Research Article

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Acute Myocardial Infarction in Young Adults: Prevalence, Clinical Background and In-Hospital Outcomes with Particular Reference to Socio-Economic Influences a Middle Eastern Tertiary Center Experience

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ABSTRACT

Background: Acute myocardial infarction in young individuals can cause death and disability in early life and has serious consequences for the patients, their family causing an increased economic burden on health system. Identifying the risk factors for acute myocardial infarction in this group of people is necessary for risk factor modification and developing cost-effective secondary prevention strategies as young. The aim of this study was to determine the prevalence, clinical background and in-hospital outcome of AMI among young (age ≤ 45 years) adults and its socioeconomic burden.

Results: All Acute myocardial infarction patients during the period from 2016-2019 were divided into two groups: young adults (age ≤ 45) and older adults (age > 45). Age data were available for 3081 patients admitted with acute myocardial infarction. Out of these 593 (19%) patients were young adults with mean age of 39 ± 6.2 whilst 2488 (80.7%) were older adults with mean age of 60 ± 9 . Young adult Patients were more of male gender (92% vs 82%, $p < 0.001$) more smoker (47% vs 30 %, $p < 0.001$) and had more prevalence of obesity (BMI ≥ 30 34% vs 27%, $p < 0.001$) but were less diabetics (43% vs 57%, $p < 0.001$) and less hypertensive (35% vs 58 %, $p < 0.001$). Young adult patients had higher level of LDL (120 ± 47 vs. 112.9 ± 41.6 , $P = 0.02$), total cholesterol (189.2 ± 54.4 vs. 173.9 ± 47.7 , $P < 0.001$) and triglycerides (157.7 ± 104.4 vs. 126.6 ± 91 , $P < 0.001$). Young adult patients had more extensive thrombus and frequently required thrombus aspiration (16 % vs. 11%, $p = 0.003$) but less common left main disease (0.9% vs 4%, $p < 0.001$) and 3 vessels disease (8% vs 18%, $p < 0.001$). Young adult patients had less deterioration of left ventricular function (EF 42.4 ± 10.4 vs. 41.1 ± 10.6 , $P = 0.04$). There was highly significant negative correlation between left ventricular ejection fraction (LVEF) and age ($P < 0.001$) but positive correlation between age and length of in hospital stay ($p = 0.02$). In-hospital complications including pulmonary edema, cardiogenic shock, cardiac arrest and mortality were similar in the two groups. Age, female gender and diabetes were found to be the independent predictors for in-hospital mortality among our patients ($P = 0.003$, 0.05 and 0.05 respectively)

Conclusion: Young adult patients presented with acute myocardial infarction are more frequently smokers, obese and dyslipidaemic. These patients also have more thrombus burden. These results underscores the importance of smoking cessation, weight reduction programs and Health education for public especially of this age. Age still showed high risk prediction for lower LVEF and prolonged in-hospital length of stay in AMI patients with more burden on the health care system although the great improvement in management of AMI patients which lead to decrease in hospital complications.

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Background

Cardiovascular disease, however, remains a major cause of

morbidity and mortality among young individuals globally, even as more steps are made in improvement in diagnosis and therapeutics [1]. Although the incidence of acute coronary syndrome (ACS) has decreased in older populations, younger men and women who present with acute myocardial infarction (AMI) have not

had similar declines (especially men) in cardiovascular events [2]. Identifying the risk factors for AMI in this group of people is necessary for risk factor modification and developing cost-effective secondary prevention strategies as early as possible. Recent studies have shown a significant rise of AMI prevalence in younger age [3].

AMI carries significant morbidity, psychological effects, and financial restrictions for the affected person and the family when it occurs at a young age. Young AMI patients present with different risk factors and Coronary angiography (CAG) features when compared with older AMI patients. Further investigations are needed to identify the biological, clinical, and social factors that contribute to the development of AMI in young patients [4].

Acute myocardial infarction in patients younger than 45 years had different clinical features and responded to different therapeutic and diagnostic approaches than acute myocardial infarction in patients over 45 years, as well as a better short-term prognosis [5]. AMI in young individuals can cause death, disability in early life and has serious costs for the patients and their family causing an increased economic burden on health system [3].

Current challenges include atypical and delayed presentation, non-adherence to treatment, and syndromes unique to this age group. For example, spontaneous coronary artery dissection (SCAD), vasospastic angina, MI with intact fibrous cap, and cocaine/methamphetamine use are more prevalent in the younger age group [6].

The aim of this study was to determine the prevalence, clinical background and in-hospital outcome of AMI among young (age ≤ 45 years) adults and its socioeconomic burden.

Method and Statistical Analysis

This is a retrospective analysis of the prospective collected data, single center study conducted at our center, Makkah during 2016-2019.

All AMI patients admitted during that period were divided into two groups: young adults (age < 45) and older adults (age ≥ 45).

Inclusion Criteria: All AMI patients during the period from 2016 to 2019 were included

Exclusion Criteria

All AMI patient who did not do CAG due to preference of patient or any limitations or contraindications.

Demographic (age, gender, body mass index (BMI), pilgrims, Arabic speaking & south Asian), clinical characteristics (diabetes

mellitus (DM), hypertension (HTN), smoking, dyslipidemia, old history of ischemic heart diseases (IHD), history of cerebrovascular stroke (CVA) & positive family history of coronary artery diseases (CAD)), ECHO data (left ventricular ejection fraction (LVEF) & LV thrombus.), laboratory data including hemoglobin on admission and on discharge, hemoglobin drop, mean platelet volume, random blood glucose, glycosylated hemoglobin, serum sodium, serum potassium, blood urea nitrogen (BUN), creatinine on admission and discharge, blood HDL, LDL, triglyceride, total cholesterol) and coronary angiography data (type of AMI, thrombolysis, contrast volume, fluoro-time, tirofiban usage, cases with left main or three vessel disease). In-hospital complications (Pulmonary edema, cardiogenic shock, ventilation, cardiac arrest, length of stay and in-hospital mortality were all obtained and recorded from medical records.

Group Definition

In this study we defined young adults as those with under the age of 45 years old. For defining the concept of “young” or “young adult”, current data varies from 40 to 55 years, an age limit increased with increasing life expectancy [7-9]. However, according to the current ESC Guidelines in the definition of acute ST-segment elevation myocardial infarction, the term “young” defines patients under the age of 45 years, while very young people are considered to be under the age of 35 [10,11].

Statistical Analysis: Was performed by use of the SPSS software package (SPSS Inc.; Chicago, Ill), version 21.0. Data are presented as mean $\pm$ SD, or as median and range according to the type of distribution of each variable. The two groups were compared using t-test and chi-squared test for continuous and categorical data respectively. Linear regression analysis was also performed. For all analyses a p value < 0.05 was considered significant and not significant if it is > 0.05 .

Our study is designed to be the part of the standard of patient care, and has received approval of the ethics committee/institutional review board of our institution.

Results

A total of 3081 patients admitted to our center with AMI, with mean age of 56 ± 11.9 years. With 593 (19%) young adult patients

Demographics and Risk Factors (Table I)

Young adults had a mean age of 39 ± 5.2 years old whilst the older adult patients had a mean age of 60 ± 8.9 years old. Young adult Patients had more prevalence of both male gender and smoking compared to older adult group (92% and 47% VS 82% and 30% respectively; $P < 0.001$).

Tables:

Table I: Comparison of demographic data and risk factors between the two groups

Variable	Young patients 593 (19 %) number %	Older adult patients 2488 (81%) number %	P value
Age Mean $\pm$ SD	39 $\pm$ 5.2	60 $\pm$ 8.9	
Male	547 92 %	2035 82 %	< 0.001
BMI (kg/m ²) Mean $\pm$ SD	28.5 $\pm$ 5.6	27.7 $\pm$ 5	< 0.001
DM	252 43%	1420 57 %	< 0.001
HTN	208 35 %	1435 58 %	< 0.001
Smoking	281 47 %	736 30 %	< 0.001
DYSLIEDMIA	89 15 %	356 14 %	0.6
CVA	29 0.5%	74 3 %	< 0.001
Old IHD	86 15 %	532 21 %	< 0.001
Positive family history of CAD	207 35 %	498 20 %	0.01
Obesity (BMI $\geq$ 30)	202 34 %	682 27 %	0.002
Pilgrims	165 28 %	886 36 %	< 0.001
Arabic speaking	373 63 %	1515 61%	0.4
South Asian	192 32 %	717 29%	0.09
BMI: Body Mass Index; DM: Diabetes Mellitus; HTN: Hypertension; CVA: Cerebro- Vascular Accidents ;IHD: Ischemic Heart Disease; CAD: Coronary Artery Disease			

Young adult patients were more obese (34% VS 27%; P<0.001) with a mean BMI of 28.5 $\pm$ 5.6 VS 27.7 $\pm$ 5 compared to older adults but there was no significant difference between the two groups regarding history of dyslipidemia (89(15%) vs 356(14.3%); p=0.6).

Diabetes and hypertension were recorded less common among young adult patients (43% and 35% VS 57% and 58% respectively; P<0.001) however, family history of ischemic heart disease was significantly positive among young adults (35% VS 20%; P=0.01) compared to older adult group of patients.

AMI pilgrim patients were notified to be less among young adult patient (28% VS 36%) compared to older adults. There were no significant recorded differences in both ethnicity and language variation between the two groups.

Comparing the Clinical Data between the Two Groups

Young adults had higher level of hemoglobin on admission (14.5 $\pm$ 1.9, 13.7 $\pm$ 2; P<0.001) and on discharge (14 $\pm$ 1.97, 13 $\pm$ 2; P<0.001) but hemoglobin drop was not significantly different between the two groups. Young adults had lower levels of random blood glucose (160 $\pm$ 76.3 VS 179 $\pm$ 79; P=0.01), creatinine on discharge (1.1 $\pm$ 0.8 VS 1.3 $\pm$ 3.6; P=0.05), sodium (136.9 $\pm$ 3.5 VS 136.5 $\pm$ 5.7; P=0.02) and BUN (14.6 $\pm$ 7.5 VS 17.9 $\pm$ 10; P<0.001), although there were no significant difference between the two groups regarding creatinine on admission, HbA1c and potassium levels (Table II).

Table II: Comparison of laboratory data between the two groups

Variable	Young patients 593 (19.3 %)	Older adult patients 2488 (81%)	P value
Hb on admission(mg\dl)	14.5 $\pm$ 1.9	13.7 $\pm$ 2	< 0.001
Hb on discharge(mg\dl)	14 $\pm$ 1.97	13 $\pm$ 2	< 0.001
Hb drop $\geq$ 3mg\dl	123 (5 %)	444 (6 %)	0.5
MPV (fl)	9.8 $\pm$ 1.98	10 $\pm$ 1.94	0.03
Glucose (mg\dl)	160 $\pm$ 76.3	179 $\pm$ 79	0.01
HbA1c	7.5 $\pm$ 3.3	7.7 $\pm$ 2.8	0.1
Creatinine on admission(mg\dl)	1.1 $\pm$ 0.8	1.3 $\pm$ 3.6	0.1
Creatinine on discharge(mg\dl)	1.1 $\pm$ 1	1.3 $\pm$ 1	0.02
BUN(mg\dl)	14.6 $\pm$ 7.5	17.9 $\pm$ 10	< 0.001
Sodium(meq\l)	136.9 $\pm$ 76.3	136.5 $\pm$ 5.7	0.05
Potassium (meq\l)	4.1 $\pm$ 0.5	4.2 $\pm$ 0.9	0.1
HDL(mg\dl)	35.5 $\pm$ 8.6	38 $\pm$ 12	< 0.001
LDL(mg\dl)	120.8 $\pm$ 47	112.9 $\pm$ 41.6	0.02

TG(mg\dl)	157.7 ± 104.4	126.6 ± 91	< 0.001
IC(mg\dl)	189.2 ± 54.4	173.9 ± 47.4	< 0.001
Hb: Hemoglobin ;MCV: Mean Corpuscular Volume; HBA1c : Glycosylated Hemoglobin; BUN: Blood Urea Nitrogen; LDL: Low Density Lipoprotein ;HDL: High Density Lipoprotein; TG: Triglyceride; IC :total Cholesterol			

Young adult patients had recorded significant higher dyslipidemia compared to older patients (120±47, 189.2±54.4 and 157.7±104.4 VS 112.9±41.6, 173.9±47.7 and 126.6±91; P=0.02, <0.001 and <0.001 respectively for LDL, total cholesterol and triglycerides) (Table II).

Frequency of anterior myocardial infarction presentation didn't differ between the two groups however, young adult more likely to receive thrombolytic therapy and had more extensive thrombus which frequently required thrombus aspiration (22% and 16% VS 18% and 11%; P=0.04 and 0.003 respectively for thrombolytic and thrombus aspiration during coronary angiogram). Young adult patients showed less deterioration of LV function post myocardial infarction compared to old patients (LVEF 42.4±10.4 VS 41.1±10.6; P=0.04) (Table III).

Regarding coronary angiography data, young patients showed less extensive coronary artery disease compared to old (0.9% and 4% VS 8 % and 18%; P<0.001 respectively for left main and three vessel disease) (Table III).

Table III: Comparison of coronary angiographic results, in-hospital outcomes and mortality between the two groups

Variable	Young patients 593 (19.3 %)	Older adult patients 2488 (80.7%)	P value
Thrombolysis	130 (22 %)	448 (18 %)	0.04
AWMI	318 (54 %)	1375 (55 %)	0.7
LVEF	42.4±10.4	41.1±10.6	0.04
Contrast(ml)	126.4± 88.8	16 ± 108.9	0.6
Fluro-time(minute)	14 ± 71	38 ± 12	< 0.001
Aspiration	400 (16 %)	67 (11 %)	0.003
Tirofiban	148 (25 %)	597 (24 %)	0.5
3VD	47 (8 %)	447(18 %)	< 0.001
LM disease	6 (0.9%)	99 (4 %)	< 0.001
LV dysfunction	379(64%)	1716(69%)	0.509
LV thrombus	17 (0.7%)	4(0.6%)	0.1
Pulmonary edema	97 (4 %)	11 (2 %)	0.01
Cardiogenic shock	114 (3 %)	(5 %)	0.5
Ventilation	7 (5 %)	3 (4 %)	0.6
Cardiac Arrest	15 (5 %)	6 (6 %)	0.7
Length of stay (days)	5.2±5.7	5.8±8.3	0.1
In-hospital Mortality	10 (1 %)	3 (4 %)	0.002
AWMI: Anterior Wall Myocardial Infarction; LV: Left Ventricular; EF: Ejection Fraction; LM: Left Main; 3VD:3 Vessel Disease.			

Comparing the In-Hospital Outcome Measures between the Two Groups

There was positive correlation between age and length of in hospital stay (p=0.02) however there was highly significant negative correlation between left ventricular ejection fraction and age (P<0.001) in patients with AMI (table IV).

Table IV: Age correlations

In-hospital length of Stay	Pearson Correlation	.041
	P value	.028
LVEF	Pearson Correlation	-.067
	P value	< 0.001

LVEF: Ejection Fraction

In-hospital complications including significant LV dysfunction, pulmonary edema, cardiogenic shock, cardiac arrest, mechanical ventilation and LV thrombus formation were all recorded similar in the two groups but the in- hospital mortality was recorded significantly less among young adult patients compared to the old group (1% VS 4%; P=0.002) (Table III).

Age, female gender and diabetes were all found to be the independent predictors for in-hospital mortality among our patients (P=0.003, 0.05 and 0.05 respectively) (Table V).

Table V: Binary regression analysis for risk factors for in-hospital mortality

Variable	B	S.E.	Wald	df	Sig.	Exp(B)
Age	.033	.011	8.876	1	.003	1.034
female	-.570	.291	3.845	1	.050	.565
BMI	.002	.025	.004	1	.951	1.002
DM	.530	.313	2.861	1	.050	1.698
Smoking	-.269	.323	.693	1	.405	.764
HTN	.129	.284	.205	1	.651	1.137
HbA1C	-.047	.058	.651	1	.420	.954

BMI: Body Mass Index; DM: Diabetes Mellitus; HTN:hypertension;HBA1C: Glycosylated Hemoglobin

Discussion

To our knowledge this is the first study reporting on STEMI in young adults in KSA. Our center is a tertiary care center in specific region of Makkah which receive most of AMI patients need revascularization with different ages, nationalities both pilgrim and residents. This gave us the chance to study age factor in a large number of patients with different background.

Clinical Background in Young Adults

The present study included 3081 patients, 593 of them were younger than 45 years of age presenting with the first AMI and all were underwent coronary angiogram. It provides a rough estimate about the prevalence of AMI in young adults to be about 20%, which is in close concordance with other studies [12] Male gender is one of the risk factors for coronary artery disease in many studies which consistent with our findings in the current study as males were highly prevalent in both groups (92% and 82% patients were being male in both groups respectively) [12-14]. Table-1 This might be attributed to male dominance, high prevalence of smoking in males and protective effects of female estrogen [13].

Smoking is the most preventable cause of death universal, as it can initiate and promote atherosclerosis [13] Smoking cessation greatly decreases the risk of CAD, and its cessation reduces the mortality risk by 90% if before the age of 40 years [13] In our study, there was a higher prevalence of smoking among the young adults compared to the older adults with AMI (47% VS 30% respectively). Table-1 most of studies were in agreement to our study [9,14].

Obesity is known to be linked with higher risk of hypertension, diabetes, dyslipidemia, and CAD. Obesity at a younger age carries a high risk to early CAD [15]. In our study, BMI was recorded higher than 30 in more than one-third of the young adult patients and more than one fourth of older adults presented with AMI (34% and 27% respectively). In-agreement with this study Iravavarapu, et al 2019 [16].

In this study, dyslipidemia was also found to be prevalent among young adult AMI patients depending on the higher recorded levels of LDL, total cholesterol and triglycerides (which was significantly higher than older adults). Also young patients showed lower

recorded levels of HDL compared to older adults. Table- 2 Various studies among young patients of AMI have reported the same high prevalence of dyslipidemia ranging from 20 to 80 % [12,13,16,18].

Moreover, well-known risk factors of CAD such as diabetes mellitus and systemic hypertension had significantly lower incidence between young adults in comparison to older adults (43% and 35% VS 57% and 58% respectively). Table-1 In agreement with this study, the prevalence of hypertension ranged between 10 and 44% in other studies [12,13,16,18]. Contrastingly to our study the prevalence of diabetes mellitus was stated to be less than 10% in many studies [12,17,18]. This variation in the prevalence of both diabetes and hypertension among our population could be explained by different background as our patients were from different countries with variable genetic and cultural characters. Also, Saudi patients showed higher prevalence of DM since younger age compared to other nationalities [19].

Association between positive family history of premature CAD and increased plaque burden in the coronary arteries is well-established. At the current study, the incidence was 35 % among young adults which was agreed by various studies contrastingly reported lower prevalence around 10% but in few studies [12,13,16,18].

Moreover, old age is considered an independent risk factor for AKI and bleeding and consequently those elderly patients with AKI have worse renal recovery and a higher mortality rates compared to than younger patients [7-9]. These studies were in agreement with our findings which showed higher level of creatinine after coronary angiography on discharge among old patients.

Imaging Data a: Echocardiography

With regards to LVEF post AMI: the mean LVEF was found to be $42 \pm 10.4\%$ (Table-3) which is more affected in older adult population. This result is agreed with other studies which recorded mild to moderate left ventricular dysfunction post AMI with recorded a mean LVEF between 37 and 55% in young patients [12,13,16]. This may be attributed to more prevalence of single vessel disease (SVD) among young adult patients and less common MVD in comparison to older adults (8% VS 18%).

Moreover, Left ventricular systolic dysfunction (LVSD) is an important cause both of mortality and morbidity in patients with ACS. In 2015 a study showed that age ≥ 70 years were found to be independent predictors of systolic dysfunction in multivariate analyses [20]. In the current study, we were found a positive correlation between age and LVEF and this was concordant with Ali et al who stated that older age may predict LV dysfunction and heart failure in patients treated with thrombolysis for STEMI [21]. Although, it was discordant with Naguib et al [22]. This discrepancy could be explained by larger sample volume included in our study, higher mean age in their study and different modalities of revascularization.

b: Coronary Artery Extent

Most studies conducted among young patients of AMI demonstrate a preponderance of SVD) and less frequent three vessel disease as observed in the present study [5,12,13,15,18,24]. Table-3 Similarly, TVD (3.3 to 6.6%) were reported to be lower prevalent by other studies as well, thereby suggesting that extensive coronary involvement is infrequent in young adults presenting with ACS [7,8,10,11,25,26].

Interestingly, young adults showed more prevalence of coronary thrombus which necessitate thrombus aspiration in about 16% than older adults (11%). This result might be attributed to less plaque burden and higher susceptibility for thrombus formation in those young patients with AMI. In concordance with our study, a high thrombus burden was also evidenced in Ge et al., study but with much higher incidence (54.9%) in young patients. Thrombus aspiration was also during their study performed in (62%) patients during PCI [27].

In-hospital Outcome Data

a: In-Hospital Length of Stay (LOS)

The ability to predict LOS as an initial assessment of patients' risk is critical for better resource planning and allocation, especially when the resources are limited, as in ICUs [28,29]. An accurate prediction of LOS can also facilitates management with higher flexibility in hospital bed use and better assessment in the cost-effectiveness treatment [30,31].

Our study showed highly significant positive correlation between in-hospital length of stay and age in patients with AMI. Similar to our finding, a study done during 2016 showed that the top three significant positive correlated variables for longer LOS were patients with heart failure, who were older and female [32]. It was consistent with the findings about factors related to prolonged LOS from literature: female, increasing age, and comorbidities such as cerebrovascular disease and diabetes mellitus [33,36].

b: In-hospital Complication and Mortality

Young adults showed lower incidence of in hospital death in comparison to older adults (1% VS 4%). Table 3, This is explained by age, multiple comorbidities, higher incidence of AKI & CVA and extensive CAD which all recorded with older age group of patients. Less prevalence of triple vessel disease in young patients might also explain why young individuals have a better prognosis than older ones after AMI [6,37-41]. Otherwise, there were observed no significant differences between young and old adults regarding post AMI complications and this reflects the equal provided high care services provided to all patients in our center.

Finally, by binary regression analysis age was detected to be an independent predictor for the in-hospital death, age is an independent predictor even after adjusting for risk factors. In

agreement with our study, Cretu et al., stated that in-hospital death for STEMI increased by almost 90% for each 10- year increase in age [14,42]. Also, the GRACE registry showed a 1.7-fold increased risk of mortality with every 10 years [43]. Mean age > 69.01 yr, femaleness and DM, are associated with higher In-hospital post-AMI mortality [44].

Socio-Economic Impact

First, our data demonstrates that one fifth of STEMI patients are younger than 45 years with a mean age of 39 ± 5.2 years. This emphasizes the importance of public health education and awareness of risk factors especially smoking and dyslipidemia, both of which are reversible. Furthermore, these patients are at their prime, and CAD greatly affects their socio-economic lives; hence emphasizing the importance of strategic implementation of cardiac rehabilitation to encourage them to return to their active lives.

Second, in-hospital length of stay post AMI is more affected with age, and this puts more burden on the health care system although the great improvement in management of AMI patients which lead to decrease in hospital complications. This finding is considered unique and highlights the use of preadmission models to predict LOS for patients in a cardiovascular unit especially with acute myocardial infarction (AMI).

Study Limitations

The study being cross-sectional without any control group, risk of each factor, and their statistical significance could not be analyzed. Long term follow wasn't studied as we had considerable percentage of our patients were pilgrims (return back to their countries and no follow up here). Also, the nature of single center. Validation in multicenter larger population with longer follow-up is highly recommended.

Conclusion

Young adult patients presented with acute myocardial infarction are more frequently smokers, obese and dyslipidemic. These patients also had more thrombus burden and more prevalence of single vessel disease. These results underscore the importance of smoking cessation, weight reduction programs and Health education for public especially among this age. Age still showed high risk prediction for lower LVEF and prolonged in-hospital length of stay in AMI patients with more burden on the health care system although the great improvement in management of AMI patients which lead to decrease in hospital complications.

Declarations

Ethics approval and consent to participate
Our study is designed to be the part of the standard of patient care, and has received approval of the ethics committee/institutional review board of the King Abdullah Medical City. The committee's reference number is (18-426). Consent to participate was taken verbally because of known conducted STEMI registry in our center (our study is a part of it) and that is approved by the committee.

Abstracts of a parts of this study was presented as poster presentation in 10th ECS together with ACC ME 19 Scientific Conference which conducted at Dubai, UAE on 10, 2019 also presented as abstract presentation at cardio-Alex, Egypt 10, 2020.

Availability of Data and Materials

The data that support the findings of this study are available on reasonable request from corresponding author but not publicly

available due to privacy.

Competing Interests

The authors declare that they have no competing interest.

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None

Authors' Contributions

GS conceived and designed the study, participated in sequence alignment and coordination, performed the statistical analysis, wrote the paper, helped in data collection and finalized the final draft of manuscript. SK participated in sequence alignment, edited and reviewed the final draft of manuscript. FA participated in sequence alignment, idea completion and participated in statistical analysis. NJ participated in sequence alignment, share in ideas and design of final draft, SA,SA,AM,RM,MA participated in data collection. All authors read and approved the final manuscript.

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