			
						I vs V	II vs V	III vs V	IV vs V
1- Pulmonary functions: - PEFR (%) (mean ± SD)	57.540 ± 15.058	71.800± 14.551	61.933± 9.958	48.400 ± 14.075	98.350 ± 0.587	0.000 HS	0.000 HS	0.000 HS	0.000 HS
- FEV <sub>1</sub> (%) (mean ± SD)	62.140 ± 15.554	85.900 ± 6.350	67.967 ± 5.236	48.400 ± 10.516	94.300 ± 19.850	0.000 HS	0.206 NS	0.000 HS	0.000 HS
3- A.E.C (mean ± SD)	731.930 ± 244.377	332.600 ± 100.603	643.467 ± 90.239	953.500 ± 122.081	121.950 ± 51.635	0.000 HS	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	46.3 ± 31.7	13.300 ± 3.683	26.850 ± 4.957	74.333 ± 30.335	6.725 ± 3.952	0.000 HS	0.000 HS	0.000 HS	0.000 HS
5- LTE <sub>4</sub> (pg/ml) (mean ± SD)	394.9 ± 287.2	110.125 ± 49.441	269.038 ± 47.010	656.333 ± 259.756	35.222 ± 5.044	0.001 MS	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate

A.E.C: Absolute Eosinophilic Count

HS: Highly significant P<0.001)

FEV<sub>1</sub>: Forced Expiratory Volume in 1 second

ABG: Arterial Blood Gases

NS: Non significant (P>0.05)

IL-5: Interleukin-5

S: Significant (P<0.05)

LTE<sub>4</sub>: Leukotriene E<sub>4</sub>

MS: Moderately significant (P<0.005)

Table (2) Patients with severe and moderate asthma showed significantly lower PEFR% and FEV<sub>1</sub>% than mild patients and also severe patients showed significantly lower PEFR% and FEV<sub>1</sub> % than moderate patients.

As regard AEC, serum IL-5 and urinary LTE<sub>4</sub>, severe and moderate asthmatics showed significantly higher values than mild patients. Also, severe patients showed significantly higher values compared to moderate patients.

**Table (2):** Pulmonary functions, A.E.C., serum IL-5 and urinary LTE4 of asthmatic children in relation to severity

	I Mild patients (n 20)	II Moderate patients (n: 60)	III Severe patients (n:60)	P. value		
				I vs II	I vs III	II vs III
1- Pulmonary functions: - PEFR (%) (mean ± SD)	71.800 ± 14.551	61.933 ± 9.958	48.400 ± 14.075	0.021 S	0.000 HS	0.000 HS
- FEV <sub>1</sub> (%) (mean ± SD)	85.900 ± 6.350	67.967 ± 5.236	48.400 ± 10.516	0.000 HS	0.000 HS	0.000 HS
3- A.E.C (mean ± SD)	332.600 ± 100.603	643.467 ± 90.239	953.500 ± 122.081	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	13.300 ± 3.683	26.850 ± 4.957	74.333 ± 30.335	0.000 HS	0.000 HS	0.000 HS
5- LTE <sub>4</sub> (pg/ml) (mean ± SD)	110.125 ± 49.441	269.038 ± 47.010	656.333 ± 259.756	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate

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Table (3) Among the studied patients *Ascaris lumbricoides* and *Toxocara* infections showed similar occurrence where they were detected in sera of 26 (18.6%), whereas *Giardia* infection was detected in

stools of 28(20%) of patients. Among 26 patients infected with *Ascaris* 15 patients have severe asthma, 9 patients have moderate asthma and 2 patients have mild asthma while among 26 patients infected with

*Toxocara* 13 patients have severe asthma, 10 patients have moderate asthma and 3 patients have mild asthma. As regard 28 patients infected with *Giardia* 15 patients have severe asthma, 12 patients have

moderate asthma and 1 patient have mild asthma. Among controls only *Giardia* infection was detected in stools of 4 (2.8%) of controls. Polyparasitism was not detected among patients or controls.

**Table (3):** Prevalence of parasitic infection among the examined asthmatic patients and controls

Parasite	Patients (n)	(n:140) (%)	Controls (n)	(70) (%)
<i>Ascaris lumbricoides</i>	(26): 15 severe, 9 moderate, 2 mild	18.6	0	0
<i>Toxocara canis</i>	(26) 13 severe 10 moderate 3 mild	18.6	0	0
<i>Giardia lamblia</i>	28 15 severe 12 moderate 1 mild	20	4	2.8
Polyparasitism	0	0	0	0

Table (4) Regarding pulmonary functions, no significant difference was found between patients who were positive and those who were negative regarding *Ascaris* infection whereas, both groups showed significantly lower values of PEFR% and FEV<sub>1</sub> % compared to controls.

Regarding AEC, patients with positive *Ascaris* infection showed significantly higher value than those

with negative *Ascaris* infection. Both groups showed significantly higher values of AEC compared to controls. Regarding serum IL-5 and urinary LTE<sub>4</sub>, patients who were positive for *Ascaris* infection showed significantly higher values than those with negative *Ascaris* infection. Furthermore, both groups showed significantly higher values of serum IL-5 and urinary LTE<sub>4</sub> compared to controls.

**Table (4):** Pulmonary functions, A.E.C, serum IL-5 and urinary LTE<sub>4</sub> in patients with +ve and -ve *Ascaris lumbricoides* infection versus controls.

	I Patients with +ve <i>Ascaris</i> infection by serology (n : 26)	II patients with -ve <i>Ascaris</i> infection by serology (n:114)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	45.385 ± 11.057	58.260 ± 15.822	98.350 ± 0.587	0.000 HS	0.000 HS	0.406 NS
- FEV <sub>1</sub> (%) (mean ± SD)	54.846 ± 12.096	63.810 ± 15.860	94.300 ± 19.850	0.000 HS	0.000 HS	0.060 NS
3- A.E.C (mean ± SD)	888.000 ± 249.733	696.330 ± 230.814	121.950 ± 51.635	0.000 HS	0.000 HS	0.010 S
4- IL-5 (pg/ml) (mean ± SD)	62.769 ± 37.468	41.272 ± 30.332	6.725 ± 3.951	0.000 HS	0.000 HS	0.031 S
5- LTE <sub>4</sub> (pg/ml) (mean ± SD)	665.833 ± 308.584	340.950 ± 253.548	35.222 ± 5.044	0.000 HS	0.001 MS	0.009 MS

PEFR: Peak Expiratory Flow Rate

FEV<sub>1</sub>: Forced Expiratory Volume in 1 second

IL-5: Interleukin-5

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NS: Non significant (P>0.05)

MS: Moderately significant (P<0.005)

Table (5) Regarding pulmonary function, no significant difference was found between patients with positive and negative *Toxocara* infection whereas, both

groups showed significantly lower values of PEFR% and FEV<sub>1</sub> % compared to controls.



Regarding AEC, patients who were positive for *Toxocara* infection showed significantly higher value than those with negative *Toxocara* infection. Both groups showed significantly higher values of AEC compared to controls. Regarding serum IL-5 and urinary

LTE<sub>4</sub>, patients who were positive for *Toxocara* infection showed significantly higher values than those with negative *Toxocara* infection. Furthermore, both groups showed significantly higher values of serum IL-5 and urinary LTE<sub>4</sub> compared to controls.

**Table (5):** Pulmonary functions, A.E.C, serum IL-5 and urinary LTE4 in patients with +ve and –ve *Toxocara canis* infection versus controls

	I Patients with +ve <i>Toxocara</i> infection by serology (n :26)	II patients with –ve <i>Toxocara</i> infection by serology (n:114)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	45.385 ± 11.057	58.260 ± 15.822	98.350 ± 0.587	0.000 HS	0.000 HS	0.406 NS
- FEV <sub>1</sub> (%) (mean ± SD)	54.846 ± 12.096	63.810 ± 15.860	94.300 ± 19.850	0.000 HS	0.000 HS	0.060 NS
3- A.E.C (mean ± SD)	888.000 ± 249.733	696.330 ± 230.814	121.950 ± 51.635	0.000 HS	0.000 HS	0.010 S
4- IL-5 (pg/ml) (mean ± SD)	62.769 ± 37.468	41.272 ± 30.332	6.725 ± 3.951	0.000 HS	0.000 HS	0.031 S
5- LTE <sub>4</sub> (pg/ml) (mean ± SD)	665.833 ± 308.584	340.950 ± 253.548	35.222 ± 5.044	0.000 HS	0.001 MS	0.009 MS

PEFR: Peak Expiratory Flow Rate

FEV<sub>1</sub>: Forced Expiratory Volume in 1 second

IL-5: Interleukin-5

LTE<sub>4</sub>: Leukotriene E<sub>4</sub>

A.E.C: Absolute Eosinophilic Count

ABG: Arterial Blood Gases

S: Significant (P<0.05)

HS: Highly significant (P<0.001)

NS: Non significant (P>0.05)

MS: Moderately significant (P<0.005)

Table (6) Regarding pulmonary functions, *Giardia* positive patients showed significantly lower PEFR % and FEV<sub>1</sub>% than patients with negative *Giardia* infection. Furthermore, both groups showed significantly lower PEFR% and FEV<sub>1</sub>% compared to controls

Regarding AEC and urinary LTE<sub>4</sub>, patients with positive *Giardia* infection showed significantly higher

values than patients with negative *Giardia* infection. Furthermore, both groups showed significantly higher values than controls. Regarding serum IL-5, patients with negative *Giardia* infection showed significantly higher value than patients with positive *Giardia* infections. Both groups showed significantly higher value than controls.

**Table (6):** Pulmonary functions, A.E.C, serum IL-5 and urinary LTE4 in patients with +ve and –ve *Giardia* infection versus controls

	I Patients with +ve <i>Giardia</i> infection (n : 28)	II Patients with –ve <i>Giardia</i> infection (n:112)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	53.930 ± 16.657	58.450 ± 14.653	98.350 ± 0.587	0.000 HS	0.000 HS	0.025 S
- FEV <sub>1</sub> (%) (mean ± SD)	68.210 ± 13.174	64.750 ± 15.088	94.300 ± 19.850	0.000 HS	0.000 HS	0.004 MS
3- A.E.C (mean ± SD)	970.360 ± 171.471	672.320 ± 223.347	121.950 ± 51.635	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	66.500 ± 40.553	93.955 ± 28.281	6.725 ± 3.952	0.000 HS	0.000 HS	0.006 MS
5- LTE <sub>4</sub> (pg/ml) (mean ± SD)	708.750 ± 352.489	305.482 ± 191.151	35.222 ± 5.044	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate

FEV<sub>1</sub>: Forced Expiratory Volume 1 second

IL-5: Interleukin-5

LTE<sub>4</sub>: Leukotriene E<sub>4</sub>

A.E.C: Absolute Eosinophilic Count

ABG: Arterial Blood Gases

S: Significant (P<0.05)

MS: Moderately significant

HS: Highly significant (P<0.001)

NS: Non significant (P>0.05)

## IV. DISCUSSION

Asthma is a chronic lung disease characterized by reversible airway obstruction, inflammation, and bronchial hyperresponsiveness<sup>11)</sup>

In this study, the relationship between *Ascaris lumbricoides*, *Toxocara canis*, *Giardia lamblia* infections and development and severity of childhood asthma has been studied. As regard the association of parasitic infections and bronchial asthma, ascariasis were detected in the sera of 26 patients (18.6%) and toxocariasis showed similar occurrence, whereas giardiasis was detected in the stools of 28 patients (20%). On the other hand only giardiasis was detected in stools of 4 (2.8%) of controls. It is possible for these parasites to be important risk factors in our communities. Our study revealed that parasitic infections with *Ascaris*, *Toxocara* and *Giardia* were more common among severely asthmatic children than among moderately and mildly asthmatics. This was supported by the finding of significantly higher levels of AEC, urinary LTE4 and IL-5 in *Ascaris*, *Toxocara* and *Giardia* positive asthmatics than negative ones. Also, pulmonary functions were insignificantly lower in the earlier than the latter (Table 4, 5, 6).

These results were in line with previous studies who reported the increased prevalence of parasitic infections and possible influence of parasitic infections on the development and severity of allergic conditions in the tropical environment<sup>12,13)</sup>.

Our results were in agreement with systematic review and met analysis of 30 cross-sectional studies found that *A. lumbricoides* infection appeared to increase asthma risk<sup>14)</sup>.

Previous studies have provided conflicting evidence regarding relationship between parasitic infections and development of asthma. These studies showed that helminth infection can inhibit<sup>15)</sup>, cause<sup>16)</sup> or is unrelated to asthma<sup>17)</sup>. The role of anti-*Ascaris* IgE in the development of asthma is not clear. One possible explanation for the relationship is that elevated anti-*Ascaris* IgE levels are associated with larval migration after re-infection, as *Ascaris* migrates through the lungs during maturation and causes pulmonary infiltrates of Th2 immunity and episodic airway obstruction associated with wheezing<sup>18)</sup>. Repeated *Ascaris* infections and larval migration due to high rate of infection could increase the risk of asthma symptoms. Another explanation is that anti-*Ascaris* IgE acts as IgE specific to common inhaled aero-antigens directly triggering mast cell activation<sup>19)</sup>. This finding was supported by two other studies<sup>20,21)</sup>. The third explanation is that the higher anti-*Ascaris* IgE levels in the wheezing group simply mean that atopic children produce more anti-*Ascaris* IgE in response to *Ascaris* infection<sup>22)</sup>. Parallel to this observation, Heukelbach et al. reported that exposure to

*Toxocara* infection was suggested to be a possible risk factor for asthma<sup>23)</sup>. One good explanation for that is, *Toxocara* species can cause allergy (asthma) in man by liberation of larval excretory/secretory antigens. Moreover, *Toxocara* was found to induce polyclonal activation of IgE producing B-cells as well as peripheral and tissue eosinophilia<sup>24)</sup>. These phenomena are commonly occurred with IgE mediated diseases such as allergy.

There is hypothesis that many zoonotic helminth infections cannot develop to maturity in the human host and therefore, larvae may migrate for prolonged periods in the tissues. Examples are infections with *Toxocara* spp, *Ascaris suum*, and dog hookworms. Such infections cause allergic type syndromes such as cutaneous and visceral larva migrans<sup>25)</sup>. Damage of these tissues can be caused by allergic inflammation directed against the migrating larvae associated with failure of immune regulation during such infections probably because host and parasite have not co-evolved.

Our results were in line with Di Prisco et al.<sup>26)</sup> who found that *Giardia lamblia* parasitized children showed significantly higher levels of both total and specific serum IgE antibodies against allergens compared both with the non-parasitized group and those infected with parasites other than *Giardia*. The investigators concluded that there was a clear relation between giardiasis and allergy, possibly because infection by this protozoan enhanced sensitization towards food antigens, due to increased antigen penetration through damaged intestinal mucosa.

It has been reported that activation of the immune system takes occurs in giardiasis. It is wider and more severe in allergy-complicated giardiasis than that of uncomplicated cases, most probably due to non-invasive character of *G. lamblia*. Enhanced IgE production pointed to Th2-type immune response and confirms its association with allergy<sup>6)</sup>.

## V. CONCLUSION

*Ascaris*, *Toxocara* and *Giardia* infections are more common among asthmatic children compared to healthy children and they were significantly associated with the disease severity therefore, infection with these parasites may be a risk factor for the development and severity of bronchial asthma among children in Upper Egypt.

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## Immunophenotyping of Acute Lymphoblastic Leukemia in Sudanese Children's Versus Egyptian Children's using Flowcytometry

By Dr. Abdelgadir Ahmed Abdelgadir, Dr. Khalid Omer Abdallah Abosalif,  
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**Abstract- Background:** Acute lymphoblastic leukemia (ALL) develops rapidly, creating immature white blood cells (WBCs) called lymphoblasts. This type of hematological malignancy is cancer involving blood and bone marrow (BM). It can affect both B and T cell lineage. Here we studied T & B markers in 100 Sudanese ALL patients attending the Radiation & Isotopes Center Khartoum versus 180 Egyptian ALL patients attending the Alksr Aani, oncology center, Cairo between October 2009 and August 2014.

*The study aimed* to detect the frequency of B and T lineages subclass in ALL among the Sudanese versus Egyptian population in correlation with their clinical symptoms, hematological parameters, gender and, age.

**Keywords:** *sudanese. egyptian children, acute lymphoblastic leukemia , flowcytometry.*

**GJMR-F Classification:** NLMC Code: QZ 350



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# Immunophenotyping of Acute Lymphoblastic Leukemia in Sudanese Children's Versus Egyptian Children's using Flowcytometry

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**Abstract- Background:** Acute lymphoblastic leukemia (ALL) develops rapidly, creating immature white blood cells (WBCs) called lymphoblasts. This type of hematological malignancy is cancer involving blood and bone marrow (BM). It can affect both B and T cell lineage. Here we studied T & B markers in 100 Sudanese ALL patients attending the Radiation & Isotopes Center Khartoum versus 180 Egyptian ALL patients attending the Alksr Aani, oncology center, Cairo between October 2009 and August 2014.

**The study aimed** to detect the frequency of B and T lineages subclass in ALL among the Sudanese versus Egyptian population in correlation with their clinical symptoms, hematological parameters, gender and, age.

**Materials and methods:** A questionnaire was used to collect geographical information and, clinical data. Initially, full blood count (FBC), peripheral blood morphology and BM examination was done to diagnose patients. FBC was done by a haematology analyzer and Leishman stain was used for the cell morphology. Immunophenotype CD markers, TdT, CD3cyt, CD3 surf, CD5, CD7, CD2, CD10, CD19, CD20, Ig, slg, and CD2 antigens were looked for to determine the B and T subclasses of ALL using flow cytometry. Mononuclear cells were prepared for flow cytometry and labeled with fluorescence conjugated antibodies. Analyzing gated populations with EPICS XL using three-color protocols, fluorescence intensity and peak width were calculated for each antigen. TdT, CD10 and, CD19 were positive in all subclasses of B lineage ALL. ((slg and Ig-cyto were detected in B ALL and Pre B ALL were detected the former was slg. In contrast, slg and Ig-cyto markers were not detected in cases with Pro B ALL. Panel T CDs were detected in thymocyte and post thymocyte ALL, as the former was positive CD3 (cyto) and the latter was positive CD3 (surf). TdT, CD2 and CD5 immunophenotypes were positive in all subclasses of T-ALL)). Pro B ALL was diagnosed in 24% (n=43) and 23% (n=23) of cases, 51% (n=91) and 29% (n=29) were Pre B ALL, 9% (n=16) and 29% (n=29) BALL, 4% (n=8) and 11% (n=11) thymocyte ALL and, post thymocyte ALL was 12% (n=22) and 9% (n=9) among Egyptian and, Sudanese patients respectively.

**Result:** Patients were classified into three categories based on their ages, (1-4 Yrs), (5-8 Yrs) and (9-12 Yrs). The frequency of ALL in the first group was significantly higher in Egyptian than

in Sudanese children ( $p < 0.05$ ), while in the last group (9-12 yrs), it was significantly higher in Sudanese children ( $p < 0.05$ ). Pre-B ALL was significantly higher in Egyptian than in Sudanese ( $p < 0.05$ ), particularly in those less than 8 Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ( $p < 0.05$ ). With regards to prevalence of T lineage ALL (thymocyte and post thymocyte) and, cases of B lineage ALL cases (Pro, Pre and B ALL), there was no significant variation ( $p > 0.05$ ) between the two ethnic groups Sudanese and Egyptian. Their significance is represented in parenthesis. You mentioned a significant difference. Regarding hematological parameters, the means of Hb concentration, TWBC, platelets and, lymphoblasts for patients with ALL were 9 and 8 g/dl,  $37 \times 10^3/\text{cm}^3$  and  $32 \times 10^3/\text{cm}^3$ ,  $64 \times 10^3/\text{cm}^3$  and  $65 \times 10^3/\text{cm}^3$  and, 75% and 72% in the Sudanese and, Egyptian respectively. The presence or absence of lymph node were stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and post thymocyte. Their presence were (12%), (13%), (14%), (5%) and (8%) in the Sudanese patients while in the Egyptians it was (19.5%), (44%), (7%), (9.5%) and (4.5%). Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL. Diverse flow cytometric parameter use helps minimize marker numbers leading to reduced cost without reduced accuracy.

**Conclusion:** Age ranging from one year to 12 years with a mean of 6.5 years. The male to female ratio was 1:37. High age group in Egyptian ethnic group was 1—4 Yrs while in the Sudanese ethnic group, the higher age group was 9-12 Yrs. In this study, B lineage origin is the most common type than T lineage origin in two ethnic groups: the T lineage had a better prognosis than B lineage. In this study, also thymocyte ALL with cytoplasmic CD3 in the pediatric group below two years showed with the high total leucocytic count. Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL. Using of certain flow cytometric parameters can help in minimization of cost without reduced accuracy. There is significant variations in ALL subclassification between Sudanese and Egyptian Patients that may be due to genetic background.

**Keywords:** sudanese. egyptian children, acute lymphoblastic leukemia, flowcytometry.

## I. INTRODUCTION

Contemporary research on childhood acute lymphoblastic leukemia (ALL) has focused on the identification of biological and clinical prognostic markers to generate better risk-adapted treatment strategies <sup>(1)</sup> The identification of several cluster

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differentiation markers and early diagnosis allowed the definition of patient subsets with distinct prognostic features <sup>(1)</sup>. Nevertheless, treatment itself remains one of the strongest prognostic factors, as has been shown in several well-designed large clinical trials <sup>(2)</sup>. Cytometry has evolved from a promising new technology to an indispensable tool in the diagnosis of hematologic malignancies. Many new antibodies, improved gating strategies, and routine use of multiparameter techniques have dramatically improved the diagnostic utility of flow cytometry. This review will focus on the use of flow cytometry in the routine clinicopathologic approach to the diagnosing of leukemias and lymphomas, emphasizing the relevant literature of the past ten years. Some of the recent advances in flow cytometric monitoring of disease and treatment are shown in the last section. We will review the use of flow cytometry in the diagnosis of major disorders highlighting the prognostically important subgroups defined either morphologically or genetically. The discussion will focus not only on the use of flow cytometry in the differential diagnosis of a particular disorder, but also correlate immunophenotypic, with clinical features, HB, TLC. Platelets and accumulation of blast cells. In the delineation of biologically important subgroups. We intend that this review supports a combined modality approach to the daily practice of hematology-oncology and hematopathology. A working knowledge of the basics of flow cytometry is assumed; thus, technical aspects of instrumentation, normal distribution of surface antigens, and methodologies. leukemia is a group of neoplastic diseases of blood- forming cells of the bone marrow, which result in the proliferation and accumulation of immature and generally defective blood cells in both the blood-stream and the bone marrow <sup>(3)</sup>. This may result in anemia, thrombocytopenia, and granulocytopenia and, infiltration of other sites such as lymph nodes, kidney, spleen, testes, and the central nervous system (CNS). The cells involved are usually leukocytes, but several different forms of the disease may be manifested; according to which leukocyte cell line is involved, the leukemia are universally fatal if untreated, generally due to complications resulting from the leukemic infiltration of the bone marrow and replacement of normal hematopoietic precursor cells. These fatal complications are usually hemorrhage and infection <sup>(3)</sup>. Leukemia is the most common childhood cancer, accounting for one- third of malignancies in children under 15 years of age in Europe and North America. The annual incidence in the United Kingdom is 3 0-40 cases per million children. About 80% are acute lymphoblastic leukemia (ALL) and 18% acute myeloid leukemia (AML) <sup>(4)</sup>. Acute lymphoblastic leukemia, with a sex ratio of 1 .2 males to females, shows a peak incidence in childhood between the ages of 3 and 5 years and is due to cases of early B cell ALL. T-ALL, with a male predominance, is more common in older

children ('Stiller and Draper 1998). Both incidence and mortality are slightly higher in males <sup>(5)</sup>. A peak of age occurs between 2-4 years (Margolin and Popolack 1997). in Egypt, acute leukemia is the most common pediatric malignancy accounting for about 40% of childhood cancer, with ALL counting for 70% of the cases <sup>(6)</sup>. the peak incidence is between 3-7 years <sup>(6)</sup>.

## II. MATERIALS AND METHODS

*Study Participants;* This study included 180 Egyptian children and 100 Sudanese children newly diagnosed with acute lymphoblastic leukemia, 158 males and 122 females. Their age ranged from one year to 12 years.

### *Sample preparation and methods*

For each patient, the following samples were collect: 4ml EDTA blood sample was collected under complete aseptic conditions for CBC and BM aspirate for morphological examination and immunophenotyping on EDTA containers. BM samples were processed within a few hours because CD antibodies monoclonal have short stabilities even if stored in the refrigerator. In most cases, samples were processed within 6 hours of collection.

*Methods; Complete blood count* Was done by using Sysmex KX-21N, Kope, Japan. *Principle of smear preparation* A small drop of blood is place near the froste end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin-film over the slide. The slide is allow to air-dry and is then stained.<sup>(7)</sup> *Staining of thin film* Leishman stain was used in staining of all blood smears and bone marrow in this study., *Flowcytometry*, The EDTA anticoagulated BM sampled were diluted 1.3 with PBS then the cells were stained by adirect immuno-florescent technique by addition of monoclonal antibodies (microwell Test Kit contains) which contains T lineage panel monoclonal antibodies (CD2, CD5, CD3 cyt, CD7 and, CD3surf) and B lineage panel monoclonal antibodies (CD10, CD9, CD20, CD19 Ig cyt and, Ig surf.) and Leukocyte marker (TdT). Sample staining be carried out as soon as possible after the nucleated cell suspension has been prepared. Delaying this step will only reduce viability and induce cell clumping, especially if the tubes holding the cell suspensions will be stored in an upright position <sup>(7)</sup>. *Direct immune fluorescence double staining* Tubes was labeled with the name of patients, type of the specimen, laboratory number and combination of fluorochrome. 100 µl of specimen (whole peripheral blood or bone marrow) was placed in a labeled tube., 2ml of phosphate buffered saline PBS, (PH 7.3) was added containing 0.02% sodium azide 0.02% Bovine albumin., Tube was centrifuged at 2000 rpm for 5 minutes and, the supernatant were removed., The cells was resuspended in 0.2-0.5 ml of fluid sheath solution (e.g . isotonic). Then the tube was readied on a flow cytometer instrument<sup>(8)</sup>. *Detection of surface Immunoglobulin,*

Surface 1g heavy and light chain can be detected using Double or triple immune staining the object in to demonstrate clonality of a B cell population. Double staining was done by Combination of an FTC labeled B cell, marker e.g, CD19 and, PE Labeled anti-light chain. The tube was labeled with the name of the patient, type of specimen, laboratory number and MCAP. 100  $\mu$ L of the specimen (whole peripheral blood or bone marrow) was pipette. 2 ml of lysine solution was added, then incubate for 10 minutes at room temperature. The tube was washed twice in PBS aside from BSA. An appropriate Volume of MC Ab was add according to the manufactures recommendation. The tube was re incubated for 10 minutes at room temperature. 2ml of PBS aside BSA or Henks Solution was added. tube was centrifuged at 2000 rpm and, the supernatant was remove. The cell was re suspended in 0.2-0.5 ml of sheath fluid (isotonic) and inserted on a flow cytometer.

*Detection of intracellular antigens*, There are several commercially available kits containing solutions to fix and stabilize cells in to detect cytoplasmic and or nuclear antigens, overall, these reagents have little or no effect on the light scatter pattern. Also, their reliability and consistency for detecting particular nuclear and cytoplasmic antigens may vary. The kits contain two solution. A is a fixing solution. B is a stabilizing agent. The tube was labeled with the name of the patient, type of specimen laboratory number and, the MC Ab. 100  $\mu$ L of the specimen was pipette into the tube. 100 $\mu$ L of Solution A (fixative) was added and incubated at room temperature for 10 minutes. Tube was washed twice in PBS and BSA by centrifuging for 5 minutes at 2000 pm. 100 $\mu$ L of solution B (stabilizing) and the appropriate amount of fluoromecen conjugated Mc Ab were added. Cell was incubated at room temperature for 15 minutes .The cell was washed twice in PBs azide BSA by Centrifuging for 5 minutes at 2000 rpm. The cell was resuspended in 0.2-0.5 ml of sheath fluid solution (isotonic) and the tube was inserted on flow cytometer.

*Statistical Analysis*: Statistical assessment was carried out with statistical package for social sciences (SPSS) version 17.0 for windows statistical software.

### III. RESULTS

#### *Age comparison within the study population*

In this study, Sudanese and, Egyptian patients with ALL were classified into three categories based on their ages, (1 to 4) Yrs, (5 to 8) Yrs and, (9 to 12) Yrs. Two hundred eighty study participants with ALL, of whom 36% (n=100) were Sudanese and, 64% (n=180) were Egyptian. The frequency of (1-4 yrs) age group was significantly higher in Egyptian than in Sudanese ( $p<0.05$ ). In contrast, the prevalence of (9-12 yrs) age group was significantly higher in Sudanese compared to Egyptian ( $p<0.05$ ). The Pre-BALL was significantly higher in Egyptian than in Sudanese ( $p<0.05$ ),

particularly in those less than 8Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ( $p<0.05$ ). With regards to the prevalence of T lineage ALL (thymocyte and post thymocyte) and cases of B lineage ALL cases (Pro, Pre and BALL), there was no significant variation ( $p>0.05$ ) between the two ethnic groups. Sudanese and, Egyptian. Their significance is represente in parenthesis . Table (1).

#### *Gender comparison within the study population*

In this study the majority of children with ALL were males n=170(61% ), 66 (66%) were Sudanese and 104(58%) were Egyptian compared to females no=80(39%), where 34 (34%) were Sudanese and 76 (42%) were Egyptian. The Pre-B ALL cases among Egyptian females (patients) were significantly higher than in Sudanese females (patients) ( $p<0.05$ ), in contraste the prevalence of B-ALL was significantly higher in Sudanese compared to Egyptian ( $p<0.01$ ). Their significance is represented in parenthesis. Table (2).

#### *Subclasses of B and T acute lymphoblastic leukemia and their frequencies within the study population*

The frequency of B lineage ALL subclasses for the two ethnic groups was 82% out of all studied samples, which was approximately five times higher than those with T lineage ALL subclasses. Pro-BALL was diagnosed in 24% (n=43) and 23% (n=23) of cases, 51% (n=91) and 29 % (n=29) were Pre B ALL, 9% (n=16) and 29% (n=29) BALL, 4% (n=8) and 11%. Table (3).

#### *Immunological findings in the study population*

TdT, CD3cyt, CD3 surf, CD5, CD7,CD2, CD10, CD19, CD20, Ig cyt and, slg antigens were investigate in the differential diagnosis of B-ALL and T-ALL. TdT, CD10 and, CD19 were positive in all subclasses of B lineage ALL. slg and Ig-cyto are crucial in identifying B ALL and, Pre B ALL as the former is slg positive and the latter is positive for Ig-cyto. In contrast, slg and Ig-cyto-markers were not detect in cases with Pro B ALL. Panel T CDs were important in identifying thymocyte and post thymocyte ALL, as the former is positive for CD3 (cyto) and the latter is positive for CD3 (suf). TdT, CD2 and, CD5 immunophenotypes were positive in all sub-classes of T-ALL. Table (4).

*Hematological parameters of study population*; Four hematological parameters were estimated; Hb, blast cells, TLC and, Plts. This study shows comparative statistics between B and T lineage ALL patients of Sudanese and Egyptian ethnicities. The results were not within the published normal range among study population, as Hb, PLt were low in both ethnic groups and TLC, blast cells were high among them. The difference in these parameters was not significant in Sudanese versus Egyptian patients  $p = >0.05$ . Table (5).



*French American British classification:* Patients were classified based on the FAB classification: 27% and 17.2% as L1; 62% and 77% as L2 and 11% and 5% as L3 in Sudanese and Egyptian respectively. Of Egyptian patients, L2 was highly significant ( $p < 0.05$ ) compared to other classes, L1, of Sudanese patients, L2 was highly significant ( $p < 0.05$ ) compared to other classes while L3 no significant variation in Sudanese and Egyptian. Table (6).

*Lymph node in study population:* To detect either presence or absence of lymph node. Stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and. post thymocyte. Their presence in Sudanese were (12), (13%), (14%), 13 (13%), (5%) and (8%) while in Egyptian were (19.5%), (44%), (7%), (9.5%) and (4.5%). table (7).

*Bleeding:* To detect either presence or absence of lymph node and, to bleed for participants. Its presence was stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and post thymocyte. table (8).

#### IV. DISCUSSION

A cross-sectional case-control study was carried out at the Radiation & Isotopes Center, Khartoum and the Alksr Aani, oncology center, Cairo to compare the prevalence of ALL immunophenotypes amongst the Sudanese and, the Egyptian population using flow-cytometry. Blood cell morphology, cytochemistry stains, cytogenetic studies and immunophenotyping are basic methods for ALL diagnosis (1, 2). These techniques have been used extensively over several years in different parts of the world and, a wide distribution of hematological malignancies found for different regions (3-5). The data report here are based on flow-cytometry results from blood samples taken from diagnosed Sudanese and Egyptian ALL patients (figure1). Flow cytometry was used to evaluate the different types of ALL, B and, T lineages in the peripheral venous blood and, the detection of their subclasses, based on the Cell 'Cluster of differentiation (CD) markers (6, 7). Here, TdT, CD3cyt, CD3 surf, CD5, CD7, CD 2, CD10, CD19, CD20, Ig cyt and, slg antigens were investigated in the differential diagnosis of B-ALL and T-ALL lineages and their immunophenotypes, as discussed in Table (4). CD markers are a helpful method to recognize a specific cell population, however; they might be express on more than one cell type (8, 9). This was also found here among study patients with T lineage, as the CD7+ was detected in 47% and 51% of Sudanese and, Egyptian, respectively (See Figure 3). Thus flow-cytometry methods have been develop for immunophenotyping cells with two or more antibodies simultaneously to diagnose subpopulations of ALL effectively (10, 11). Of these markers, slg, CD10, CD19, CD20 and Ig cyto were used in this study is the differential diagnosis of B

lineage ALL immunophenotypes. Similar markers have been used in reported studies in the literature review (7, 12, 13). The immunophenotypes of B lineage ALL can be differentiate by analyzing the results for just five CD markers, slg, Ig-cyto, CD10, CD19 and, TdT. As the TdT is positive in all immunophenotypes of ALL, the current study used the first four CD markers in differentiating subclasses of B lineage ALL as follows: CD10+ and CD19+ were found in all subclasses of B lineage-ALL, but they were not found in all immunophenotypes of T lineage ALL; slg+ was found only in B-ALL and Ig-cyto+ was found only in Pre B ALL. In contrast, slg+ and Ig-cyto+ markers were not found in cases with Pro B ALL, as discuss in Table .4. This classification is important for the identification of the outcome of the ALL immunophenotypes (14, 15). The immunological sub classification of B and T lineages ALL is important in diagnosis in correlation with clinical features and molecular cytogenetic for management of patients for instance pro-BALL in children is associated with t(4,11) (16), pre-BALL consider coarse prognosis when is accompanied with t(9;22) & t(1;19) (Philadelphia chromosome) (17) and, BALL with translocation of (8;14) (Burket lymphoma) (18, 19). Regarding panel T lineage CD markers in the current study, CD2+ and CD5+ were found in both thymocyte and post thymocyte ALL sub populations but CD3 (cyto) + was found to be positive only for thymocyte ALL and CD3+ (surf) was positive only for post thymocyte ALL (See Table 4). In contrast, this finding was similar to the study by Yoneda, N. and co-workers concerning the presence of the CD2+ and CD5+ in T lineages ALL subpopulations (7). Of the study samples with T ALL, CD7+ was found in approximately half cases of Sudanese and Egyptian. This marker was found in all cases with bad outcomes for T ALL subpopulations, thymocyte and, post thymocyte (13, 20). T lineage ALL (thymocyte & post thymocyte) also has considered with bad prognosis when is associated with t(11;14) & t(10;14) respectively (19, 21). All cases had TdT at the time of initial diagnoses, but other CDs marker significantly increased during the staging of the diseases, such as Ig and CD7. Immunophenotypes also appear to affect the prognosis of ALL (figure 1). It is hoped this study may act as a pilot study to highlight the need to implement a flow cytometry for ALL and other hematological malignancies in Sudan. In the current study, B lineage ALL subclasses were detected more than T lineage ALL, but the former was a little bit more in Egyptian compared to Sudanese, 84% and 80%, respectively. Controversially, T lineage ALL subclasses were detected higher among Sudanese (20%) than Egyptian ethnicities (16%). (See Figures 2 and 3). This finding is in disagreement with other ethnicities, as in patients with ALL from Brazil and Japan. In Brazil, the B lineage ALL was detected in lower frequency 56.7% and the T lineage ALL in higher frequency 43.3% (22), while

in Japan, the T-lineage ALL accounted for lower frequency (13%) and B-lineage ALL accounted for higher frequency (87%)(37) compared to the current study groups. The higher percentage of B lineage ALL in this study might explain that the outcome of ALL might worsen in Egyptian and Sudanese; as it was reported to have a significantly poor event; low survival compared with patients with B lineage ALL (20). Accurate immunophenotyping of ALL is essential to evaluate the value of treatment in early diagnosis and to individualize treatment protocols, as described in the literature review (22), the frequency of ALL subclasses constitutes the theme of this study, using CDs markers (See Table 4). Pro-B-cell ALL in all age groups is associated with an unfavorable prognosis. Patients with pro-B cell phenotype had a more favorable prognosis compared to those patients with pre-B cell phenotype, based on their clinical symptoms (36). Another study reported that there was a significant correlation between immunophenotyping at diagnosis and higher complete remission rate and longer survival (24). Of Egyptian patients with B- lineage ALL, the commonest immunophenotype was pre-BALL compared to other B- lineage ALL. In contrast, out of Sudanese patients with B- lineage ALL, the prevalence of B- lineage ALL immunophenotypes was approximately similar with some varying degrees (See Table 3). Accordingly, most Egyptian cases with B- lineage ALL may have bad prognosis, as most of their cases were pre B ALL as opposite of Sudanese cases with B- lineage ALL. Therefore, from the above-mentioned studies and the current findings, the immunophenotyping at diagnosis may predict the a good outcome. The quantity of CD marker was used to evaluate the outcome and staging of ALL, it found that cases with positive CD10 had a good prognosis (25). In this study, the CD10 was detect positive qualitatively in all cases with B lineage ALL. Hence, further researchers might be useful to detect CD10 quantitatively in these ethnicities. The flow cytometry technique was preferred over alternative techniques in this study because of its accuracy and reliability. It is currently reported as one of the most reliable methods for hematological malignancies; it also has the advantage of diagnosing patients with ALL and other hematological malignancies (15, 24). Several antibodies must be used together to evaluate unique cell markers (35), Therefore eleven CD markers were use in the current study to immunophenotype ALL, B and T populations and subpopulations, as demonstrated in Tables 3 and 4. Although flow-cytometry is currently the best available method for ALL immunophenotypes determination, it is expensive of equipment purchase and maintenance. The study is also concern with the high prevalence of ALL in Sudan, as reported previously (25, 26). The data collected was also intended to instigate the relationship between immunophenotypes of ALL between Sudanese and,

Egyptian patients (See Table 3). Interviews and questionnaires were designed to collect demographic: age, sex, ethnicity (tribe), family history and clinical data (See Appendix-1). This information was taken from the patients' parents and, information on clinical symptoms, family history and geographical data. ALL is very prevalent in Sudan and Egypt with, high mortality and morbidity rates (25-28). It is worth searching of ALL in Sudan, as it is a fertile and , virgin area due to a lack of researches and the last international published study was done approximately three decades ago by Ahmed and, co workers (25). Thus, the current research intended to identify the frequency of B and T lineages ALL and their subpopulations in Sudanese children with ALL. Of Sudanese cases here, 17% had T lineage ALL and 81.5% had B lineage ALL (See Table 3). In contrast, this finding was similar to the study by A. Redall and co-workers, concerning the widespread presence of the B and T lineages ALL in Italy, the United States (US), Switzerland, and Costa Rica, where ALL was report with the highest incidence (29). Furthermore, in the current study, the prevalence of ALL subclasses among Sudanese was slightly different from the Egyptian findings, 20% were T ALL and 80% had B ALL (See Table 3). The former was marginally higher in Egyptian than in Sudanese, whereas the latter was vice versa. Generally, in both ethnic groups, Sudanese and, Egyptian, the frequency of ALL was nearly similar with some varying degrees compared to the previously reported studies (29, 30). In general, the prevalence of B lineage ALL was higher than T lineage ALL phenotypes (31) (32), as a study found that its frequency was 76.8% pre B and 6% as pro B and 2.3% as T ALL (19) what about the rest ?? and , another study was detected the T ALL only 1.3% in newly diagnosed ALL patients which was slightly lower than the previous study. In the current study, T ALL was 20% in Sudanese and 16% in Egyptian, which was higher compared to the above two studies (12) (15) and a little bit than in a study reported by Pieter Van and coworkers (31). BALL was 80 % in Sudanese and 84% in Egyptian, which was slightly lower than were found in the previous studies (31) (20) (16). This prospective study included all newly diagnosed children with ALL less than 15 years of age registered from October 2009 to August 2014 at Radiation & Isotopes Center Khartoum versus 180 Egyptian ALL patients attending the Alksr Aani, oncology center, Cairo. Besides epidemiological data, the objective of the current study was to look for the age, sex, clinical features and, laboratory findings at presentation and compare it with reported literature. Approximately 75% of ALL cases are in children and its after other nervous system and brain tumors (29). The relationship between ALL and, age was report from the published sources found within this Literature Review (30, 32, 33). As the ALL is the most common leukemia among children and the second most common cancer of childhood after

other nervous system and brain tumors, the current study was carried out in Sudanese and Egyptian children with ALL (29). A higher mortality rate was reported among children with ALL, who were younger than two Yrs and older than 10 Yrs (34). Another study found that 2-6 Yrs children with ALL survived more than those who were less than 2 Yrs and older than 10 Yrs (Ref). ALL in pediatric is treated based on risk factors, which is defined by laboratory and clinical features, therapeutic approach can be provided for patients who have a lower probability of long-term survival (14, 32), so that this study highlights and interprets the frequency of ALL subclasses among Sudanese and Egyptian population in correlation with their ages, as discussed in (Table 1). With regards to B lineage, the prevalence of BALL in 9-12 yrs group was significantly higher in Sudanese compared to Egyptian ( $p < 0.05$ ), whereas the frequency of Pre B subclass among those who were less than 8 Yrs was higher in Egyptian than in Sudanese ( $p < 0.05$ ) (See Table 3.2). This finding is similar to a reported study by (Smibert 1996 and co-workers, (34) 2-6 Yrs children with ALL were survival more than those who were less than 2 Yrs and older than 10 Yrs (34). This poor outcome in infants may be related to the common occurrence of other poor prognostic features in this group of patients, such as higher leucocytic counts, higher incidence of hepatosplenomegaly and immunophenotype (13). Patients under the age of three years were found to have significantly lower intelligence quotients than patients who received the same treatment at an older age and a group of healthy children matched for age, sex and, parental occupation. As the intensity of treatment required for favorable outcome varies substantially among subsets of children with ALL, the participant's ages of the two ethnic groups were compared to identify the prognosis of the ALL in each one.

With regards to the ages of patients with T lineage ALL (thymocyte and post thymocyte), there was no significant variation ( $p > 0.05$ ) between the two ethnic groups, Sudanese and Egyptian. (Table 2) summarized gender differences in incidence rates of childhood B- and T precursors ALL. Of B lineage ALL, males had higher susceptibility to having ALL rather than females in both ethnic groups, still, in T lineage ALL, the frequency was nearly the same in males and, females of both ethnic groups without significant variation ( $p\text{-value} > 0.05$ ) [See Table 2]. Ching-Hon Pui and , coworkers were reported that boys had higher susceptibility to having T-cell ALL than girls (20.9% v 10.7%) (35). This is in agreement with the current data, as males were more likely to have T-cell ALL than females (66% v 34%,  $P < .001$ ), in both subclasses of T-lineage ALL, thymocyte and post-thymocyte. Acute lymphoblastic leukemia (ALL) develops at a rapidly, creating immature white blood cells (WBCs) called lymphocytes. This type of hematological malignancy is cancer involving blood

and bone marrow (BM) (36). With regards to laboratory data, the Egyptian population showed severe anemia ( $Hb < 8.8$  g/dl), thrombocytopenia ( $68 \times 10^3/\text{cm}^3$ ) and, ~ %69 of them had blast cells. Interestingly, the B-ALL subclass had a higher number of blast cells than other subclasses of ALL and, the thymocyte subclass had a higher percentage of blast cells rather than the post thymocyte subclass (See Table 3.5). This data was in agreement with a study of (14), as they found a high percentage of blast cells in cases with thymocyte & pre B ALL compared with other subclasses of ALL. This may reflect that Egyptian patients with ALL have severe clinical symptoms, as they might have a bleeding tendency because of low platelet and anemia as due to low Hb concentration. Recurrent infection is not unexpected in those patients as they had a high number of blast cells in their blood circulation. In contrast, Hb and platelets were found low among Sudanese cases,  $< 9$  g/dl and  $65 \times 10^3/\text{cm}^3$ , respectively which were the same as in the Egyptian population without significant variation ( $p > 0.05$ ). Blast cells were detected slightly higher among the Sudanese compared to the Egyptian, ~ %78 without significant variation ( $p > 0.05$ ) [See Table 5]. The presence of lymphadenopathy is usually found in cases with poor prognosis and, patients who have a diagnostic problem (37). Thus it is important to distinguish between cases of ALL with lymphadenitis from others. Clinical features such as lymph node (LN) enlargement and bleeding were taken from each patient using a questionnaire. The presence of LN enlargement was detected significantly higher ( $p\text{-value} < 0.05$ ) in Egyptian 85% ( $N=153$ ) than in Sudanese 52% ( $N=52$ ). Of Egyptian cases, its presence in B lineage was found high pre B ALL phenotype 44% followed by pro B ALL 19.5% ( $N=35$ ) and B ALL 7% ( $N=14$ ) and its presence in T lineage ALL was detected in all cases with post thymocyte and, its approximately three-quarters of cases with thymocyte. Regarding lymphadenopathy among Sudanese, it was found that its presence and absence were nearly the same, 52% and 48%, respectively. There was no significance variation in the presence of lymph node enlargement between the B lineage ALL phenotypes in Sudanese. Interestingly, patients with post thymocyte are possible to have lymph node enlargement, as found that all Egyptian with post thymocyte had lymphadenopathy and Sudanese patient with post thymocyte had lymphadenopathy except one case (See Table 3.6). Therefore, patients with thymocyte ALL phenotype might have a better outcomes in comparison with post thymocyte ALL phenotype. The presence of bleeding was nearly equal in ethnic groups, 53% in Sudanese and 54% in Egyptian. Regarding to bleeding in the study, population, the high frequency was found mostly in the early phenotypes of B lineage ALL in Egyptian and, vice versa in Sudanese patients. Of T lineage ALL, the presence of bleeding was common among Sudanese in both phenotype equally,

thymocyte and post thymocyte, but its presence in Egyptian was found mostly in thymocyte (See Table 7). In this study Sudanese and Egyptian patients with ALL were classified into three categories based on their ages, (1-4 Yrs), (5-8 Yrs) and the (9-12 Yrs). This Table describes two hundred eighty study participants, with ALL, of whom 36% (n=100) were Sudanese and 64% (n=180) were Egyptian. The frequency of (1-4 yrs) age group was significantly higher in the Egyptian than in the Sudanese ( $p < 0.05$ ). In contrast, the prevalence of (9-12 yrs) age group was significantly higher in Sudanese compared to Egyptian ( $p < 0.05$ ). The Pre-BALL was significantly higher in Egyptian than in Sudanese ( $p < 0.05$ ), particularly in those less than 8Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ( $p < 0.05$ ). With regards to the prevalence of T lineage ALL (thymocyte and post thymocyte) and cases of B lineage ALL cases (Pro, Pre and, BALL), there was no significant variation ( $p > 0.05$ ) between the two ethnic groups Sudanese and Egyptian. Their significance is represent in parenthesis.

## V. CONCLUSIONS

This study concluded that:- Age ranging from one year to 12 years with a mean of 6.5 years. The male to female ratio was 1:37 High age group in Egyptian ethnic group was 1—4 Yrs while in the Sudanese ethnic group the , higher age group was 9-12 Yrs. In this study, B lineage origin is most common type than T lineage origin in two ethnic groups; In the T lineage had a better prognosis than B lineage. In this study, also thymocyte ALL with cytoplasmic CD3 in the pediatric group below two years showed with a high total leucocytic count. Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL. using of certain flow cytometric parameters can helps in minimization of cost without reduced accuracy. There is significant variations in ALL sub classification between Sudanese and Egyptian Patients that may be due to genetic background.

List of abbreviations

AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
ANLL	Acute nonlymphocytic leukemia
ASlp75	Alternative splice I
ASNS	Asparagine synthetase gene
ATRA	All-trans-retinoic acid
BAK	Proapoptotic Bcl-2 family member
BAL	Biphenotypic acute leukemia
BASP1	Brain acid-soluble protein 1
BFM	Berlin-Frankfurt-Munster
BMT	Bone marrow transplantation
CALLA	CD 10 common ALL antigen
CASP8AP2	Caspase 8—associated protein 2
CBF	Core binding factor
CBP	Creb binding protein
CCR5	Chemokine receptor 5
cDNA	Complementary deoxyribonucleic acid
CG	Control gene
CLL	Chronic Lymphocytic Leukemia
CML	Chronic myeloid leukemia
CN	Copy number
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
Ct	Cycle threshold
CT	Cytotoxic T lymphocyte
Cyt 1gM	Cytoplasmic immunoglobulin M
DFS	Chronic Leukemia
DIC	Disease free survival
DLBCL	Diffuse large B-cell lymphoma

dNTPs	Deoxyribonucleotide triphosphates
EAC	Europe Against cancer
EBV	Epstein-Barr virus
EGFR	Epidermal growth factor receptor
EGIL	European group for the immunological classification leukemia (EGIL)
FAB	French-American-British classification
FACS	fluorescence-activated cell sorter
FIST/HIPK3	Fas-interacting serine/threonine kinase
GSTM1	Glutathione transferase
GVHD	Graft-versus host disease
GVL	Graft-versus-leukemia
HES	Hypereosinophilic syndrome
HR	Hematological relapse
HRG	High risk group
HSCT	Hematopoietic stem cell transplantation
UBI9	Human ubiquitin conjugating enzyme 9
I-BFM-SG	International BFM Study Group
IGITCR)	gene Immunoglobulin and T cell receptor
IGF-I rec	Insulin-like growth factor I receptor
IGFII	Insulin-like growth factor II
IL-15	Interleukin- 15
IPSS	International prognostic scoring system
ITD	Internal tandem duplications
KD	Kilodalton
KTS	Lysine-threonine-serine
LDH	Lactate dehydrogenase levels
MALT	Mucosa-associated lymphoid tissue
M-CSF	Macrophage colony-stimulating factor
MDS	Myelodysplastic syndrome
MFC	Multiparameter flow cytometry
MHC	Major histocompatibility
MIC	Morphology, immunophenotyping and cytogenetics
MLL	Mixed lineage leukemia gene
MPO	Myeloperoxidase
MRD	Minimal residual disease
DLI	Donor leukocyte infusion
MRDv	MRD value
MRG	Medium risk group
MTHFR	Methylene tetra hydrofolate reductase
MTRR	Methionine synthase reductase
NCN	Normalized copy number
NK	Natural killer
NOS	Non otherwise specified
NSE	Non specific esterase stains
PAS	Periodic acid-schiff
PAX-2	Paired-box gene
PB	Peripheral blood
PBMNC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PDGF-a	Platelet-derived growth factor (α-chain
PG	Profile gene
PNH	Paroxysmal nocturnal haemoglobinemia
PTD	Partial tandem duplications
PTLD	Post-transplant lymphoproliferative disorders
RA	Refractory anemia



RAR-o	Retinoic acid receptor- $\alpha$
Reo	Reactive eosinophilia
RISC	RNA-induced silencing complex
RNAi	RNA interference
ROC-curve	Receiver operator characteristic curve
RRM	RNA recognition motif
RT	Reverse transcription
RT-qPCR	Reverse transcriptase- quantitative- polymerase chain reaction
SB	Sudan black
SCT	Stem cell transplantation
SE	Specific Esterase
SENSV	Sensitivity
SF-1	Steroidogenic factor 1
siRNA	RNA-small interfering sequence.
SREBPs	Sterol-responsive element-binding proteins
SRG	Standard risk group
SRY	Sex-determining region Y
Taq	Thermus aquaticus
TCR	T-cell receptor
TdT	Terminal deoxynucleotidyl transferase
TGF- $\beta$	Transforming growth factor- $\beta$
TNF	Tumor necrosis factor
TYMS	Thymidylate synthetase
WHO	World Health Organization
WT	Wild type
WT1	Wilms' tumor gene
WTIP	WT 1-interacting protein
ZF	Zinc finger
p-ME	3-Mercaptoethanol

## Declarations

### Ethical approval and consent to participant:

Approval of This study was obtained from the hematology department of medical laboratory science (MLS), Omdurman Islamic University and, the ministry of health issued by the local ethical committee Khartoum State, Sudan. Written consent was taken from each member of the study.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets generated during and analyzed in this study are not publicly available due to Bahri hospital centers ethical policy to protect participant confidentiality.

### Competing interest

The authors declare that they have no competing interests.

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### Authors contributions

AA and KO and AH contributed in literature search and manuscript writing. AA had the main idea of the study and contributed in manuscript writing, KO

contributed to clinic work, AH contributed in statistical analysis. KO and AA supervised the study and critically reviewed the manuscript. All authors read and approved the final draft of the manuscript.

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#### Figure Legends

**Figure 1:** Shows T lineage acute lymphoblastic leukaemia

**Figure 2:** Shows B lineage acute lymphoblastic leukaemia

**Figure 3:** Result of CD7 in study population



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## The Prevalence of CKD of Unknown Etiology

By Dr. A. S. M. Julfekar Helal

**Abstract-** Chronic kidney disease (CKD)—or chronic renal failure (CRF), as it was historically termed—is a term that encompasses all degrees of decreased kidney function, from damaged—at risk through mild, moderate, and severe chronic kidney failure. Epidemics of CKD of uncertain etiology (CKDu) are emerging around the world. Highlighting common risk factors for CKD of uncertain etiology across various regions and populations may be important for health policy and public health responses. Prevalence of CKD of unknown (CKDu) etiology is being increasingly considered as an emerging etiology, especially in the developing countries, with environmental predisposition to hot humid climate, dehydration and toxic metal contaminations.

**Keywords:** *chronic kidney disease (CKD), CKD of unknown (CKDu) etiology. chronic kidney failure.*

**GJMR-F Classification:** *NLMC Code: WJ 340*



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## I. INTRODUCTION

Chronic kidney disease (CKD) means your kidneys are damaged and can't filter blood the way they should. The disease is called "chronic" because the damage to kidneys happens slowly over a long period of time. This damage can cause wastes to build up in body. CKD can also cause other health problems. The kidneys' main job is to filter extra water and wastes out of blood to make urine. To keep body working properly, the kidneys balance the salts and minerals—such as calcium, phosphorus, sodium, and potassium—that circulate in the blood. Your kidneys also make hormones that help control blood pressure, make red blood cells, and keep bones strong.

Causes of chronic kidney disease include diabetes, high blood pressure, glomerulonephritis, and polycystic kidney disease.<sup>1-2</sup> Risk factors include a family history of chronic kidney disease.<sup>[2]</sup> Diagnosis is by blood tests to measure the estimated glomerular filtration rate (eGFR), and a urine test to measure albumin.<sup>3</sup> Ultrasound or kidney biopsy may be performed to determine the underlying cause.<sup>4</sup> Several severity-based staging systems are in use.<sup>5-6</sup>

Screening at-risk people is recommended.<sup>7</sup> Initial treatments may include medications to lower blood pressure, blood sugar, and cholesterol.<sup>8</sup> Angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (ARBs) are generally first-line agents for blood pressure control, as they slow progression of the kidney disease and the risk of heart disease.<sup>9</sup> Loop diuretics may be used to control edema

and, if needed, to further lower blood pressure.<sup>10-11</sup> NSAIDs should be avoided.<sup>12</sup> Other recommended measures include staying active, and certain dietary changes such as a low-salt diet and the right amount of protein. Treatments for anemia and bone disease may also be required. Severe disease requires hemodialysis, peritoneal dialysis, or a kidney transplant for survival.<sup>13</sup>

Chronic kidney disease affected 753 million people globally in 2016: 417 million females and 336 million males.<sup>[1]</sup> In 2015 it caused 1.2 million deaths, up from 409,000 in 1990.<sup>6</sup> The causes that contribute to the greatest number of deaths are high blood pressure at 550,000, followed by diabetes at 418,000, and glomerulonephritis at 238,000.<sup>6</sup>

## II. WHAT IS CKDU AND WHO GETS IT

CKDu or Chronic Kidney Disease of Unknown etiology/Uncertain cause is a type of chronic kidney disease that mainly affects marginalized agricultural communities in specific areas of the world where a large number of people develop an unexplained, deadly form of kidney disease.

The common clinical features of CKDu are impaired kidney function in the absence of diabetes, evidence of primary glomerulonephritis (either on renal biopsy or clinically), or structural abnormality. The limited number of kidney biopsies performed in affected persons show scarring of a type that might be the consequence of a wide range of insults.<sup>6</sup>

Although there have been numerous case reports, it is only now that evidence from population-based surveys is beginning to emerge showing that CKDu exists in India and Sri Lanka,<sup>14-15</sup> in addition to Central America.<sup>1,9-11</sup> It also may be occurring in other tropical/subtropical parts of the world, including Saudi Arabia, Egypt, and Senegal, but standardized data are not available for comparison. Valid comparisons cannot be made using renal replacement therapy registry data because of varying access. Therefore, valid prevalence estimates can currently be obtained only by identifying renal impairment in random population surveys.

## III. RISK FACTOR OF CKDU

CKD in general, and CKDu in particular, increases markedly with age. It is noteworthy that a high proportion of the participants had hypertension, diabetes, or proteinuria, in contrast with CKDu hotspots in Central America, and therefore it is more challenging to separate CKDu from CKD in this setting. Although CKDu is associated with agricultural work, it is not

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particularly strongly associated with farming (odds ratio 1.39) or with pesticide use (odds ratio 0.85), unlike many studies in Sri Lanka.

The authors conjecture that “there might be a new etiological factor or multiple factors responsible.” This is similar to findings in other cross-sectional studies in South Asia and Central America, which consistently find that increased risks, if they exist, occur primarily (but not exclusively) in agricultural populations, but that these do not seem to be clearly linked to exposures such as heat stress or pesticides. This may reflect the absence of any causal association, inadequacies with the exposure questions used, or the more general inadequacies of cross-sectional studies.<sup>12</sup>

In Asia, drinking water was the most studied risk factor, with heavy metals, agrochemicals, and food also frequently mentioned in many reports. Several studies report that drinking water in the dry region of Sri Lanka is very hard (high calcium), with elevated fluoride concentrations, salinity, and dissolved organic carbon and suggest that the interaction of magnesium and fluoride are potentially nephrotoxic.

Unique hydro geochemistry of Sri Lanka features hard or very hard water, and fluoride, iron, manganese, sodium, and lead have been reported to occasionally exceed applicable World Health Organization or U.S. Environmental Protection Agency drinking water standards. One geochemical study found that in regions where surface water recharged groundwater was commonly consumed, there was a low incidence of CKDu, while in regions where groundwater followed natural flow paths or had stagnant groundwater had increased incidence of CKDu, pointing to a potential geochemical source of contamination.

One study found trace levels of lead and high silicon concentrations in Indian ground-water, leading researchers to examine the potential cytotoxic risks of these chemicals. The resulting laboratory study showed that long-term exposure to both lead and silica at concentrations comparable to those found in the groundwater were found to be cytotoxic in in-vitro cytotoxicity-assays on human-kidney-cell-lines.

In Anuradhapura, in the North Central Province of Sri Lanka, the local government has decided to prioritize clean drinking water in light of media coverage and research into the CKDu epidemic. Results from one interventional study in Sri Lanka show that replacement of drinking water supplies in CKDu patients with bottled water resulted in diminished disease progression; however, the study did not measure differences in water chemistry.

In farmers, the presence of increased dichlorodiphenyltrichloro-ethane (DDT) concentrations was associated with a greater drop in eGFR levels compared to those with low levels of DDT over 10 years. Exposure to pesticides has been implicated in the development of CKDu, but because of the cross-

sectional nature of the studies and the widespread use of many nephrotoxic pesticides across the globe, it is difficult to attribute CKDu to pesticide exposure alone. Exposure to glyphosate, a weed killer that has been found to chelate metal ions and has been banned in many European Union countries because of concerns about linkages to cancer, has been proposed as contributing to the development of CKDu, especially with hard water.

In the presence of glyphosate, cadmium and lead are more likely to leach from soils in rice paddies. Contamination of naturally sourced fertilizers with heavy metals has been suggested as contributing to the development of CKDu in Sri Lanka, although at least one study has suggested that this factor may not be observed in endemic areas. A few studies suggest that pesticides may be involved in the development of CKDu in both Nicaragua and Sri Lanka.<sup>13-14</sup>

Other studies researched the presence of metals in other environmental media, such as soil and food. In some cases, arsenic, cadmium, lead, and mercury exceeded U.S. soil screening levels. Additionally, studies on environmental contamination in foods suggest that some may be consuming hazardous amounts of lead and cadmium in a typical diet of the region, while others could not find significant levels of heavy metals in food, water, or urine from the area. Analysis of hair and toenail samples revealed a deficiency of selenium in both patients and controls, but no difference in cadmium or lead concentrations between CKDu patients and healthy individuals. Other environmental exposures have also been hypothesized as possible CKDu risk factors in Asia.

Mycotoxins, metabolites produced by fungi that grow in improperly stored rice, corn, groundnuts, wheat, and other products, have been linked with kidney disease and other adverse health outcomes, including cancer. Aflatoxins, ochratoxins, and fumonisins are several well-known classes of mycotoxins that have been characterized with respect to kidney disease. One study investigated the presence of mycotoxins in urine from CKDu patient, and another found elevated ochratoxins, aflatoxins, and fumonisins. Leptospirosis (or Weil's disease), malaria, leprosy, and hantavirus have been suggested as potential risk factors contributing to CKDu in several endemic regions, suggesting that viral and bacterial infections may play a role in the development of CKDu.

Despite an increasing global awareness of CKD and CKDu, many of the risk factors remain unknown. In South Asia, family history, agrochemical use, and heavy metal exposures were studied most frequently, whereas altitude and temperature were studied only in Central America. However, many similarities also exist. Heavy metals, heat stress, and dietary exposures were reported in studies across all geographic regions, and family history, temperature, altitude, dietary exposure,

ochra-toxin A, herbal use, and snake bite were frequently reported in both South Asia and Central America.

Given the similarities and the differences observed in studies across the regions, the growing CKD burden may, in part, be driven by factors that are common across regions as well as unique within regions. Pathologic exposures can affect disease outcomes by interacting with a wide range of factors, including source emissions, transport and transformation, human contact, bioavailability, early expression of disease, and/or health effects.

In low-income countries, for example, rapid urbanization has led to poor sanitation, unplanned infrastructure, overcrowding, and environmental pollution. For CKD, these exposures may interact with other urban risk factors, such as high rates of noncommunicable and communicable diseases, to increase CKD prevalence. Likewise, in rural, low-income areas, extreme poverty and agricultural-based economies expose people to other CKD risk factors, such as dehydration, snake bite, water contamination, heavy metals, and agrochemicals, which can also interact with noncommunicable and communicable diseases as well as genetic factors to increase CKD risk. As such, the regional variation in reporting of risk factors for CKDu may reflect a complex interplay between different global and regional exposures and local factors, such as environment and lifestyle. One example of regional variation in the reporting of risk factors for CKDu was observed with dehydration. Although dehydration is increasingly being posited as a potential etiologic factor for CKDu in endemic communities, assessment of the associations between dehydration and CKDu across studies was limited by inconsistent reporting of the measurements for assessing dehydration.

For example, whereas all studies that measured variation in altitude and seasonal temperature reported a significant association between dehydration and CKDu, studies reporting heat stress infrequently reported an association between dehydration and CKDu. This

highlights the importance of designing standardized measurements to consistently and comparatively assess the role of dehydration in the etiology of CKDu across the world. Additionally, genetics may also play a role in the observed regional heterogeneity in the reported epidemiology of CKDu. Genetic differences in ethnicities are known to have a strong effect on the prevalence and risk of progression of CKD.

In the United States, for example, Americans of African descent who carry the APOL1 genotype have higher rates and faster progression of CKD, especially when exposed to other augmenting factors, such as diabetes and hypertension. In India and Sri Lanka, there are specific polymorphisms and single-nucleotide mutations that have been associated with CKDu in some endemic communities, and in the Balkan states, particular chromosomal aberrations have been associated with Balkan Endemic Nephropathy. Therefore, more studies should be conducted to explore the role of genetic predisposition in the CKDu disease mechanism.

Additionally, the causal association between heavy metals and CKDu is not well established, and but some studies observed significant variability in the reported associations. For example, cadmium was inconsistently reported to be associated with CKDu, whereas arsenic was consistently associated with CKDu. Another study also observed regional heterogeneity in the reporting of heavy metals as a risk factor for CKDu. Only one study from Central America reported heavy metals, whereas six studies from South Asia reported it. It is likely that heavy metals do play a role in the development of CKDu in endemic areas, where levels are often present in the microenvironment and multiple other risk factors coexist. However, because the microenvironment and human levels of heavy metals from CKDu-endemic regions have been reported to be below the upper limits of detection, exploring mechanisms of interaction between heavy metals and other risk factors may be important in advancing CKDu research.

**Table-1:** Five most frequently studied CKD of uncertain etiology risk factors by region

CKDu Risk Factors by Region	
South Asia (n=11; 42%):	n
Heavy metals	6
Occupation (farmer)	6
Family history	5
Agrochemical use	4
Smoking	4
Central America (n=8; 31%)	n
Age	7
Men	7
Agrochemical use	6

Occupation (farmer)	5
Heat stress	3
Other regions (n=7; 27%)	n
Body mass index	3
Heavy metals	2
Age	2
Dietary exposure	1
Heat stress	1

However, still do not know, at least on a global level, who gets this disease, or even if the disease is the same in different parts of the world.<sup>15-17</sup>

This lack of basic epidemiological information also has important implications for health care. World Kidney Day 2019 has a theme of "health care access for all," but health care access cannot be provided for a disease that is unrecognized, undiagnosed, and (even when diagnosed) often un-treated. CKDu is clinically silent in the early stages and, in many of the affected areas, carries a poor prognosis. It is usually diagnosed late, and no data are available on factors predicting progression. Renal replacement therapy is un-available in many low- and middle-income countries, and even if present, it is often in accessible to most of the population, meaning that end-stage renal disease is usually fatal. In addition to the young lives lost, CKDu has a substantial negative impact on social and economic development of affected countries, through jeopardizing the economic development of the affected communities, and straining the poorly resourced health systems of the affected countries.

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## The Origin of Viruses and Somatic Diseases

By Vladyko A.S.

**Abstract-** A main genome is the genome of bacteria or, more precisely, the genome of single-celled prokaryotic microorganisms that appeared on earth 3.5 billion years ago, which marked the beginning of biological life on the earth planet. How the bacteria appeared - there are many assumptions, but the main one is that the earth, like other planets in universe, participated in this creative process. One of the intermediate biological products of the bacterial genome is a human. The action of creating multicellular mammals from single-celled microorganisms that have "gray" matter and are capable of thinking has been going on for millions of years and is still going on today. The question remains: where does this process go - from Homo sapiens to Homo degrading or Homo natural? The significance of the relationship and interdependence between the main genome and the human genome has become particularly acute with the appearance of the SARS-CoV-2 coronavirus (disease X?). Here we will discuss the mechanisms of the emergence of coronavirus as one of the crucial stages of the adaptation process of virus formation from adaptogens and its impact on the further development of mammals, including humans.

**Keywords:** *main genome, SARS-CoV-2, disease X, CRISPR/Cas, adaptogens, vaccine, diagnosis, infection prognosis, concept.*

**GJMR-F Classification:** *NLMC Code: WM 90*



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# The Origin of Viruses and Somatic Diseases

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## INTRODUCTION

The virus has been initially discovered in the late 19th century as the causative agent of an infectious disease in the tobacco plant<sup>1</sup>. After this discovery, all subsequent findings of viruses from plants and animals have been related to the pathologies that they caused. As a result, if a new virus has been discovered, it was necessarily associated with a plant or mammalian disease. It has been thus established that the virus is an etiological factor that causes the disease and that it must will be fought as a source of infection. However, the virus cannot be the source of infection since it is its consequence or marker, that is, an indicator of violation of certain protective functions of the whole organism in a mammal, including in humans<sup>2,3</sup>. Thus, the virus is a signal of what has already happened and is spreading. It have been assumed that the virus appears from adaptogens (Fig.1) circulating in prokaryotes (bacteria, archaea, etc.) in the form of complexes of molecular motifs designed to adapt mammals to changing environmental conditions. Prokaryotes, as is known, in addition to the external environment, live in the mammalian body - in the intestines, on the skin and mucous membranes, including the respiratory tract<sup>4</sup>. In humans, the number

of prokaryotes is being determined by the ratio: one human gene – some million genes of bacteria, archaea, etc.<sup>4,5</sup>. If we take into account that bacteria multiply within a few minutes and are also under the control of the immune and hormonal systems (a kind of filter) of the macro-organism, they both adapt (improve) and are a source of pathogens (viruses, bacteria), as well as somatic diseases (Fig.2). Thus, the origin of both infectious and somatic diseases of mammals are adaptogens or molecular motifs located in prokaryotes.

Bacterial molecular motifs adjusted due to external factors genetically adapt the mammalian immune and hormonal systems to the environment, which, as a rule, does not manifest itself as a disease (Fig.2, left half). Adaptive events occur constantly, according to the biological laws of development, and allows us to modify by changes in the environment, i.e., to adapt. At the same time, the damaged or altered immune (anti-infectious) and hormonal (anti-"somatic") systems of mammals are not able to fight and utilize prokaryotic molecular motifs or adaptogens at the genetic level (Fig.2, right half). In turn, the inability to adapt normally leads to the appearance of infectious disease and, in the future, to somatic diseases. How do disorders occur in the immune and hormonal systems of mammals? These are, as a rule, xenobiotic factors of both natural and anthropogenic origin, including medical, which often goes against the biological laws of nature development<sup>3</sup>.

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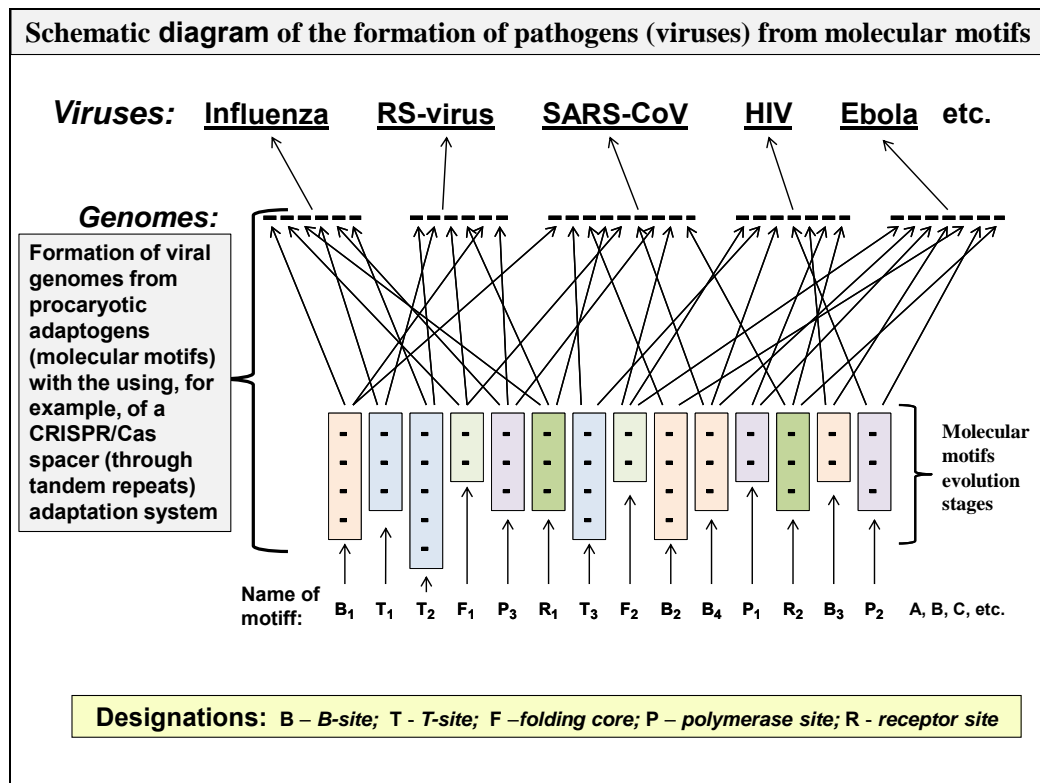


Fig. 1: Scheme for constructing of viruses from molecular motifs

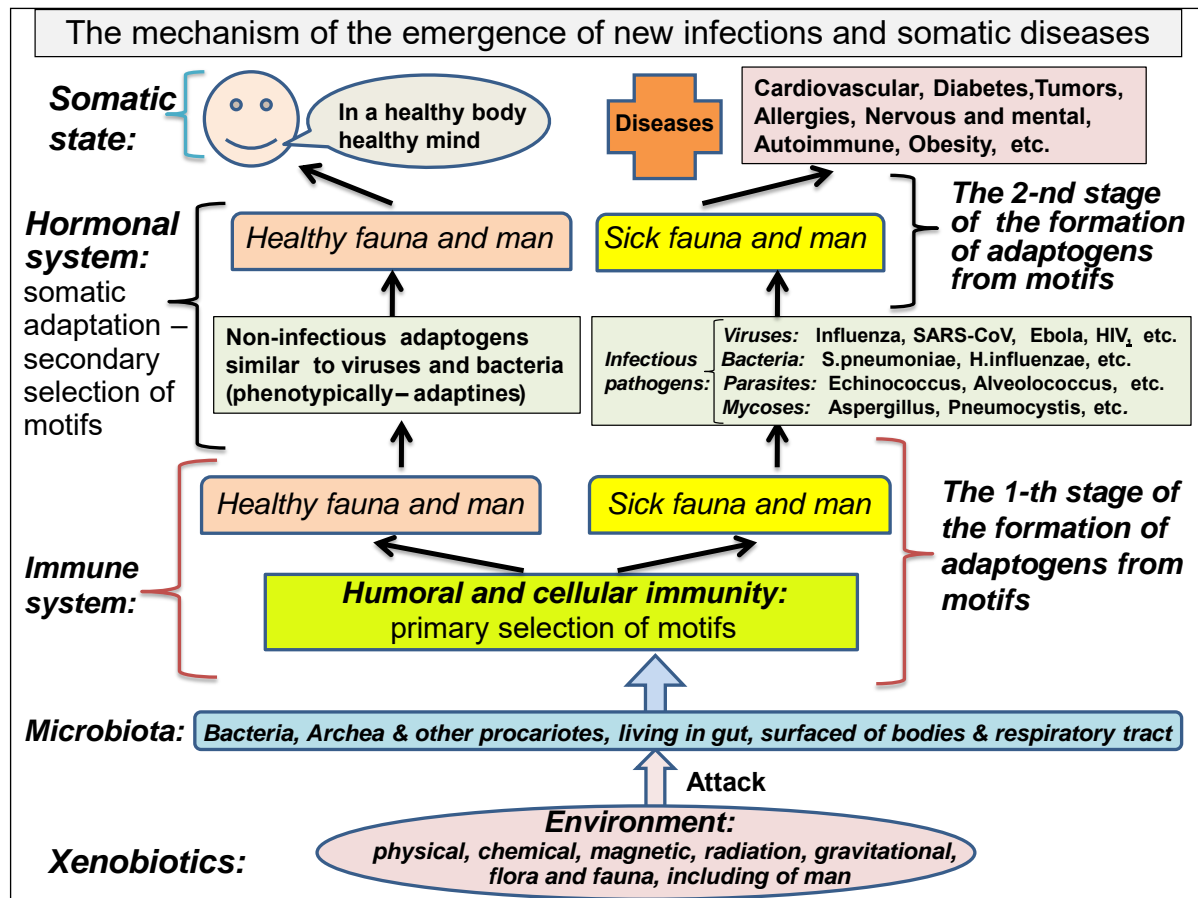


Fig. 2: Mechanism of appearance of infections and somatic diseases

The biological significance of the system of interrelation and interdependence between prokaryotes and multicellular (eukaryotic) organisms (flora and fauna) is the adaptation of eukaryotes to external environmental factors through the use of the prokaryotic microbiome. The mechanism by which modified molecular motifs are being transferred from prokaryotes to eukaryotes (from bacteria to mammals) is carried out, for example, by the CRISPR/Cas spaser adaptation system according to the retrovirus-like cut-and-paste principle<sup>6,7</sup>. The loci where the molecular motif shall have been inserted are being marked by tandem repeats in the genomes of bacteria and mammals<sup>8</sup>. These repeats are being also found in viruses<sup>9,10</sup>.

The main task of medicine is to learn the biological laws and use them for medical purposes. To realize this, it is necessary to correct the immune and hormonal systems of a particular person based on their characteristics. At the same time, the features of the immune and hormonal systems of each person depend on the prokaryotes that live in the intestine, on the surfaces of the skin and mucous membranes, as well as in the respiratory tract<sup>4</sup>. In this sense, prokaryotes manipulate molecular motifs (Fig. 1). Given that the ratio of molecular motifs in adaptogens (prokaryotes) and molecular motifs in even the largest viruses is always in favor of adaptogens, it is necessary to prevent the appearance of infectious and somatic pathology at the stage of pathogen formation.

Before attempting to carry out the genetic correction of prokaryotes at the level of molecular motives, it is necessary to determine the phenotypic markers of the immune and hormonal systems of a particular person. Objective methods of analysis (blood tests, ultrasound, computed tomography, biopsy, heredity, etc.) are being widely used to study the hormonal or "somatic" systems. But this is not enough to determine the existing somatic structural changes at the genetic level. A method for identifying somatic molecular markers will have been discussed below. As for the immune system, it is necessary to compare the molecular motifs of prokaryotes living in the macroorganism with the humoral and cellular defense systems adapted to them. Available diagnostic technologies make it possible to construct personal immunoantigenograms (IAG) based on the use of, for example, microarrays<sup>11</sup>. Each microdroplet represents one molecular motif. The result is a unique matrix for each region or person, which can be used to analyze the infectious and somatic state of the environment or each person in specific geographical conditions<sup>12</sup>.

With a high degree of probability, it can be assumed that the technology presented above for the analysis of the immune and hormonal systems of mammals, including humans, is relatively accessible due to its simplicity and has already been implemented in several closed laboratories over the past 15-20 years.

Medicine, whose task is to prolong the comfortable biological life of an individual person, does not always follow an individual approach. If this principle is still observed in medical (somatic) practice, then about prevention (vaccination), an individual approach has not yet been developed. The highest vaccination coverage is still considered preferable. In this case, as a rule, the vaccine is used against a specific infection, and all are vaccinated simultaneously (herd immunization) without taking into account the state of the immune and hormonal systems of a particular person. Moreover, vaccination of the population to eliminate the causative agent of the infection is inappropriate because the virus is not the cause but only the consequence of the disease. Thus, the fight against the result, and not against the cause, as a long-term practice has shown, exacerbated the medical problems declared in the form of "disease X"<sup>13</sup>.

One example of "disease X" is the coronavirus. This virus should have appeared because the ecological niche<sup>14</sup>, artificially created by humans for more than two centuries in the population of mammals, led to a gradual filling with molecular motifs that formed a prokaryotic adaptogen that manifested itself in the form of coronaviral infection. A feature of this virus that distinguishes it from other viruses, such as HIV, is that SARS-CoV-2 causes local immunodeficiency in the respiratory tract<sup>15,16</sup>. As a result, chronic "smoldering" infections show their activity. In our opinion, these activated chronic bacterial infections cause a "cytokine storm." In this regard, it is clear why in the first wave of infection, older people get sick and die, and, with rare exceptions, children do not get sick. As a result of the current of the first wave, favorable conditions had been created to create a natural vaccine. Sexually mature young people who were asymptomatic with the coronavirus should have been a source of prokaryotic adaptogens for the elderly. At the same time, the closer the family ties and the same sex, the more effective the vaccine should have been<sup>12</sup>.

Since this natural vaccination was not controlled for several reasons (isolation, antibiotics, antiseptics, etc., which prolonged and worsened the situation), the spread through the respiratory tract of inadequate adaptogens formed in sick and dying older adults in the form of genetically modified variants of coronavirus caused a second, more extensive wave of infection, involving middle-aged people and children. Subsequent waves of infection will mostly affect children and middle age, not so much in terms of the severity of the disease, but in terms of somatic consequences (Fig. 2, top right).

Vaccines developed using traditional technology based on neutralization and removal of the pathogen circulation from the population, even using different platforms, will have not a significant impact on preventing the development of coronavirus infection, especially with regard to somatic complications. The



harmlessness and immunogenicity of the preparation are not yet determining indicators of their effectiveness. Even medically effective vaccines against smallpox, measles, polio, yellow fever, etc., will eventually return to the human population in new infectious and somatic pathogens. Thus, epidemiological or ecological niches, artificially created as a result of vaccination using traditional technology and inconsiderate interference with the biological laws of nature, are filled with new infectious pathogens or their complexes, for example, disease X. In addition, the practice has shown, vaccines against AIDS, hepatitis C, and other infections cannot be developed using this technology.

However, the closest antigenic composition of the currently available adaptogen vaccines for SARS-CoV-2 is pneumococcus, so the available pneumococcal vaccine for individuals 60+ is currently more promising than the set of limited molecular motifs contained in the coronavirus itself<sup>17</sup>.

In the future, the obtained personal IAG will serve as a basis for introducing a new vaccine technology. The IAG encoded on electronic carriers, obtained during the analysis of blood serum during annual preventive examinations, can be used at a convenient time for a person to receive an aerosol (for the respiratory group of infections) or a yogurt (intestinal group) cocktail as a preventive vaccine against infectious and somatic pathology<sup>3,12</sup>.

Therefore, the vaccine should not be viral against viruses and bacterial against bacteria, but prokaryotic adaptogenic or adaptive to correct the human immune and hormonal systems to changing environmental conditions. The number of molecular motifs involved in the construction of IAG are significantly limited due to the existence of common structures or molecular motifs in nature (Fig.1). Here the analogy with music is appropriate, where there are only seven notes and an infinite number of melodies.

As for the importance of using monospecific diagnostic genetic engineering and serological methods developed without considering the molecular motifs of adaptogens, the result, in most cases, cannot provide the complete and necessary information. It is especially evident with SARS-CoV-2 infection when the virus suppresses innate and acquired immunity in the respiratory tract and chronic bacterial infections (for example, pneumococci, enterococci, etc.) begin to activate, stimulating antibodies against other molecular motifs that do not coincide with motives of the virus (false-negative results). The situation is complicated when the diagnostic molecular motifs of the coronavirus and diagnostic motifs, for example, pneumococcus, are the same. For example, one of the immunodominant antigenic determinants localized in the lysine-rich domain of the SARS-CoV-2 phosphoprotein - at the position of amino acids 369-375 (KKDKKKK) - coincides with a large number of similar antigen-significant B-sites

located in the proteins of pneumococci, enterococci, staphylococci, and Klebsiella. Thus, it is not known which B-epitopes: coronavirus or bacterial stimulate antibodies? If it is bacterial, which most often happens with a coronavirus infection, then the treatment tactics based on the choice of an antibiotic will give a positive result, and there is no need to select antiviral drugs for a long time.

The possibility of analyzing the hormonal (somatic) state through the use of molecular motifs in the IAG had been confirmed by the publication of Tilson et al.<sup>18</sup>, where it has been shown that antibodies specific to the structural protein of the Ebola virus was detected in patients with aortic aneurysm. Thus, firstly, confirms the position that there are common antigenic structures and, consequently, common molecular motifs in viruses and humans. Secondly, it confirms the ability to diagnose the somatic state by serological methods. In Figure 2 (left part), adaptogens (the formed complex of molecular motifs) involved in the hormonal (somatic) adaptation of a healthy person manifest themselves as adaptins capable of inducing antibodies that serve as a marker of the hormonal (somatic) state. In result, if infectious pathogens are markers of disease, then adaptins are markers of the hormonal (somatic) state of mammals, including humans. It has to be assumed, molecular motifs found in animals in the form of 827,000 viruses (the data presented at the 68th Annual International Conference "Association of Wildlife Diseases," held on August 4-9, 2019 in Tahoe City, California (USA), Zoe Grange, et al.: "SpillOver - a new web tool to assess spillover risk of wildlife viruses") were non-infectious adaptogens or adaptins.

The main task of advanced diagnostic technologies is the epidemiological forecasting of new infections and somatic changes based on the construction of regional and personal immunoantigenograms with subsequent correction of the human immune and hormonal systems based on the use of biological laws of nature. In this plan, it is necessary to combine the existing methodological developments and improve them for the new tasks arising from the concept: "In nature, small fragments of genetic information (molecular motifs) evolve into large, structured formations called viruses. At the same time, the same motive can be found in different micro- and macro-organisms, that confirms the unity of the biological world, its close relationship and interdependence"<sup>3</sup>.

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## Comprehensive Overview of 473 Cases of COVID-19: Outcome Experiences of a Dedicated Hospital in Dhaka, Bangladesh

By Perveen RA, Nasir M, Ferdous J, Murshed M, Nazneen R & Rahman MA

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**Methods:** This retrospective study was conducted from May to September 2020 in 720 bed Holy Family Red Crescent Medical College Hospital. Four hundred seventy-three patients included in this study, diagnosed by RT-PCR of the nasopharyngeal swab, were divided into four groups. The mild group includes 254 patients, the moderate group has 82 patients, 38 patents in the severe group, and the critical group who were admitted to ICU, 99 patients. Demographic data, available investigation reports of individual patients, obtained from hospital records manually and compared between all four different categories of patients.

**Keywords:** COVID-19, biomarkers, co-morbidities, clinical features, severe, critical, Bangladesh.

**GJMR-F Classification:** NLMC Code: QW 168.5.C8



*Strictly as per the compliance and regulations of:*



# Comprehensive Overview of 473 Cases of COVID-19: Outcome Experiences of a Dedicated Hospital in Dhaka, Bangladesh

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**Results:** Among 473 patients, the majority were male (359) compares with female (115). The mean age of the mild group was 39.04(±12.24) years, the moderate group was 52.35(±11.92) years, the severe group was 56.81(±15.51) years and 61.08 (±12.76) years in critical cases. The severe and the critical group had the higher percentages (23.68% and 33.34%) of the patients in the 60 - 69 years of age group. Average 10- 12 days of hospital stay were observed in all four groups of patients. The mortality rate was 08.46%, 39 in the critical and only one in the severe group. Diabetic Mellitus (35.09%) and hypertension (32.55%) were the predominant comorbidities. The highest percentage of symptoms were shortness of breath (40.38%), fever (33.61%), cough (27.06%) followed by anosmia (10.57%), lethargy (08.03%), diarrhea (06.34%), myalgia (05.71%), loss of aste (04.44%) and sore throat (03.59%). Total WBC count, NLR, d-NLR, PLR, Platelet, CRP, SGPT, PT, INR levels were statistically significant. Changes in the renal and metabolic (Serum creatinine, HbA<sub>1c</sub>, lipid profile) biomarkers were statistically unremarkable. Chi-

square test for qualitative variables and one-way ANOVA for quantitative variables were done by SPSS version 21.0, and all values were two-tailed, with  $p < .05$  considered as statistically significant.

**Conclusions:** The preliminary observations and outcomes of disease severity in the case of COVID-19 included age, the presence of co-morbid conditions, and changes in hematological, inflammatory, and hepatic biomarkers in the blood. However, the symptoms were dominated by fever, cough, breathlessness in severe and critical case, whereas anosmia was a common predictor in mild cases. The clinical correlation and management strategy should be adopted with the pace of viral mutation and immune response of the population at a larger scale and time.

**Keywords:** COVID-19, biomarkers, co-morbidities, clinical features, severe, critical, Bangladesh.

## I. INTRODUCTION

More than a year has passed since the first diagnosed SARS-CoV-2 infection in Wuhan; China was announced in December 2019. This was an unprecedented year with more than 15 billion documented infections and more than 3.2 million deaths worldwide due to SARS-CoV-2<sup>1</sup>. This large number of infected patients with a case fatality ratio ranges from 0.1% to 25% in different countries demonstrates that the coronavirus disease is extremely contagious<sup>2</sup>. on 11th March 2020, WHO declared COVID-19 a pandemic situation. Near this announcement, Bangladesh reported their first case of COVID-19 on 8th March 2020. From then to 2nd May 2021, Bangladesh deals with 7,60,584 confirmed cases and 11,510 death<sup>3</sup>. Besides Bangladesh, COVID became a concern in the densely populated South Asian region with more than 8 million confirmed cases and 1.2 million deaths up to 17th February, 2021<sup>4</sup>.

SARS-COV-2 is a single-stranded enveloped RNA virus that produces symptoms like fever, myalgia, non-productive cough, fatigue, shortness of breath, diarrhea, and many others in affected patients<sup>5</sup>. COVID-19 patients were categorized into mild, moderate, severe, and critical cases for proper management. Mild cases represent Influenza-like illness (ILI), moderate with pneumonia, severe patient with severe pneumonia, sepsis, and with ARDS, septic shock developed in those, considered as critical<sup>6</sup>.

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As the pandemic continues, global biomedical researchers are working urgently to identify coronavirus risk factors. Older age and underlying co-morbidities – particularly cardiovascular disease, diabetes, respiratory disease, chronic kidney disease, and many more are at high risk of severity<sup>7</sup>.

Besides symptoms and co-morbidities, change in some biomarkers level also reflects the disease severity. Though COVID-19 is a novel disease, Evidence shows severe inflammatory response, which contributes to weak adaptive immune response, thereby resulting in immune response balance in the patient body. Therefore, circulating biomarkers representing inflammation and immune status are potential predictors for the prognosis of COVID-19 patients<sup>8</sup>. Among hematological parameters, disease severity is associated with lymphopenia. Non-survivors of COVID 19 have had significantly less amount of lymphocyte counts than survivors<sup>9</sup>- other blood cells – including white blood cells, neutrophils, and platelets, were partial predictors to differentiating mild cases from severe COVID-19. Other than these, NLR, d- NLR. PLR are indicators of systemic inflammatory response<sup>10,11</sup>.

Besides hematological markers, increased liver and cardiac biomarkers, which reflect dysfunction of these organs, were also observed in the critical group of patients than those with milder disease<sup>12,13,14</sup>. C-reactive protein, serum ferritin level, levels of plasma D-dimers, and fibrin degradation products of COVID patients also correlate with disease severity<sup>15,16,17</sup>.

As this is a novel virus, scientific research is going on throughout the world to know more about how we can manage the patients affected by it. So, we conducted this retrospective study on 473 different categories of admitted COVID-19 patients to highlight their difference between a demographic profile, symptoms, comorbidities, and change on the biomarkers in a tertiary care dedicated hospital.

## II. MATERIALS AND METHOD

**Study design:** This observational study was conducted in Holy Family Red Crescent Medical College Hospital (HFRCMCH) from May 17th to September 9th, 2020. HFRCMCH was a 720-bed tertiary care hospital located in Dhaka, Bangladesh. This hospital was assigned responsibility for treating patients with COVID-19 by the People's Republic of Bangladesh on May 15th May 2020, for five months. All RCT-PCR positive (by nasopharyngeal swab) patients treated in HFRCMCH within the period of the study were included. Patients who have insufficient information and discontinued or unavailability of any data, excluded from the study.

**Data collection method:** The researcher screened all 1348 hospital record files of admitted patients. All the data recorded in a customized form. Researcher divided 473 patients' record files into four groups, the mild group

includes 254 patients, the moderate group has 82 patients, the severe group has 38 patients, and the critical group have 99 patients.

**Case definition:** National Guideline of Bangladesh published on 5th November 2020 categorized the confirmed COVID-19 cases. Mild cases present with fever, cough, sore throat, malaise, headache, muscle pain without shortness of breath, or abnormal imaging. Moderate group of patients have clinical sign of pneumonia with oxygen saturation of more than 90% at ambient air. The severe group of patients have 30 breaths/ minute and finger oxygen saturation less than 90% at rest. The critical group of patients admitted in ICU with respiratory failure or any other organ failure or shock and requiring mechanical ventilation. Though the clinical categories of the patients were discrete by the Triage zone (the zone where sorting of patients occur according to the urgency of their need for care), attending doctors, and attending critical care physicians.

**Ethical declaration:** The hospital authority and the institutional ethics board of Holy Family Red Crescent Medical College approved the study. Though it is a retrospective study, formal consent was not taken from the patients. However ethical measures were taken throughout the study period to maintain a high standard of confidentiality of patient's hospital record files.

**Data acquisition and statistical analysis:** We categorized age into eight groups with ten years' interval. We observed demographic data (age, gender, hospital stay, mortality), co-morbidities (DM, HTN, CKD, IHD, Bronchial asthma, Thyroid disease, cancer), symptoms (inflammatory and neurological), and laboratory biomarkers (hematological, inflammatory, hepatic, renal, metabolic). We expressed categorical variables like age range, comorbidities, and symptoms as the counts and percentage and continuous variables like age, hospital stay, and biomarkers as mean and standard deviation. We used SPSS version 21.0 for statistical analysis (chi-square test for qualitative variables and one-way ANOVA for quantitative variables), and all values were two-tailed, with  $p < .05$  considered as statistically significant.

## III. RESULT

Among 473 patients admitted in the hospital with COVID-19, the mean age of the mild group was  $39.04(\pm 12.24)$  years, gradually increasing in  $52.35(\pm 11.92)$  moderate group,  $56.81(\pm 15.51)$  in severe group and  $61.08(\pm 12.76)$  in critical group, with an age range from 18 to 91 years. Most of the severe and critical patients were in 60-69 years (23.68% and 33.34%), the moderate group were 50- 59 years (42.68%), and the mild group were 30- 39 years (31.89%). Out of all patients, 359 were male, and 115 were female. The male: female ratio was 1:3.12. Thirty-nine patients (39.39%) in ICU and only one patient

(2.63%) admitted in the general ward have died.  
(Table 1)

**Table 1:** Demography of different cases of COVID patients

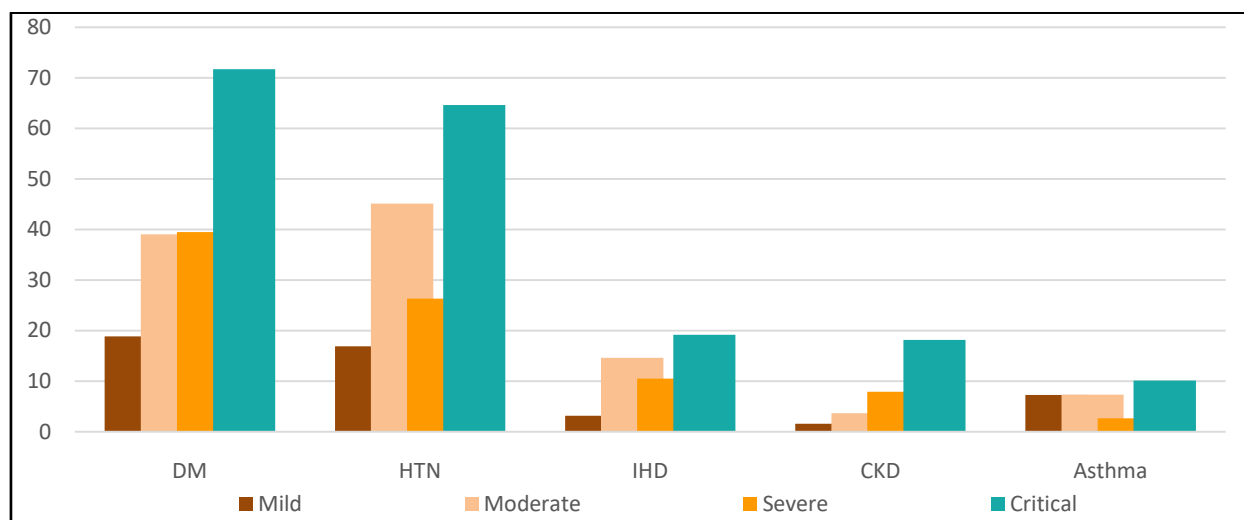
	Mild case (n= 254)	Moderate case (n=82)	Severe case (n= 38)	Critical case (n=99)
Mean age	39.04± 12.24	52.35± 11.92	56.81± 15.51	61.08± 12.76
10-19years	03/ 254 (0.79%)	-	-	-
20-29years	63/ 254 (24.80%)	02/ 82 (02.44%)	01/ 38 (02.63%)	01/ 99 (01.01%)
30- 39 years	81/ 254 (31.89%)	12/ 82 (14.63%)	03/ 38 (07.89%)	06/ 99 (06.06%)
40- 49years	57/ 254 (22.44%)	13/ 82 (15.85%)	07/ 38 (18.42%)	08/ 99 (08.08%)
50- 59years	36/ 254 (14.17%)	35/ 82 (42.68%)	10/ 38 (26.31%)	27/ 99 (27.28%)
60- 69years	12/ 254 (04.72%)	14/ 82 (14.07%)	09/ 38 (23.68%)	33/ 99 (33.34%)
70 and above	04/ 254 (01.57%)	06/ 82 (07.32%)	08/ 38 (21.05%)	24/ 99 (24.25%)
Male/ Female	213/ 42	48/34	27/ 11	71/ 28
Hospital stay in days	12.19± 05.26	12.24± 07.29	10.96± 07.10	12.44± 10.22
Mortality (%)	-	-	01/ 38 (02.63%)	39/ 99 (39.39%)

Regarding co-morbidities, the highest number of patients in all four groups presents with diabetes Mellitus (35.09%) and hypertension (32.55%) than other co-morbidities like ischemic heart disease (09.09%), chronic kidney disease (03.81%), bronchial asthma (05.07%), thyroid-related disorder (02.32%) and neoplasm (01.06%). Among all four groups, the highest

number of co-morbidities present in critical patients (71.72%, 64.65%, 19.19%, 18.19%, 10.10%) in comparison with the other three groups, which were statistically not significant. Patients with thyroid-related disorder in lowest percentage (0.79%, 04.88%, 02.63%, 04.04%) in all four groups and cancer (02.63%, 04.04%) in severe and critical patients. (Table: 2, Fig: I)

**Table 2:** Comparison of presence of co-morbidities among different stages of COVID patients

Characteristics	Mild case (n= 254)	Moderate case (n=82)	Severe case (n= 38)	Critical case (n=99)	Statistical Significance
DM	48/ 254 (18.89%)	32/ 82 (39.02%)	15/ 38 (39.47%)	71/ 99 (71.72%)	Chi-square = 48.981. $p < 0.00001$ .
HTN	43/ 254 (16.93%)	37/ 82 (45.12%)	10/ 38 (26.31%)	64/ 99 (64.65%)	
IHD	08/ 254 (03.14%)	12/ 82 (14.63%)	04/ 38 (10.52%)	19/ 99 (19.19%)	
CKD	04/ 254 (01.57%)	03/ 82 (03.66%)	03/ 38 (07.89%)	18/ 99 (18.19%)	
Bronchial asthma	07/ 254 (07.25%)	06/ 82 (07.32%)	01/ 38 (02.63%)	10/ 99 (10.10%)	Result is highly significant at $p < .001$ .



**Figure-1:** Co-morbidities of different stages of COVID patients

The presenting symptoms of the patients were variable. The highest percentage of symptoms were shortness of breath (40.38%), fever (33.61%), cough (27.06%) followed by anosmia (10.57%), lethargy

(08.03%), diarrhea (06.34%), myalgia (05.71%), loss of taste (04.44%) and sore throat (03.59%). These symptoms were compared between four groups of patients and were not statistically significant. Fever

(18.50 %), anosmia (17.71%), and cough (14.96%) were the most common in the mild group of patients. Whereas, SOB (57.32%), cough (46.34%), and fever (45.12%) in the moderate group of patients. The severe group of patients complain about similar symptoms in a

higher percentage (76.31%, 52.63%, and 31.51%). SOB (85.85%) was the most common symptom, followed by fever (66.66%), cough (32.32%) and anosmia was absent in ICU admitted patients (Table: 3, Fig: II)

Table 3: Symptoms of different stages of COVID patients

Symptoms		Mild case (n= 254)	Moderate case (n=82)	Severe case (n= 38)	Critical case (n=99)	Statistical Significance
Inflammatory	Fever	47/ 254 (18.50%)	37/ 82 (45.12%)	12/ 38 (31.51%)	63/ 99 (63.64%)	Chi-square = 43.2556. $p=.00002$ . Result is highly significant at $p < .001$
	Cough	38/ 254 (14.96%)	38/ 82 (46.34%)	20/ 38 (52.63%)	32/ 99 (32.32%)	
	SOB	30/ 254 (11.81%)	47/ 82 (57.32%)	29/ 38 (76.31%)	85/ 99 (85.85%)	
	Sore Throat	10/ 254 (03.94%)	04/ 82 (04.88%)	02/ 38 (05.26%)	01/ 99 (01.01%)	
	Diarrhea	10/ 254 (03.94%)	12/ 82 (14.63%)	04/ 38 (10.52%)	04/ 99 (04.04%)	
Neurological	Myalgia	13/ 254 (05.12%)	08/ 82 (09.76%)	03/ 38 (07.89%)	03/ 99 (03.03%)	Chi-square = 76.9569. $p< .00001$ Result is highly significant at $p < .001$
	Lethargy	05/ 254 (01.97%)	12/ 82 (14.63%)	08/ 38 (21.05%)	12/ 99 (12.12%)	
	Anosmia	45/ 254 (17.71%)	04/ 82 (04.88%)	01/ 38 (02.63%)	-	
	Loss of taste	07/ 254 (02.75%)	06/ 82 (07.32%)	02/ 38 (05.26%)	06/ 99 (06.06%)	

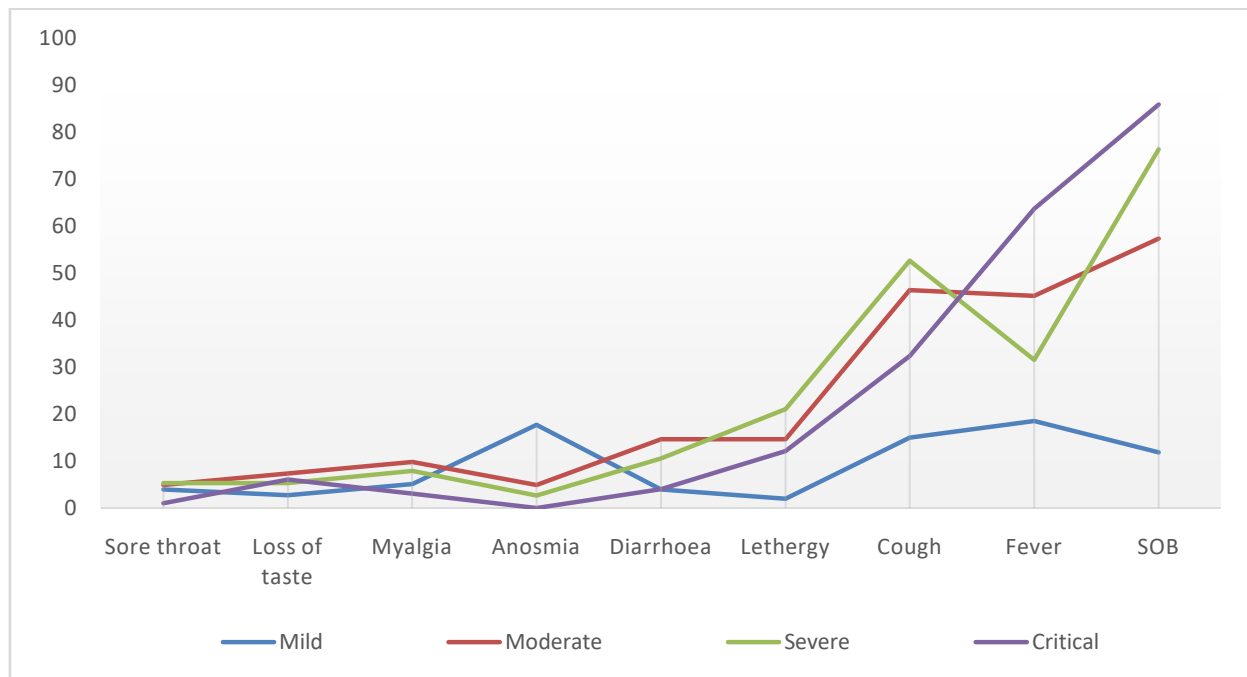


Figure-2: Symptoms of different stages of COVID patients

Table 4: Changes in the biomarkers in different/ respective/ distinct stages of COVID patients

Biomarkers		Mild case (n= 254)	Moderate case (n=82)	Severe case (n= 38)	Critical case (n=99)	Statistical Significance Test
Hematological	Hb%	13.28± 2.32	12.12± 1.67	12.55±1.33	12.33±2.15	$p = .385118$ .
	Total WBC	6,622± 2,432	7,778± 3,059	8,766±3,641	10,532±4,174	$^{**}p = .005149$ .
	NLR	2.18± 2.37	04.48± 03.17	05.09±03.23	07.56± 5.43	$^{***}p = < .00001$ .
	d- NLR	1.68± 1.65	03.36± 02.03	03.93±02.38	05.68± 4.60	$^{***}p = < .00001$ .
	PLR	128.35± 62.84	216.81±131.48	206.99±78.99	266.92±178.18	$^{***}p = .000018$ .
	Platelet (10 <sup>9</sup> /LX)	253 X 10 <sup>9</sup> ± 71 X 10 <sup>9</sup>	287X 10 <sup>9</sup> ± 103X 10 <sup>9</sup>	295X 10 <sup>9</sup> ± 83X 10 <sup>9</sup>	298X 10 <sup>9</sup> ± 99X 10 <sup>9</sup>	$^{*}p = .037806$ .
Inflammatory	HCT	41.13± 6.83	37.93± 4.96	39.47± 4.32	38.69± 5.38	$p = .073442$ .
	CRP (mg/ L)	9.70± 10.57	17.39± 13.76	33.56± 28.42	35.49± 27.55	$^{***}p = .000226$ .
	D dimer (mg/ L)	0.21± 0.59	0.72± 01.73	0.91± 01.38	01.43± 02.05	$p = .106931$ .
Hepatic	Ferritin (ng/ml)	295.39±322.41	561.34±560.36	761.43±1020.33	897.20±644.04	$^{**}p = .006706$
	SGPT (IU/ L)	49.78± 36.71	57.73± 45.28	87.50± 83.06	61.82± 44.28	$^{*}p = .042316$ .
	Prothrombin time (Sec)	13.97± 2.13	14.49± 02.86	14.64± 02.10	15.97± 02.66	$^{***}p = .000023$ .
Renal	INR	1.07± 0.17	01.10± 0.13	01.17± 0.22	01.20± 0.27	$^{***}p = .000065$ .
	S. creatinine (mg/ dl)	1.23± 1.19	01.15± 0.31	01.77± 03.33	01.76± 01.91	$p = .432518$ .
Metabolic	HbA <sub>1</sub> C (%)	6.12± 1.19	06.45± 1.52	06.39± 1.05	07.45± 01.04	$p = .336891$ .
	Total Cholesterol	160.99± 38.77	149.23± 42.57	138.45± 48.22	138.34± 71.32	$p = .658324$ .
	Triglyceride	230.28±160.01	189.51±130.99	142.6±71.48	225.54± 94.59	$p = .677266$ .
	HDL	31.61± 9.08	34.08± 12.51	34.63± 14.89	28.42± 10.01	$p = .079309$ .
	LDL	83.83± 31.71	79.89± 29.76	77.64± 39.61	73.32± 41.65	$p = .699251$ .

\* stands for significance  $p < .05$ , \*\* stands for significance  $p < .01$ , \*\*\* stands for significance  $p < .001$

Different categories of COVID-19 patients show change in the level of biomarkers. Most of the biomarkers showed significant change except Hb%, HCT, Serum Creatinine, HbA<sub>1</sub>C, and serum lipid profile level. (Table 4)

#### IV. DISCUSSION

The retrospective study revealed the difference in demographic data, age groups, gender, clinical symptoms, and change in the biomarkers in admitted four different clinical categories of COVID-19 patients. Data were recorded from May to September 2020 in the pick of the pandemic to distinguish the relevant factor of disease severity.

The number of male patients (359) admitted to the hospital was much higher than the number of the female (114), which was similar to the other studies worldwide, including Bangladesh<sup>13,18,19,20</sup>. Patients mean age increased from 39 years to above 60 years according to disease severity. The severe and critical group of patients were above 60 years, found to be similar among the same categories patients in other studies<sup>18,19,20,21</sup>.

COVID-19 patients who have co-morbid conditions such as diabetes mellitus (DM), hypertension (HTN), ischemic heart disease (IHD), chronic kidney disease (CKD), and bronchial asthma lead to disease severity, thus increases ICU admission and risk of

mortality. Other observational studies of Bangladesh<sup>22,23,24</sup>, and china<sup>7,14</sup> support similar findings. In our study, mild category patients present with a lower percentage of co-morbid conditions than moderate to critical ones. A lower percentage of patients without comorbidities have a lower case fatality rate (0.9%)<sup>25</sup>.

In this study, patients present with various inflammatory and neurological symptoms, which were almost similar in many studies. But the predominant symptoms vary in different categories of patients. Fever, anosmia, and cough were the most frequent symptoms in the mild group of patients. Whereas shortness of breath, cough, and fever was common and increased in percentage in the other three groups. Anosmia was absent in the critical group. Several studies in Bangladesh<sup>19,20,22,23</sup> and worldwide<sup>7,14</sup> show patients with similar symptoms.

In this study, we observed and compared several biomarkers level like hematological, inflammatory, hepatic, renal, and metabolic between different clinical categories of the COVID-19 patients to focus on disease severity. We found a statistically significant rise of total WBC, NLR (neutrophil-lymphocyte ratio), d-NLR, PLR (platelet-lymphocyte ratio), and total platelet count, but Hb% and HCT were not statistically remarkable in all four groups of patients. These hematological findings were associated with disease severity, clearly support our study findings<sup>7,9,14</sup>.

Specially platelets, NLR, d- NLR. PLR were also discriminating mild cases from severe COVID-19<sup>10,11</sup>.

Among the inflammatory biomarkers (CRP, d-Dimer, and ferritin), we observed a statistically significant change in CRP levels in different clinical categories of COVID-19 patients. Several studies stated raised levels of the inflammatory marker has a clear connection with the severity of illness<sup>15,16,17</sup>. We found a significant difference in increased SGPT, prothrombin time, and INR between all four categories of COVID-19 patients. Patients with severe COVID-19 appear to have more frequent signs of liver dysfunction than those with milder disease<sup>12,14,17,26</sup>. Changes in the renal and metabolic (Serum creatinine, HbA<sub>1c</sub>, lipid profile) biomarkers were also unremarkable.

## V. CONCLUSION

The pragmatic observations and outcomes of the study guides, age, co-morbid conditions, and changes in hematological, inflammatory, and hepatic biomarkers, influences the disease severity in COVID-19 cases. However, the commonly observed symptoms were fever, cough, breathlessness in severe and critical cases, whereas anosmia was the common predictor in mild cases. This clinical experience and correlation helped us adopt the management strategy, with the new variant and immune response against it, in our population.

## VI. LIMITATIONS

The study has few limitations, including a short period, and data were not representing the information of all socioeconomic classes of the country.

## ACKNOWLEDGMENTS

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### Conflict of Interest

None of the co-authors declared any conflict of interest.

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<b>Certificate</b> , LoR and Momento 2 discounted publishing/year <b>Gradation</b> of Research 10 research contacts/day 1 GB Cloud Storage GJ Community Access	<b>Certificate</b> , LoR and Momento <b>Unlimited</b> discounted publishing/year <b>Gradation</b> of Research <b>Unlimited</b> research contacts/day 5 GB Cloud Storage <b>Online Presense</b> Assistance GJ Community Access	<b>Certificates</b> , LoRs and Momentos <b>Unlimited</b> free publishing/year <b>Gradation</b> of Research <b>Unlimited</b> research contacts/day <b>Unlimited</b> Cloud Storage <b>Online Presense</b> Assistance GJ Community Access	GJ Community Access



# PREFERRED AUTHOR GUIDELINES

## **We accept the manuscript submissions in any standard (generic) format.**

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from <https://globaljournals.org/Template>

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at [submit@globaljournals.org](mailto:submit@globaljournals.org) or get in touch with [chiefeditor@globaljournals.org](mailto:chiefeditor@globaljournals.org) if they wish to send the abstract before submission.

## BEFORE AND DURING SUBMISSION

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct*, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

## **Declaration of Conflicts of Interest**

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

## POLICY ON PLAGIARISM

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

## AUTHORSHIP POLICIES

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

### Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

### Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors' research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

### Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

### Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

### Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

## PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



### ***Manuscript Style Instruction (Optional)***

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

### ***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



## FORMAT STRUCTURE

***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

### **Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

### **Author details**

The full postal address of any related author(s) must be specified.

### **Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

### **Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

### **Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

### **Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

### **Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

### **Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.





## Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

### PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

### TIPS FOR WRITING A GOOD QUALITY MEDICAL RESEARCH PAPER

**1. Choosing the topic:** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

**2. Think like evaluators:** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

**3. Ask your guides:** If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

**4. Use of computer is recommended:** As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

**5. Use the internet for help:** An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



**6. Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

**7. Revise what you wrote:** When you write anything, always read it, summarize it, and then finalize it.

**8. Make every effort:** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

**9. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

**10. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

**11. Pick a good study spot:** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

**12. Know what you know:** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

**13. Use good grammar:** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

**14. Arrangement of information:** Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

**15. Never start at the last minute:** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

**16. Multitasking in research is not good:** Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

**17. Never copy others' work:** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

**18. Go to seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**19. Refresh your mind after intervals:** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



**20. Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

**21. Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

**22. Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

**23. Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

## INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

### Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

### Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

### The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

### General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.



### *Mistakes to avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

### **Title page:**

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

*Reason for writing the article—theory, overall issue, purpose.*

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

### **Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

### **Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

#### **Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

#### **Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

#### **Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

#### **Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

#### **Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

#### **What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.





**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
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