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Male gender and aging as predictors of severe COVID-19: Studying the genetic, laboratory, radiologic and clinical findings among Egyptianadults

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Abstract:
COVID-19 poses a serious risk, especially to the elderly. Additionally, COVID-19 has demonstrated a blatant sex-specific bias, with men exhibiting a more severe reaction and greater fatality rate. The aim of this study is to assess the genotypic and allelic frequencies of the chemokine ligand CXCL12 rs2839693 and its receptor CXCR4 rs2228014 according to age and sex. Also, to evaluate the interaction between age and sex and other risk variables affects how severe COVID-19 is.The present study conducted on 131 female and 169 male COVID-19 Egyptian patients who admitted to Assiut University Quarantine Hospital during the period from July to November 2022, the mean age of patients was 64.31±11.002 years old, patients with age > 65 years were 156 and < 65 years old were 144. Real-time PCR was used to identify the polymorphisms of CXCL12 rs2839693 and CXCR4 rs2228014 using a TaqMan test probe.No significant differences in CXCL12 rs2839693 and CXCR4 rs2228014 genotypic and allelic frequencies according to age and sex.Severe/critical illness was more prevalent in males than females. Also, severe and critical illness was more prevalent in patients with age >65years than patients with age ≤65.Of the statistics that are usually obtained upon admission, age and sex were significant indicators of the severity of the condition. Severe instances were more likely to occur in males and elders. Particular care needs to be paid to older male individuals, as well as individuals with comorbid diseases.

Key-words: CXCR4, CXCL12, COVID-19, SARS-CoV-2, male, female.

INTRODUCTION

Coronaviruses were first identified in the beginning of the 1930s, after a serious respiratory condition in domesticated chickens proved to be triggered by avian infectious bronchitis virus (IBV). The earliest coronaviruses that affect humans (HCoVs) were identified in the 1960s. The word coronavirus (Latin: corona, crown) was used to describe these organisms, which have a distinctive fringing morphology in the electron microscope(Peiris, 2012).

In the last 20 years, coronaviruses (CoV) have become a major hazard to healthcare due to global outbreaks in 2002 due to severe acute respiratory syndrome (SARS)and in 2012 due to middle east respiratory syndrome (MERS)(Pourbagheri-Sigaroodi et al., 2020).

The coronavirus disease 2019 (COVID-19) pandemic began in Wuhan, China, in early December 2019 and quickly spread around the world. The etiology of COVID-19 was identified as novel coronavirus (2019-nCoV) which known as severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*), a betacoronavirus that had not been discovered before(Yang et al., 2020).Around 6.9 million deaths and over 769 million confirmed cases had been recorded worldwide as of August 6, 2023(Ikejezie et al., 2024).

For COVID-19 patients, routine testing typically entails a complete blood count (CBC), assays investigating the coagulation and fibrinolysis pathways (activated Partial Thromboplastin Time(PTT), prothrombin Time(PT), and D-dimers), and factors related to inflammation (C.Reactive Protein(CRP), Erythrocyte Sedimentation Rate(ESR), procalcitonin, andferritin)(Wang et al., 2020).

The clinical presentation of COVID-19 might vary from the absence of symptoms to the malfunctioning of numerous organs. Severe COVID-19 can result in consequences such as pneumonia, blood clots, coronary artery disease, renal damage, acute respiratory distress syndrome (ARDS), sepsis, and other infections caused by viruses and bacteria that are not associated with coronavirus(Sivaselvi et al., 2023).

An increasing amount of studies has demonstrated that COVID-19 affects organs other than the lungs, such as the olfactory, neurological, cardiovascular, hepatobiliary gastrointestinal, endocrinologic,dermatological, and kidneys(Tsai et al., 2021).

Eighty percent of COVID-19 patients start off with a mild disease that may normally be treated without the need for hospitalization. For moderate-to-severe disease, 15% of patients require hospitalization(Wu & McGoogan, 2020).

Numerous changes brought about by aging in the human body might impact COVID-19 pathogenesis. There are several factors that contribute to lung aging, including oxidative stress, mitochondrial dysfunction, NK cell cytotoxicity, immunosenescence linked to immune surveillance, and enhanced cellular senescence. Immune alterations associated with aging include altered neutrophilic infiltration, IFN-γ signaling, decreased numbers of CD4+ or CD8+ T cells and naïve B cells, increased production of pro-inflammatory cytokines, and activation of alveolar macrophages, all of which may deteriorate with age. COVID-19 advancement(Schneider et al., 2021).

There is a correlation between specific demographic characteristics and an increased likelihood of a severe clinical course of COVID-19.Among them, advanced age is regarded as a crucial component in the suggested clinical severity risk ratings as it is a significant predictor of death(Cecconi et al., 2020).

A greater risk of severe disease is seen with older COVID-19 patients. Understandably, the primary cause of the higher death rate seen in this age range is age-associated comorbid diseases. A patient may be classified as elevated or minimal-risk based more on age instead of real health that might result in inaccurate risk evaluation, inefficient use of resources, and poor patient care(Statsenko et al., 2022).

There are several pulmonary viral infections that improperly impact men and women. Men tend to have lower respiratory tract infections more frequently than women do, and they also tend to get more severe disease from SARS-CoV, respiratory syncytial virus (RSV), andinfluenza A and B viruses. Males are also more likely to die from *SARS-CoV-2,*received admission to the critical care unit (ICU), and require hospitalization, even though their infection rates seem to be comparable to those of females. Estimates of the infection fatality ratio broken down by sex show that male mortality rates are consistently higher across all age groups(Zsichla & Müller, 2023).

An increasing amount of research suggests that infectious illnesses may impact men and women differently. Economic and social status,sex disparities, job-related exposure, and a gender-specific gap in immunological reactions are the causes of these gender disparities(Morgan & Klein, 2019).

Sex variations in morbidity and mortality from illnesses may be partially explained by certain demographic variables, including work force involvement, lifestyle traits like cigarette smoking. Furthermore, a number of biological variables, including obesity, insulin resistance or insufficiency, elevated cholesterol levels, and estrogen production, are important in the pathogenesis of many illnesses. There's proof that women visit

doctors more frequently for acute, self-limited ailments, but they receive different treatment than males for prevalent chronic conditions. But in the majority of populations, ladies live longer than males(Falagas et al., 2007).

Chemokines are cytokine derivatives called for their chemotactic involvement in the migration of certain immune cells. It is believed that chemokines and their receptors are crucial for a number of physiological functions, including immunological responses, wound healing, and cancer development. Chemokines impact both the adaptive and innate immune responses. They play a function in the inflammatory process by directing the chemotaxis of white blood cells, which causes neutrophils and monocytes to migrate to the site of tissue damage or infection(Jia et al., 2022).

CXCL12 rs2839693 contributed to the emergence and severity of COVID-19. Rather than mild or moderate sickness, Patients having a higher risk T allele or TT genotype had serious or life-threatening illnesses(Korayem et al., 2023).

The CXCR4 gene is situated at location 21 on the long arm of chromosome 2, and rs2228014 is found on CXCR4 exon 2. Complex diseases like as pulmonary disease, HIV, warts disease, pulmonary artery hypertension (PAH), and cancer are associated with CXCR4 rs2228014(Kawaguchi et al., 2019).

This study aims to evaluate the genotypic and allelic frequencies of the chemokine ligand CXCL12 rs2839693 and its receptor CXCR4 rs2228014 among males and females, COVID-19 patients with age over 65 years old and below 65 years old. As far as we are aware, this is the first research to show that. Also, to evaluate the interaction between age and sex and other risk variables affects how severe COVID-19 is.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study was conducted on 131 female and 169 male COVID-19 Egyptians with real time PCR-confirmed COVID-19 who admitted to Assiut University Quarantine Hospital during the period from July 2022 to November 2022, the patients mean age was 64.31±11.002 years old, patients with age over 65 years old were 156 and below 65 years old were 144. In this study, more patients were male and more patients were more than 65 years. In accordance with WHO standards (Organization, 2020), the diagnosis of COVID-19 is contingent upon the achievement of positive findings from reverse transcription polymerase chain reaction (RT-PCR) on nose or pharyngeal swabs.

Criteria for Inclusivity and Exclusion

The research included all Egyptian adults (aged from 18 to 85 years) that had been diagnosed with COVID-19. The exclusion criteria were: (1) Individuals under the age of 18 or over the age of 85. (2) The research eliminated individuals who had inadequate charts, such as lacking clinical and hematological information, as well as those lacking clinical outcomes records. (3) Individuals that were referred from other medical centers following a long period of hospital admission but did not have an admission full blood count (CBC), at the initial center. (4) Individuals with the following conditions: coronary heart disease, HIV-1 2, cancer, systemic lupus erythematosus(SLE), COPD,celiac disease, asthma, and neurological conditions.

Data collection

We examined all individuals' clinical files, nursing documents, laboratory results, and chest CT images. Information gathering forms from electronic medical files were used to capture epidemiological, clinical, laboratory, and radiological parameters, as well as outcome and therapy information. Demographics, exposure history, underlying comorbidities, chest CT image, signs and symptoms, time of first symptoms, laboratory values at hospital admission,time from first symptom to dyspnea, ICU admission, complications,treatments, and prognosis were all included.

Accurate records were kept regarding the date of death, the first COVID-19 diagnosis, and the development of symptoms. The "onset survival time" refers to the period of time between the onset of certain symptoms and signs and the moment of death. Two researchers examined the data collecting forms separately in order to improve the accuracy of the data that was gathered. Additionally, the attending physicians spoke with the patients or their families directly upon admission to the hospital in order to get information on symptoms and epidemiology.

Laboratory and imaging methods

D-dimers levels were measured using an immunoturbidimetric assay. A chest computed tomography (CT) was implemented for all participants. Routinely, CBC, coagulation profiles, liver and renal functions, electrolytes, creatine kinase, myocardial enzymes, procalcitonin, andCRP were collected upon admission.

Severity assessment

According to the National Health Commission of the People's Republic of China COVID-19 individuals can be clinically classified as mild, moderate, severe, or critical.

(i) Mild: individuals with minor symptoms, and no pneumonia is shown on pulmonary imaging.

(ii) Moderate: individuals with Fever and breathing problems, with pneumonia evident on imaging; no dyspnea or other consequences.

(iii) Severe: Individuals with dyspnea,oxygen saturation ≤93%, respiratory rate (RR) ≥30 beats/min, and partial pressure of arterial oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≤300 mmHg. Pulmonary imaging indicates that lesions have advanced by more than 50% within 24 to 48 hours.

(iv) Critical: Individuals who suffer from shock, multiorgan failure necessitating ICU monitoring and care, or respiratory failure needing mechanical ventilation(Medicine, 2020).

Sample Size calculation

Sample size calculated conferring to Takazawa & Morita., 2020 (Takazawa & Morita, 2020)and based on the work of Belperio et al., 2004 (Belperio et al., 2004). The following assumptions were made while determining the sample size using Epi Info STATCALC: - Eighty percent power and ninety five percent two-sided confidence. The final sample consisted of 140 people, and the odds ratio, with a 5% error margin, was evaluated at 1.115.

Specimen collection

Two milliliters of blood from the vein were extracted from each COVID-19 Egyptians via venipuncture and put in an EDTA vacutainer tube for the genotyping assay of the CXCL12 rs2839693 and CXCR4 rs2228014. Samples of blood were frozen at -20°C in order to extract DNA.

Genotyping analysis
DNA extraction

Thermo Fisher Scientific's Genomic DNA Purification Kit was utilized in accordance with the primary procedure to isolate DNA from COVID-19 patients' blood. K0512 is the catalog number

Genotyping of CXCR4 rs2228014 and CXCL12 rs2839693

Thermo Fisher's Genotyping TaqPath 1-Step Multiplex, Cat. No. A28521, was the master mix utilized for DNA amplification. The amplification steps are as follows: The

enzyme was activated for 10 minutes at 95 degrees, followed by 35 cycles of denaturation for 15 seconds at 95 degrees, annealing for 1 minute at 60 degrees, and elongation for 1 minute at 72 degrees.

Thermo Fisher's TaqMan ready-made SNP assay (catalog number 4351379) was utilized for CXCR4 rs2228014 and the context sequence [VIC/FAM] was

GCCTCTGACTGTTGGTGGCGTGGAC[A/G]ATGGCCAGGTAGCGGTCCAGACT GA.

The context sequence [VIC/FAM]: for CXCL12 rs2839693 was

GAAGGGGACGACAGGATGCTCTAGG[C/T]ACCTGGGGAGGGGAGAATGGAG AGC utilizing the TaqMan ready-made SNP assay (Thermo Fisher; Catalogue no. 4351379).The PCR mix was made up of twenty μL by adding the following ingredients: distilled water (11.5 μL), SNP assay (0.5 μL), extracted DNA (3 μL), and Master Mix (5 μL). Equipment for real-time PCR (Applied Biosystems 7500) was used.

STATISTICAL ANALYSIS

The IBM SPSS software program version 20.0 was utilized to input and analyze data. Using $\chi 2$ test, the categorical variables were characterized as numbers and percentages.The use of the Kolmogorov-Smirnov test was used to make sure the distribution was normal. Range, which includes the lowest and greatest values, to describe quantitative data, the mean and standard deviation were utilized. To compare categorical variables amongst several groups, the chi-square test was utilized(Kirkpatrick, 2015).

The best cutoff value, sensitivity, and specificity of D-dimersand ferritin for all-cause mortality and disease severity were determined using receiver operating characteristic (ROC) curve analysis, which was also utilized to evaluate the prediction ability of D-dimersand ferritin on mortality and severity. The findings were presented as 95% confidence intervals (CI) and area under the curve (AUC). By contrasting the incidence of mortality or serious illness in two distinct sets of independent factors, the odds ratios were computed. Factors with a p-value < 0.05 were considered statistically significant for both disease outcome and severity. Multivariable logistic regression was performed, and a 95% confidence interval for an Adjusted Odds Ratio (AOR) was calculated.

RESULTS

1. **Gender stratification as regarding CXCR4 rs2228014.**

Our data showed no statistical significant differences between males and females COVID-19 patients according to CXCR4 rs2228014genotypic and allelic frequencies.

Table (1): Gender stratification as regarding CXCR4 rs2228014.

CXCR4 rs2228014	Male		Female		OR (95%CI)	P value
	No.	%	No.	%		
Genotypes						
GG	153	90.5	118	90.1	0.95(0.44-2.05)	&1.000
GA	13	7.7	13	9.9	1.32(0.59-2.96)	&0.539
AA	3	1.8	0	0	0.56(0.51-0.62)	&0.260
GA+AA	16	9.5	13	9.9	1.05 (0.49-2.28)	&1.000
Alleles						
G	319	94.4	249	95.0	1.14(0.55-2.36)	&0.855
A	19	5.6	13	5.0		

&Fisher's Exact test. OR: Odds ratio. CI:confidence interval. *p* values represent statistical significance < 0.05

2. Gender stratification as regarding CXCL12 rs2839693

According to table 2, no statistical significant differences between males and females COVID-19 patients according to CXCL12 rs2839693 genotypic and allelic frequencies.

Table (2): Gender stratification as regarding CXCL12 rs2839693.

CXCL12 rs2839693	Male		Female		OR (95%CI)	P value
	No.	%	No.	%		
Genotypes						
CC	113	66.9	96	73.3	1.36(0.82-2.25)	&0.256
CT	52	30.8	32	24.4	0.73(0.43-1.22)	&0.245
TT	4	2.4	3	2.3	0.97(0.21-4.40)	&1.000
CT+TT	56	33.1	35	26.7	0.74(0.45-1.22)	&0.256
Alleles						
C	278	82.2	224	85.5	0.79(0.51-1.22)	&0.317
T	60	17.8	38	14.5		

&Fisher's Exact test. OR: Odds ratio. CI:confidence interval. *p* values represent statistical significance < 0.05

3. Gender stratification as regarding Clinical course, CT findings, and outcomes

Table (3) demonstrationsa statistical significant difference between males and females as regard to clinical course where severe/critical illness was more prevalent in males than females (p value 0.017). No statisticalsignificant difference between males and females as regarding pneumonic consolidation (p value 0.332) while a highly statistically significant differences between them as regard to Bilateral GGO was detected where Bilateral GGO was more prevalent in males than females (p value 0.024). No statisticalsignificant difference between males and females as regarding home management, hospitalization without ICU, ICU, and death (p value 0.792, 0.599, 0.486, and 0.863 respectively)

Table (3): Gender stratification as regarding Clinical course, CT findings, and outcomes

	Male		Female		OR (95%CI)	P value
	No.	%	No.	%		
Clinical course						
Mild/moderate illness	38	24.4	54	37.5	0.54(0.33-0.88)	&0.017*
Severe and critical illness	118	75.6	90	62.5		
CT findings						
Normal	12	7.1	15	11.5	1.69(0.76-3.75)	&0.224
Bilateral GGO	125	74.0	80	61.1	0.55(0.34-0.90)	&0.024*
Pneumonic consolidation	56	33.1	51	38.9	1.29(0.80-2.07)	&0.332
Outcomes						
Home management	45	26.6	33	25.2	0.93(0.55-1.56)	&0.792
Hospitalization without ICU (yes)	124	73.4	98	74.8	1.08(0.64-1.82)	&0.792
ICU (YES)	78	46.2	66	50.4	1.19(0.75-1.87)	&0.486
Death	23	13.6	16	12.2	0.88(0.45-1.75)	&0.863

&Fisher's Exact test. CI:confidence interval.OR: Odds ratio. *p* values represent statistical significance < 0.05. ICU: Intensive care unit. GGO: Ground-glass opacity. CT: Computed Tomography.

4. Age stratification as regarding CXCR4 rs2228014

Subsequently, we compared CXCR4 rs2228014 genotyping between COVID-19 patients with age ≤65 years and >65 and it shows no statistical significant differences between the two groups according to CXCR4 rs2228014 genotypic and allelic frequencies (Table 4)

Table (4): Comparison according to genetic (CXCR4 rs2228014) findings of COVID-19 patients age ≤65 years vs >65 years.

CXCR4 rs2228014	≤65		>65		OR (95%CI)	P value
	No.	%	No.	%		
Genotypes						
GG	133	92.4	138	88.5	0.63(0.29-1.39)	&0.329
GA	9	6.3	17	10.9	1.84(0.79-4.26)	&0.217
AA	2	1.4	1	0.6	0.46(0.04-5.11)	&0.609
GA+AA	11	7.6	18	11.5	1.58 (0.72-3.46)	&0.329
Alleles						
G	275	95.5	293	93.9	0.73(0.35-1.50)	&0.468
A	13	4.5	19	6.1		

&Fisher's Exact test. OR: Odds ratio. CI:confidence interval. *p* values represent statistical significance < 0.05

5. Age stratification as regarding CXCL12 rs2839693

Table (5) displays no statistical significant differences between COVID-19 patients with age ≤65 years and >65 according to CXCL12 rs2839693 genotypic and allelic frequencies

Table (5): Comparison according to genetic (CXCL12 rs2839693) findings of COVID-19 patients with age ≤65 years vs >65 years.

CXCL12 rs2839693	≤65		>65		OR (95%CI)	P value
	No.	%	No.	%		
Genotypes						
CC	101	70.1	108	69.2	0.96(0.59-1.57)	&0.900
CT	42	29.2	42	26.9	0.90(0.54-1.48)	&0.700

TT	1	0.7	6	3.8	5.72(0.68-48.10)	&0.123
CT+TT	43	29.9	48	30.8	1.04(0.64-1.71)	&0.900
Alleles						
C	244	84.7	258	82.7	1.16(0.75-1.79)	&0.510
T	44	15.3	54	17.3		

&Fisher's Exact test. OR: Odds ratio. CI:confidence interval. *p* values represent statistical significance < 0.05

6. Age stratification as regarding clinical course, CT findings, and outcomes

Table (6) presents statistically significant variations in the clinical course between COVID-19 patients aged 65 and above. Patients aged 65 and older were more likely to have severe and critical illness (p value 0.017). Regarding bilateral GGO and pneumonic consolidation, there was no statistically significant difference between patients who were 65 years of age or older (p values of 1.000 and 0.620, respectively). There was no statistically significant difference in hospitalization without intensive care unit (ICU) and ICU between patients who were 65 years of age or older (p values of 0.598 and 0.107, respectively). There were statistically significant differences found regarding home management and mortality, with home management being more common in patients under 65 than in those over 65 (p value 0.013)while death was more prevalent in patients with age >65 than patients with age ≤65 (p value<0.001).

Table (6): clinical course, CT findings and outcomes of COVID-19 patients with age ≤65 years vs >65 years.

	≤65		**>65**		**OR (95%CI)**	**P value**
	No.	**%**	**No.**	**%**		
Clinical course						
Mild/moderate illness	54	37.5	38	24.4	0.54(0.33-0.88)	&0.017*
Severe and critical illness	90	62.5	118	75.6		
CT findings						
Normal	15	10.4	12	7.7	0.72(0.32-1.59)	&0.427
Bilateral GGO	96	66.7	109	69.9	1.16(0.71-1.89)	&0.620
Pneumonic consolidation	51	35.4	56	35.9	1.02(0.64-1.64)	&1.000
Outcomes						
Home management	47	32.6	31	19.9	0.51(0.30-0.87)	&0.013*
Hospitalization without ICU (yes)	97	67.4	125	80.1	1.95(1.16-3.30)	&0.013*
ICU (YES)	62	43.1	82	52.6	1.47(0.93-2.31)	&0.107
Death	4	2.8	35	22.4	10.12(3.50-29.30)	&<0.001*

&Fisher's Exact test. OR: Odds ratio. CI:confidence interval. *p* values represent statistical significance < 0.05.ICU: Intensive care unit. CT: Computed Tomography.GGO: Ground-glass opacity

7. ROC curve of D-dimersregarding clinical stage of COVID19

Table (7) shows ROC curve, cut value with sensitivity and specificity of D-dimersregarding clinical stage of COVID19 (mild/moderate and sever/ critical) and it show that at cut off value of >1 and sensitivity of 45.8% and specificity of 85.9% it can predict severity of the patients.

Table (7): ROC curve of D-dimersregarding clinical stage of COVID19

	Area Under the Curve	P value*	95% Confidence Interval	
			Lower Bound	Upper Bound
D dimer	0.694	<0.001*	0.638	0.745

**p* values represent statistical significance < 0.05.*Significant.

8. ROC curve of Ferritin regarding clinical stage of COVID19

Table (8) shows ROC curve, cut value with sensitivity and specificity of Ferritin regarding clinical stage of COVID19 (mild/moderate and sever/ critical) and it show that at cut off value of >540 and sensitivity of 53.5% and specificity of 73.1% it can predict severity of the patients.

Table (8): ROC curve of Ferritin regarding clinical stage of COVID19

	Area Under the Curve	P value*	95% Confidence Interval	
			Lower Bound	Upper Bound
ferritin	0.663	<0.001*	0.606	0.716

p values represent statistical significance < 0.05.*Significant.

DISCUSSION

This study was carried out on 131 female and 169 male COVID-19 Egyptians with real time PCR-confirmed COVID-19 who admitted to Assiut University Quarantine Hospital from July 2022 to November 2022, the mean age of patients was 64.31±11.002 years old, patients with age over 65 years old were 156 and below 65 years old were 144. No significant differences between males and females COVID-19 patients according to CXCL12 rs2839693 and CXCR4 rs2228014 genotypic and allelic frequencies. Also, no significant differences between COVID-19 patients with age ≤65 years and >65 according to CXCL12 rs2839693 and CXCR4 rs2228014 genotypic and allelic frequencies. A highly significant difference between males and females as regard to clinical course where severe/critical illness was more prevalent in males than females (p value 0.017). Significant difference between COVID-19 patients with age ≤65 years and >65 as regard to clinical course where severe and critical illness was more prevalent in patients with age >65years than patients with age ≤65 (p value 0.017).

CXCR4 rs2228014 and CXCL12 rs2839693genotyping according tosex

Regarding gender stratification our present data showed non-significant difference between male and female as regarding genotyping and allelic frequencies of CXCR4 rs2228014 and CXCL12 rs2839693.

Sex differences in clinical course,CT Findings, and outcomes.

Gender stratification as regarding clinical course revealed a significant difference in the frequency of mild/moderate illness and severe/critical illness between males and females. Males have increased severe/critical ratio and lower mild/moderate ratio than females.These data in agreement with data reported from 332 articles by Acheampong et al., 2020 and reported that severe COVID-19 has significant gender differences, with increased mortalities usually described amongst males than females(Acheampong et al., 2020).

The current study shows that among adult Egyptians, there is a notable sex difference in lung involvement. Consistent with Dangis et al., 2020 results whoreported that male COVID-19 patients had more extensive lung disease on CT than female patients did(Dangis et al., 2020), our data showed that men had a more severe lung engrossment than women; that is, men were found to have bilateral GGO more commonly than women (p 0.024).

Regarding the outcomes between males and females our data showed non-significant difference between males and females as regarding home management, hospitalization, ICU, and death. In contrast to these findings of the 5,700 hospitalized COVID-19 participants in the New York City trial, 70% of patients hospitalized in the ICU were men, and fatalities for men were greater than women (Kragholm et al., 2021).

In most medical specialties, gender variations in the frequency and severity of different diseases are regarded as fundamental epidemiologic data. However, the reason that sex differences in the onset and course of illnesses are not well known is partly because many pathophysiology and disease preventive research conducted in the past have only included or mostly included male participants(Theobald et al., 2006).

There have been several reports reported that ACE2 mutations exhibiting sex-specific expression. Men will be more negatively impacted by illness or risk mutations since ACE2 is an X chromosome encoded gene. It is hypothesized that missense ACE2 variations may have an impact on spike protein binding, which would therefore have an impact on COVID-19 development(Pradhan & Olsson, 2020).

Different hormonal environments affect men and women differently. It has been demonstrated that estrogen enhances immunity whereas testosterone suppresses it(Taneja, 2018). Estrogen protects against infections by having antiviral characteristics in several viral illnesses such as HIV, hepatitis C virus, Ebola, and human cytomegalovirus. Estrogen has been found to limit influenza A virus multiplication in cultured nasal epithelial cells derived from female mice(Peretz et al., 2016).

CXCR4 rs2228014 and CXCL12 rs2839693 genotyping according to age

Similarly, age stratification as regarding CXCR4 rs2228014 and CXCL12 rs2839693 revealed non-significant difference between patients with age more than 65 years old and patients with age less than 65 years old as regarding genotyping and allelic frequencies of both SNPs.

Age differences according to clinical course, CT Findings, and outcomes.

Age stratification as regarding clinical course revealed a significant difference in the frequency of mild/moderate illness and severe/critical illness between patients with age more than 65 years old and patients with age less than 65 years old. Patients with age more than 65 years old have increased severe/critical ratio and lower mild/moderate

ratio than patients with age less than 65 years old. These data in agreement with Gao et al., 2021 who reported that age is a risk factor for severe COVID-19(Gao et al., 2021).

One CT finding that is very common is GGO. Air bronchograms, consolidation, adjacent pleura thickening, and interlobular septal thickening were among the other CT findings in COVID-19 cases. GGO, consolidation, and thickening of the surrounding pleura were observed in over half of the patients. The bulk of the imaging results were in the peripheral portions of the lungs and related to the bilateral lungs. Both lobes may be affected by the illness, albeit the bilateral lower lobes are more frequently affected(Bao et al., 2020).

Our results revealed a non-significant difference between patients with age ≤65 years vs >65 years as regarding pneumonic consolidation and bilateral GGO. A study by Li et al, 2020 showed no noticeable change in the CT feature among different age groups(Li et al., 2020).Contrary, Song et al., 2021 reported that younger individuals had more GGOs, whereas older patients had more consolidations and included lung regions(Song et al., 2020).

Outcomes revealed that the frequency of home management was significantly higher in patients with age less than 65 years old than between patients with age more than 65 years old. Moreover, the mortality rate among patients with age more than 65 years old was significantly higher than in patients with age less than 65 years old. These data in agreement with Kang et al., 2020 who reported that elder COVID-19 patients have increased mortality rate(Kang & Jung, 2020)**.**

ROC curve of D-dimers and ferritin regarding clinical stage of COVID19

When plasmin cleaves fibrin to break up clots, one of the pieces that is formed is D-dimers. The synthesis of D-dimer, a fibrin degradation product that can only occur when hemostasis and fibrinolysis pathways are active at the same time, requires a large number of cross-linked D domains and/or E domains from the original fibrinogen molecule. D-dimers testing has been an essential part of clinical studies that have been proven in the diagnosis, exclusion, and prognostication of venous thromboembolism (VTE) and disseminated intravascular coagulation (DIC)(Lippi et al., 2023)**.**

As a "acute phase reactant," serum ferritin reflects the intensity of both acute and chronic inflammatory responses in the body.A monocyte-macrophage system that is active is indicated by a greater ferritin level. Hyperferritinaemia is a recognized

biomarker for several diseases, including rheumatologic illnesses, malignancies, and inflammatory diseases(Kernan & Carcillo, 2017).

In our study we revealed ferritin and D-Dimers levels as indicators of serious outcome in the COVID-19 patients participated in our study. For mortality in COVID-19 patients, the area under the curve (AUC) values were 0.627 for CRP, 0.773 for D.dimer, and 0.603 for serum ferritin. In a study conducted by Arshad et al AUC values were 0.711 for CRP, 0.803 for D-dimers and 0.714 for ferritin(Arshad et al., 2020)**.** An observational study on 386 COVID-19 patients by Yousaf et al, AUC values were 0.737 for CRP, 0.758 for D-dimers and 0.747for ferritin (Yousaf et al., 2022)**.**

According to the findings of Lu J's, a mortality risk score including age can forecast COVID-19 death(Lu et al., 2020). In a retrospective cohort analysis by Zhou et al., 2020 multivariable regression revealed that older age and D-dimers levels more than 1 g/mL were related with an increased risk of in-hospital mortality(Zhou et al., 2020). Our results reveled thatD-dimers, and serum ferritin levels associated with the risk of severe disease and mortality in participants of our study.

The current results may provide new insights into the many factors affecting the COVID severity, which may affect outcomes and treatment strategies.Based on the previously mentioned data, the effect of testosterone and ACE2 receptor could affect the disease severity and may be responsible for the variation in disease severity between male and female. Moreover, comorbidities could affect the disease severity in older patients.

The majority of studies conducted to date have examined clinical, laboratory, and CT results based on the age and gender of COVID-19 patients; this is the first to assess the genetic signature in the aforementioned categories. The findings that are being presented in our study not only add to our understanding of the immunological processes that these patient groups experience, but they may also be utilized as prognostic biomarkers to track the progression of COVID-19 patients' diseases and as possible targets for future treatments.

LIMITATIONS:

The difficulties we encountered carrying out this inquiry are described in the part that follows. First, the patient population was smaller since it was difficult to get samples

from patients in the advanced stages of the disease, those who were asymptomatic, or those who had fully recovered. Secondly, certain cases with unfavorable smear findings or comorbidities that weren't relevant to the study were excluded from the analysis.

RECOMMENDATIONS:

Investigating additional chemokine ligands and their receptors, which may have an association with the X chromosome and influence the severity of sex-related diseases. The mechanism via which these chemokine variations affect COVID-19 severity has to be investigated further. It also recommends increasing the number of research participants. However, larger studies in different cultures with an emphasis on gene expression levels will help us understand the mechanism's possible applicability.

CONCLUSION

Age and sex were the most important markers of the severity of the disease among the statistics that are typically gathered upon admission. Men, the elderly, and people with various medical conditions were more likely to experience severe cases. Males who are older and/or have many medical conditions require special attention.

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Data availability: The datasets generated and/or analysed during the current study are available at biosample depository, SubmissionID: SUB13604667 https:// ncbi.nlm .nih.gov /subs/biosample/SUB13604667

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval

After they gleefully and freely consented to take part in the current study, all participants were recruited. The protocol was carried out in conformity with the

Declaration of Helsinki (ethical guidelines for medical research involving human subjects), and it was approved by the Institutional Review Board of the Faculty of Medicine, Assiut University, Assiut, Egypt (local approval number: 17200716, date of approval: 24 April 2022). We acquired informed permission from each participant or legal guardian.

Consent for participate

All subjects and/or legal guardians gave their informed consent to participate in our research.

Abbreviations

°C: degree Celsius; ACE 2: Angiotensin-converting enzyme II; ARDS: Acute respiratory distress syndrome; COPD: Chronic obstructive pulmonary disease ; COVID-19: Coronavirus disease 2019; CT: Computed tomography; ICU: Intensive care unit; IFN-γ: Interferon-gamma; PCR: L: liter; mL: millileter, mg/L: milligram/Liter, µg/L: microgram/Liter, Polymerase chain reaction; RT-PCR: reverse transcription polymerase chain reaction, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SpO2: Oxygen saturation; WHO: World Health Organization

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