Body mass index have no effect in hospital mortality or intensive care outcomes in an inner city population

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Abstract

Background: The relationship between body mass index and outcome in the intensive care unit is controversial. The objective was to evaluate the incidence and effect of different body mass indexes on mortality, duration of mechanical ventilation, intensive care unit and hospital length of stay among critically ill medical patients.

Methods: Retrospective, single center study in a medical intensive care unit of an inner city teaching hospital. All patients with available BMI levels on admission were included.

Results: There were 1,988 patients admitted to the intensive care unit between January to December 2008; 1,797 (90.3%) were analyzed. Based in body mass index, 162 (9%) patients were underweight, 598 (33%) normal, 774 (43%) overweight/obese and 263 (14.6%) severe obesity. Patients with obesity/severe obesity were more likely to be female ($p < 0.0001$); to have hypertension ($p < 0.0001$), diabetes mellitus ($p < 0.0001$) and obstructive sleep apnea ($p < 0.0001$) as comorbid conditions. Severely obese patients had lower APACHE IV scores (66 ± 33 vs. 71 ± 32 , $p = 0.035$) and higher like hood of mechanical ventilation (105 (40%) vs. 180 (28%), $p < 0.0007$) when compared to normal. Underweight patients were sicker (APACHE IV 80 ± 32 vs. 71 ± 32 , $p = 0.002$) and more likely to have HIV infection (55 (36.6%) vs. 164 (26%), $p = 0.008$) and cancer (22 vs. 51, $p = 0.017$) compared to normal. Neither underweight, obesity nor severe obesity were associated with an increased risk of hospital mortality, (odd ratio=1.162; 95% confidence interval, 0.57-2.34 for underweight, odd ratio=1.04; 95% confidence interval, 0.62-1.75 for overweight/obese, odd ratio=1.23; 95% confidence interval, 0.64-2.39 for severe obesity). There were no differences in hospital length of stay or days on mechanical ventilation between the different body mass index groups. However, intensive care unit length of stay were significantly longer in the severe obese group by 1.2 days (95% confidence interval, 1.00-1.09; $p = 0.0015$) compared with all the other groups.

Conclusion: Body mass index in critically ill inner city patients is not associated with increased mortality. Severely obese patients have a longer intensive care unit length of stay, but not hospital stay. In our inner city MICU population, the prevalence of obesity and severe obesity is higher than reported.

Keywords: Intensive care unit, obesity, underweight, body mass index, mortality, length of stay, mechanical ventilation

Background

Obesity is a health epidemic of industrialized countries and has been associated with substantial morbidity and mortality in the general population. The 2010 National Health and Nutrition Examination Survey (NHANES) survey [1] shows that the prevalence of obesity in the United States has increased steadily in both men and women. The association of increased body mass index (BMI) with hypertension, diabetes mellitus, cardiovascular and pulmonary diseases and cancer is well reported [2-5]. At the other extreme, low BMI have been associated with higher mortality on patients with cardiovascular and peripheral vascular disease [6,7]. Population based studies suggest a U or J shaped curve indicating a higher mortality at the extremes of BMIs [8-10]. The association of BMI with mortality in the intensive care unit (ICU) is controversial with reports suggesting no relation [11-13], increased [14-16] or decreased hospital mortality [17]. The role of BMI

for length of stay (LOS) on mechanical ventilation (MV) and ICU LOS is equally inconsistent [11,18-23].

There are no studies evaluating the effect of BMI in ICU outcomes in an inner city population. The prevalence of overweight and obesity in this population have been reported to be closer to 40%, probably due their unique demographics. Additionally, inner cities have a higher rate of immigrants, uninsured and low income patients with a higher mortality rates from almost all diseases [24].

The effect of BMI on the outcome of this high risk group of patients who need intensive care admission is unknown. This study examines the prevalence of different categories of BMI in patients admitted to our inner city medical ICU and the association with hospital and ICU mortality.

Materials and Methods

Study design and setting

This was a retrospective study conducted in a 26-bed

closed adult medical ICU of a 970-bed hospital. Medical records of patients admitted to the unit between January and December 2008 were included.

Methods

All patients admitted to the ICU with available BMI levels on admission were included in the study. Exclusion criteria included: a) height or weight not available at the time of ICU admission and b) readmission to the ICU during the same hospital admission.

Determination of BMI

Height and weight data were routinely measured immediately on an admission to ICU. Body mass index, determined as weight (kg)/height (m²), was calculated for all patients. As per ICU policy, BMI was validated if there was a difference of more or equal to 10% in subsequent measurements. Other validation measures included admission dietary assessment notes.

Definition and classification of obesity

The classification system of obesity by BMI developed by the World Health Organization Obesity Task Force and adopted by the Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, of the National Heart, Lung, and Blood Institute of the National Institutes of Health was used [25,26]. Based on BMI, patients were classified into five groups: underweight (BMI ≤ 18.4 kg/m²); normal (18.5 to 24.9 kg/m²); overweight (25 to 29.9 kg/m²); obese (30 to 34.9 kg/m² and severely obese (BMI ≥ 35 kg/m²). The overweight and obese categories were combined into one group for analysis.

Baseline demographics included age, gender and race, pre-existing conditions like hypertension (HTN), diabetes mellitus (DM), infection with Human immunodeficiency virus (HIV), end stage renal disease (ESRD)-defined as those patient undergoing dialysis, obstructive sleep apnea (OSA), cancer and history of tobacco, alcohol and substance use. Information was obtained from admission medical records. The acute physiology and chronic health evaluation (APACHE) IV score-derived risk of death during hospitalization was determined from the worst values obtained within 24 hours of ICU admission. Laboratory variables were obtained during the first 24 hours of admission to ICU and included hematocrit (HCT), serum creatinine, albumin, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), and international normalized ratio (INR). Right ventricular systolic pressure (RVSP) and ejection fraction (EF) from echocardiogram when available for that admission were obtained. Days on mechanical ventilator, ICU and hospital LOS, and mortality were abstracted from medical records. The primary outcome measure was hospital mortality. Secondary outcomes included requirement for mechanical ventilation and ventilator days, ICU and hospital LOS and

tracheotomy rates. The study was approved by the hospital institutional research review board, and the need for informed consent was waived.

Statistical analysis

Data analysis was conducted using the Statistical Analysis System software 9.2 (SAS Institute, Cary, NC). Discrete variables are expressed as counts (percentage) and continuous variables as means $\pm$ standard deviations (SD). For the demographic and clinical characteristics of the patients, differences between groups were assessed using the chi-squared test and Fisher's exact test for categorical variables; wilcoxon rank sum used for all continuous comparisons for data non-normally distributed; p values listed for mean values across BMI categories were derived using analysis of variance (ANOVA). We determined which variables to include in the logistic regression model by looking for significant associations between these potential confounders and mortality (primary outcome) and primary independent variable BMI category. If there was a significant association in either assessment, we included that variable in the stepwise selection model in logistic regression. A p value less than 0.05 was considered statistically significant.

Results

Between January and December 2008, a total of 1,988 patients were admitted to the medical ICU, 191 patients were excluded due to either no BMI available or readmission to the ICU; 1,797 (90.3%) patients were analyzed. The distribution of the BMI was as follows: 162 (9%) patients were underweight, 598 (33%) normal, 774 (43%) overweight/obese and 263 (14.6%) severely obese (**Figure 1**). The mean BMI of the cohort was 27.5 ± 8.5 kg/m² range 13.3-96.4 kg/m². Distribution of BMIs can be seen in **Figure 2**. Comparison of baseline characteristics for different BMI groups revealed that patients with obesity and severe obesity were more likely to be female, to have a lower APACHE IV score and to suffer from diabetes and hypertension. Patients in the underweight group were more likely to have HIV infection, malignancy and active use of tobacco, alcohol and recreational drugs. Anemia, hypoalbuminemia and low EF was also most commonly found in this group (**Table 1**). None of our patients had history of bariatric surgery or organ transplantation.

Data analysis revealed that neither underweight, overweight/obesity nor severe obesity were associated with an increased risk of hospital mortality compared to patients with normal BMI (**Table 2**). Analysis for secondary outcomes showed that patients with a non-normal BMI were more likely to require mechanical ventilation when compared to normal, ($p=0.006$). No differences for ventilator days, tracheotomy rates and hospital LOS were found between the spectrums of BMIs. However, ICU LOS were significantly longer in the groups with higher compared to normal BMI (severe obesity 5.3 ± 5.1 and overweight/obesity 4.4 ± 4.5

compared with 4.1 ± 3.9 in normal, $p=0.0015$) (Table 3).

Univariate analysis on predictors of mortality is shown in Table 4. Logistic regression revealed association of the following variables with mortality in our population: mean age (OR 1.019), APACHE IV score (OR 1.029), history of cancer (OR 4.261), mechanical ventilation (OR 4.767), RVSP (OR 1.015), albumin levels (OR 0.414), and history of alcohol use (OR 0.48, 95% CI 0.257-0.894), however there was no

association of different BMI groups and mortality (Table 5).

Discussion

We found no association between BMI and mortality in our single center cohort. In addition, BMI had no impact in any of the secondary outcomes in our inner city population.

Reported prevalence for severe obesity and underweight in the ICU is between 6% to 15% and 10% to 16% respectively

Table 1. Admission characteristics between different BMI groups.

	Underweight BMI ≤ 18.5 n=162	Normal BMI 18.5 –24.9 n=598	Overweight /Obese BMI 25- 34.9 n=774	Severe Obesity BMI ≥ 35 n=263	P
Male Gender n (%)	90 (55.6%)	324 (54.2%)	356 (47.1%)	85 (32.3%)	<0.0001
Age, y (mean ± SD)	56.9 ± 16.0	56.7 ± 18.5	57.3 ± 17.6	55.7 ± 16.8	0.65
APACHE IV (mean ±SD)	77.9 ± 30.7	71.6 ± 31.8	67.3 ± 33	65.1 ± 31.9	<0.0001
Tobacco use n (%)	59 (36.4%)	211 (35.3%)	210 (27.8%)	78 (30%)	0.01
Alcohol use n (%)	33 (20.4%)	125 (20.9%)	151 (20%)	35 (13.3%)	0.06
Drug use n (%)	40 (24.7%)	129 (21.6%)	119 (15.7%)	41 (15.6%)	0.004
Hypertension n (%)	61 (37.7%)	295 (49.3%)	444 (58.7%)	160 (60.8%)	<0.0001
Diabetes n (%)	30 (18.5%)	148 (24.8%)	287 (38%)	100 (38%)	<0.0001
HIV n (%)	61 (37.7%)	153 (25.6%)	132 (17.5%)	31 (11.8%)	<0.0001
ESRD n (%)	11 (6.8%)	47 (7.9%)	46 (6.1%)	15 (5.7%)	0.54
Cancer n (%)	22 (13.6%)	48 (8%)	64 (6%)	15 (6%)	0.006
OSA n (%)	0	0	5 (0.66%)	18 (6.8%)	n/a
INR (mean ± SD)	1.2 ± 0.6	1.3 ± 1.2	1.4 ± 1.3	1.3 ± 0.9	0.37
Hematocrit (mean ± SD)	30.9 ± 8.7	31.4 ± 8.4	32.9 ± 7.6	33 ± 7.8	0.0005
Creatinine (mean ± SD)	2.3 ± 2.5	2.2 ± 2.8	1.9 ± 2.3	2.2 ± 3.6	0.09
Albumin (mean ± SD)	2.9 ± 0.8	3.1 ± 0.8	3.1 ± 0.9	3.2 ± 0.8	0.003
SGPT (mean ± SD)	54.5 ± 99.9	57.9 ± 255.0	50.2 ± 168	45.4 ± 111.4	0.8
SGOT (mean ± SD)	106.9 ± 399.3	83.2 ± 242.1	99.8 ± 422.1	72.5 ± 252.4	0.6
ECHO performed	96 (59%)	386 (64%)	465 (60%)	193 (73%)	-
RVSP (mean ± SD)	40.2 ± 15.5	41.5 ± 16.6	39.3 ± 13.5	41.1 ± 23.1	0.4
EF (mean ± SD)	57.8 ± 15	58.5 ± 15.6	61.4 ± 13.2	60.8 ± 15.7	0.01

Table 2. Primary outcomes between different BMI groups.

	Non survivors n=425 (%)	Survivors n=1372 (%)	P	Odds Ratio (95% CI)
Underweight BMI ≤18.4, n=162	42 (26)	120 (74)	0.28	1.2 (0.8 - 1.9)
Normal BMI 18.5–24.9, n=598	131 (22)	467 (78)	-	-
Overweight/Obese BMI 25-34.9, n=774	181 (23)	593 (77)	0.5	1.1 (0.8 - 1.4)
Severe Obesity BMI ≥ 35, n=263	71 (27)	192 (73)	0.1	1.3 (0.9 - 1.8)

[27,28]. In our population, we found higher prevalence of obesity and morbid obesity and lower prevalence of underweight patients when compared with other reports [11,21,28 and 29]. In agreement with the findings of Martino *et al.*, [29], we found an association between BMI categories and the increased requirement for MV and prolonged ICU stay. Our finding that overweight/obese and severely obese patients were more often to be female when compared with underweight and normal weight patients has been rarely reported for ICU populations [28].

There is evidence that social and demographic factors contributes to obesity. These complex and dynamic “socio-demographic factors” include race/ethnicity, gender,

socioeconomic status, psychosocial factors, neighborhood characteristics and safety [30-33]. NHANES data reported that prevalence of obesity among non-Hispanic black males was 38.8%, compared to 36.6% among Mexican Americans and 36.2% for non-Hispanic whites. When examined by gender, female non-Hispanic African Americans were more likely to be overweight (58.5%) compared to Mexican Americans (44.9%) and non-Hispanic white (32.2%) [33]. Socio economic status is an additional cause for obesity as evidenced by increased association of obesity in the lower socio economic class [32]. Other interesting associations for obesity include physical inactivity due to building environments and lack of space seen in low-income public housing [34]. Additionally concerns about safety in neighborhoods may influence willingness for physical activity [35-37].

Our hospital serves the low income inner-city population, mainly African Americans and Hispanic community with a lower social economic status. We conclude that the higher incidence of obesity seen in our study most probably reflects the socio-demographic factors that are unique in our catchment population.

Obesity and severe obesity have been found to be a protective factor for mortality compared to patients with normal BMI in various disease processes including cardiovascular diseases, congestive heart failure, ESRD and

Table 3. Secondary outcomes between different BMI groups.

	Underweight BMI ≤ 18.5, n=162	Normal BMI 18.5–24.9, n=598	Overweight /Obese BMI 25– 34.9, n=774	Severe Obesity BMI ≥ 35, n=263	P
Patients on Mechanical ventilation n (%)	61 (38.4)	169 (29.7)	260 (36)	105 (41)	0.006
Tracheotomy n (%)	8 (11.1)	61 (10.2)	75 (9.9)	24 (9.1)	0.9
ICU LOS (mean ± SD)	4.3 ± 3.9	4.1 ± 3.9	4.4 ± 4.5	5.3 ± 5.1	0.0015
Hospital LOS (mean ± SD)	17.7 ± 15.5	16.9 ± 23.2	15 ± 17.8	17 ± 19.6	0.18
Ventilator Days (mean ± SD)	9.2 ± 14.8	9.9 ± 15.2	11.1 ± 19.7	12.3 ± 18.9	0.6

Table 4. Univariate indicators of mortality.

Variable	Non survivors n=425	Survivors n=1372	P value
Male Gender (n=855) %	51	47	0.16
Age, Mean (SD)	63.3 (15.8)	54.8 (17.8)	<0.0001
APACHE IV, Mean (SD)	99.2 (34.9)	60.1 (25.2)	<0.0001
Tobacco use n (%)	96 (22.8%)	462 (34%)	<0.0001
Alcohol use n (%)	45 (10.7%)	299 (22%)	<0.0001
Drug use n (%)	63 (15%)	266 (20%)	0.03
Hypertension n (%)	235 (56%)	725 (53%)	0.38
Diabetes n (%)	128 (30%)	437 (32%)	0.5
HIV n (%)	119 (28%)	258 (19%)	<0.0001
ESRD n (%)	28 (7%)	91 (7%)	0.97
Cancer n (%)	51 (12%)	80 (6%)	<0.0001
OSA n (%)	3 (0.7%)	20 (1.5%)	0.32
INR (mean ± SD)	1.6±1.4	1.2 ± 1.0	<0.0001
Hematocrit (mean ± SD) %	29.1 ± 7.3	33.2 ± 8.0	<0.0001
Creatinine (mean ± SD) mg/dl	2.5 ± 2.7	2.0 ± 2.7	<0.0001
Albumin (mean ± SD) g/dl	2.4 ± 0.8	3.3 ± 0.8	<0.0001
SGPT (mean ± SD) unit/l	74 ± 207.4	45.8 ± 185.5	0.003
SGOT (mean ± SD) unit/l	161.2 ± 578.6	69 ± 225.1	<0.0001
RVSP (mean ± SD) mmHg	43.6 ± 19.4	39.3 ± 15.3	0.0007
EF (mean ± SD)	58.3 ± 16.8	60.7 ± 13.7	0.12
Intubated n (%)	294 (74.6%)	301 (22.9%)	<0.0001
Ventilator days (mean ± SD)	12.4 ± 19.9	9.3 ± 15.6	0.03
Tracheotomy n (%)	49 (11.7%)	129 (9.5%)	0.19
ICU LOS (mean ± SD)	6.2 ± 6.1	3.9 ± 3.5	<0.0001
Hospital LOS (mean ± SD)	21.9 ± 23.6	14.4 ± 18.2	<0.0001

Table 5. Multivariate logistic regression analysis for mortality.

Variables	Odds Ratio (confidence interval)
Underweight versus Normal BMI	1.162 (0.576-2.343)
Overweight/Obese versus Normal BMI	1.047 (0.627-1.751)
Severe Obesity versus Normal BMI	1.239 (0.642-2.391)
Cancer	4.261 (2.083-8.716)
History of alcohol use	0.480 (0.257-0.894)
Mechanical ventilation	4.767 (2.930-7.755)
Apache IV	1.029 (1.021-1.038)
Age	1.019 (1.005-1.033)
Albumin	0.414 (0.313-0.549)
RVSP* ≥25 mmHg	1.015 (1.003-1.028)
ICU LOS**	1.051 (1.005-1.099)

* Right ventricular systolic pressure, ** MICU Length of stay

obesity and possibly underweight were associated with an increased all-cause mortality [43]. Finkelman *et al.*, [44] reported that a BMI<18.5 was associated with increased mortality in both, post-operative (OR=2.14, 95% CI, 1.39 to 3.28) and non-operative (OR=1.51, 95% CI, 1.13 to 2.01) patients. In addition the LOS was increased in post-operative patients with low BMI. Other studies report no correlation between low BMI and mortality or LOS in the ICU [27,28]. In our study, 9% of our patients were underweight and our results support published data regarding no effect of BMI in outcomes.

The impact of BMIs in the requirement for MV and the outcome for those patients are mixed with most of the reports addressing the obese population [11,19,45-49]. Pulmonary physiological changes related to elevated BMI includes a decreased lung compliance with a lower total lung capacity, functional residual capacity and residual volume and an increased airway resistance. These changes can lead to atelectasis and hypoxemia. Clinically this will translate in a higher risk for aspiration and difficult airway [50]. Some studies suggest that critically ill patients with elevated BMIs would have an increased need for MV and prolonged mechanical ventilation [11,29,48 and 49]. Other studies have shown that BMI have no impact on days of MV, however, a difficult airway and a higher incidence of post extubation stridor have been reported in the morbidly obese patients [45,46,51]. A lower BMI was associated with

HIV/AIDS [22,38]. The protective role of obesity have been postulated to be due to factors like increased nutritional reserve, younger age, role of adipose tissue and higher levels of immune modulating anti-inflammatory adipokines [39-42]. There are few studies addressing outcomes for the underweight patient in the ICU. A large study, not restricted to ICU patients that included 1.46 million white (non-Hispanic) adults and 160,087 deaths showed that overweight,

increased mortality in mechanically ventilated patients with acute respiratory distress syndrome [47]. The effect of low BMI on patient requiring MV is unclear, but it has been suggested that a low BMI could be associated with increased malnutrition and muscle weakness leading to prolonged MV. In our study the extremes of BMI groups were more likely to require MV compared to normal BMI. However, days on mechanical ventilation and tracheotomy rates were similar between BMI groups.

Similar to inconsistent relationship mortality and MV, the data for ICU and hospital LOS varies as well. Some studies report a higher ICU and hospital LOS across all BMI groups [20,23] while others report no correlation [17]. In our study although the Hospital LOS were similar between BMI groups, The ICU LOS was higher in severely obese patients. We could speculate that the ICU LOS in severely obese patients in our institution is related to intensivists practice. Most of the severely obese patients require additional care and increase monitoring; it is not uncommon that patients are kept longer in the ICU until they are deemed very stable to be transferred to floor at which time the discharge period is shorter.

In our study there was no association between BMI and pulmonary hypertension in critically ill patients; however the presence of pulmonary hypertension as assessed by elevated RVSP, was a predictor of mortality. The association of BMI with pulmonary HTN remains controversial with some studies suggesting an association [52,53] while others did not report this association [54]. Common causes of pulmonary hypertension in the critically ill include chronic cardiac or pulmonary diseases or acute pulmonary hypertension due to critical illness like acute respiratory distress syndrome, sepsis or pulmonary embolism. Despite right sided heart catheterization being the accepted standard approach in determining pulmonary artery pressure [55], Doppler echocardiography is widely used in the ICU to screen for pulmonary HTN. Estimates of pulmonary artery systolic pressures by Doppler echocardiography are usually inaccurate in patients with pulmonary hypertension and should not be relied solely to make the diagnosis [56]. We admit that these associations may be simply a detection bias as patients without echocardiograms (36%) during the hospitalization were assumed to have normal echocardiograms.

Our work validates the association of mortality with a low serum albumin [57,58] and admission APACHE IV scores [59] irrespective of BMI groups. There is no severity of illness assessment tool specifically designed for the obese or underweight, and any existing assessment tool, including APACHE IV, may not be accurate to reflect mortality risk in extremely obese patients due to "hidden" factors not accounted for in the assessment. The inverse association of alcohol use and mortality may be explained by reporting bias.

The associations between extremes of hemoglobin

concentration and mortality in acute stroke have been reported, with some studies showing an association between mortality and higher hematocrit in patients with stroke [60,61]. The association between low hematocrit and mortality in acute coronary syndromes are well established [62]. In our study there was a linear correlation between BMI categories and admission hematocrit. Patients with higher BMI had a higher hematocrit when compared to patients with a lower BMI. Univariate analysis revealed that patients with lower hematocrit had a higher mortality compared to patients with higher hematocrit. However this association was not validated by multivariate analysis and therefore our study found no association between admission hematocrit and mortality.

There are several strengths in our study. First, availability of admission height, weight and BMI data in majority of our patients. Second, this study describes an inner city population with a high prevalence of obesity and co-morbid conditions specially HIV infection. Third, the comparison of outcomes across all BMI groups. Fourth, in our ICU, there are weaning and sedation practice protocols which decrease individual variations and effect in weaning and LOS on MV.

Limitations of our study include all limitations of retrospective observational studies and single center study. In addition, we did not account for recent weight gain or loss prior to hospital admission and there are no measures of waist-to-thigh ratio in both gender and waist-to-hip ratio in women which have been positively associated with mortality in middle-aged adults [52]. Volume resuscitation in the ED in patients with severe sepsis and septic shock would have resulted in pseudo obesity in a small group of patients, however we feel this effect would affect all BMI groups. We did not adjust for the co-morbid conditions that could have masked a true association with mortality.

Conclusion

In conclusion, our study demonstrates that there is no association of BMI with mortality or any other outcomes in critically ill inner city population. Severely obese patients have a longer ICU LOS, but a similar hospital LOS.

Key messages

There is no association of BMI with mortality in critically ill patients in an inner city population. ICU length of stay is longer for the severely obese patients. Prevalence of obesity and severe obesity is higher in our inner city population.

Abbreviations

NHANES: National Health and Nutrition Examination Survey
BMI: Body mass index
ICU: Intensive care unit
LOS: Length of stay
MV: Mechanical ventilation
HTN: Hypertension
DM: Diabetes mellitus
HIV: Human immunodeficiency virus

ESRD: End stage renal disease
OSA: Obstructive sleep apnea
APACHE: Acute physiology and chronic health evaluation
HCT: Hematocrit
SGOT: Glutamic oxaloacetic transaminase
SGPT: Glutamic pyruvic transaminase
INR: International normalized ratio
RVSP: Right ventricular systolic pressure
EF: Ejection fraction
SD: Standard deviations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SV conceived the study, and participated in the analysis of data and revising the manuscript critically for important intellectual content. VR participated in the acquisition and interpretation of data and drafting the manuscript. MA participated in the acquisition and analysis of data and drafting the manuscript. GDF participated in the design of the study and revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Publication history

Editor: Francesca Pistoia, University of L'Aquila, Italy.

Received: 24-Mar-2013 Revised: 19-Apr-2013

Accepted: 22-Apr-2013 Published: 01-May-2013

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Citation:

Venkatram S, Rapaka V, Anwer M and Diaz-Fuentes G: **Body mass index have no effect in hospital mortality or intensive care outcomes in an inner city population.** *Internal Medicine Inside* 2013, **1**:1.
http://dx.doi.org/10.7243/2052-6954-1-1