Regression analysis in patients with thallasemia induced osteoporosis

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ABSTRACT

Introduction: Thalassemia Major (TM) is a hereditary disease caused by defective globin synthesis. Although, it is considered a disease which is well understood both from the clinical as well as the biological aspect, it is a complex phenomenon and still remains under thorough investigation. One of the recent findings, include the fact the due to the significant increase in life expectancy, TM patients suffer from various secondary health conditions, including endocrinopathies and low bone mineral density. **Aim:** The aim of the present study was to analyze biochemical factors, collected from TM patients, with the application of regression analysis in order to find probable causative relations for TM-induced osteoporosis. **Methods:** Sixty-four patients with TM (32 men and 32 women) participated in a cross-sectional study design. The patients were recruited from "Aghia Sofia" Children's Hospital and evaluated using dual-energy X-ray absorptiometry (DXA) of the lumbar spine and femoral neck and with markers of bone remodeling including receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), C-terminal telopeptide (CTX), and sclerostin. **Results:** Regression analysis, manifested significant relations in TM patients with respect to calcium, phosphorus, testosterone, luteinizing hormone, follicle stimulating hormone and DXA.
Conclusions: In TM patients, several factors appeared to be linearly correlated, which indicates that these factors play an important role in TM-induced osteoporosis.

KEY WORDS: Regressions, Thalassemia Major, Osteoporosis

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Introducton

Thalassemia Major (TM) is a hereditary disease, which is a result of defective globin synthesis [1]. These patients are depended on frequent blood transfusion, causing iron overload and are in need of several medications. The combination of transfusion and chelation therapy extended the life expectancy of TM patients. Thus, many health conditions, usually present in the aging general population, are becoming also common in TM patients [2]. In particular, some endocrinopathies are present in these patients and among them low bone mineral density has become a topic of wide interest. In addition, we have previously reported that TM patients manifest altered bone metabolic factors [3, 4].

Recent discoveries have highlighted the role of numerous factors that affect bone metabolism and thus osteoporosis. Such factor include, but are not limited to, calcium [5, 6], phosphorus [7-9], testosterone [10-12], Follicle Stimulating Hormone (FSH) in female patients [13], Luteinizing Hormone (LH) [13], Parathyroid Hormone (PTH) [14-17], 25-Hydroxy Vitamin D [18] and Ferritin [15]. Other molecular factors affecting bone metabolism are known to be the receptor activator of nuclear factor-kB ligand (RANKL) [9, 19], a tumor necrosis factor-family member, and its decoy receptor osteoprotegerin (OPG) widened the therapeutic spectrum [19]. An imbalance of the RANKL/OPG ratio has been described in both postmenopausal women and β-thalassemia major-induced osteoporosis.

One of the main problems in the treatment of osteoporosis is that it usually becomes evident due to an accidental fracture. In that sense, studies that can assist towards the understanding and prognosis of bone metabolic disease are of great importance. The main problem with osteoporosis and its secondary pathologies, is that it affects the patient's quality of life as well as it is a burden for the health system. The consequences and complications of osteoporosis impose a significant amount of morbidity, mortality and economic burden on patients and societies worldwide. The various treatment options are targeted in maintaining bone health and reduce the risk of fractures.

The present study aims at investigating the correlation of biochemical and bone metabolic factors in patients with TM.

Materials And Methods

Study design

In a previous study we have reported a cross-sectional study of 63 patients with TM [4]. Patients were collected from the Thalassemia Unit of "Aghia Sofia" Children's Hospital during the period of July 2016 until March 2017. 63 subjects (mean age 37.63±5.65 years, 28 men and 35 women) were evaluated with DXA of the lumbar spine and femoral neck. Serum levels of biochemical factors were evaluated, which included Ferritin (ng/ml), 25OHD (ng/ml), PTH (ng/lt), Ca+2 (Urine) (mmol), Ht (%), DXA (Lumbar Spine), DXA (Hip), FT4 (ng/dl), TSH (mU/ml), LH (IU/lt), FSH (IU/ml), Testosterone (ng/dl), E2 (pg/ml), Ca+2 (mg/dl) and P^{+4} (mg/dl). Transfusion Frequency (every/days) was also accounted for. This cohort was also screened with dual-energy X-ray absorptiometry (DXA) of the lumbar spine and hip. Osteoporosis was defined according to WHO criteria as a T-score of <-2.5 standard deviations (SD).

Measurement of Biomarkers

Markers of bone metabolism were assessed by using ELISA methodology (Biomedica Medizinprodukte, No. BI-20412, Gesellschaft GmbH, Wien, Austria). Bone Mineral Density (BMD) was determined using Dual-Energy X-ray Absorptiometry.

Availability of Data

All data are available upon reasonable request.

Statistical analysis

Multiparameter analyses were performed with MATLAB® simulation environment (The MathWorks, Inc., Natick, MA, USA). Continuous data are presented as mean standard deviation (SD) and the categorical data are presented as a man to woman ratio Correlations between variables were calculated using the Pearson's correlation coefficient. Linear regressions were performed using the y=ax+b form and curves were estimated using a least-chi-squared approach. A value of $p<0.05$ (two-tailed) was set as the level of significance.

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TABLE 1

Totals (n=63)	Mean±StDev	Median	Min	Max
Age	37.63±5.65	38.00	22.00	56.00
Transfusion Frequency (every/days)	15.51±3.14	14.00	12.00	27.00
Ferritin (ng/ml)	1275.14±1196.22	874.00	182.00	5121.00
25OHD (ng/ml)	28.56±12.48	28.19	7.90	49.80
PTH (ng/lt)	24.58±17.51	20.24	6.54	79.25
Ca+2 (Urine) (mmol)	5.57±3.03	5.65	0.40	10.10
Ht (%)	27.73±1.45	28.00	25.00	31.00
DXA (Lumbar Spine)	-2.38±0.82	-2.45	-3.90	-0.30
DXA (Hip)	-2.31±0.74	-2.20	-4.00	-1.20
FT4 (ng/dl)	1.01±0.22	0.95	0.75	1.70
TSH (mU/ml)	1.83±0.81	2.08	0.50	3.70
LH (IU/lt)	5.85±6.08	3.82	0.34	19.00
FSH (IU/ml)	4.14±3.40	2.89	0.59	10.90
Testosterone (ng/dl)	497.50±149.67	519.00	267.00	685.00
E2 (pg/ml)	67.90±16.90	67.90	51.00	84.80
Ca+2 (mg/dl)	9.18±0.41	9.10	8.40	10.00
P+4 (mg/dl)	3.77±0.72	3.80	2.50	5.00

Males (n=28)	Mean±StDev	Median	Min	Max
Age	37.79±6.48	38.00	22.00	56.00
Transfusion Frequency (every/days)	15.26±3.18	14.00	12.00	27.00
Ferritin (ng/ml)	987.91±1068.77	500.00	182.00	3620.00
25OHD (ng/ml)	27.49±11.66	28.13	7.90	49.80
PTH (ng/lt)	27.15±22.22	20.77	6.54	79.25
Ca+2 (Urine) (mmol)	5.02±2.66	5.65	0.40	8.60
Ht (%)	27.47±1.63	28.00	25.00	31.00
DXA (Lumbar Spine)	-2.62±0.65	-2.50	-3.60	-1.65
DXA (Hip)	-2.48±0.89	-2.50	-4.00	-1.30
FT4 (ng/dl)	1.04±0.24	0.94	0.77	1.70
TSH (mU/ml)	1.62±0.78	1.25	0.50	2.74
LH (IU/lt)	3.07±1.87	3.24	0.34	5.46
FSH (IU/ml)	2.06±0.98	2.20	0.59	3.26

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Testosterone (ng/dl)	497.50±149.67	519.00	267.00	685.00
E2 (pg/ml)	NaN	NaN	NaN	NaN
Ca+2 (mg/dl)	9.21±0.43	9.20	8.40	10.00
P+4 (mg/dl)	3.75±0.71	3.80	2.50	5.00

Females (n=35)	**Mean±StDev**	**Median**	**Min**	**Max**
Age	37.51±4.87	37.00	28.00	48.00
Transfusion Frequency (every/days)	15.77±3.08	14.00	12.00	24.00
Ferritin (ng/ml)	1562.36±1246.84	1100.00	457.00	5121.00
25OHD (ng/ml)	30.05±13.39	28.25	10.20	48.91
PTH (ng/lt)	20.98±4.69	19.70	15.60	27.19
Ca+2 (Urine) (mmol)	6.40±3.35	6.80	1.90	10.10
Ht (%)	27.95±1.21	28.00	26.00	30.00
DXA (Lumbar Spine)	-2.29±0.86	-2.40	-3.90	-0.30
DXA (Hip)	-2.17±0.53	-2.10	-3.10	-1.20
FT4 (ng/dl)	0.96±0.14	0.96	0.75	1.16
TSH (mU/ml)	2.22±0.74	2.20	1.23	3.70
LH (IU/lt)	11.40±7.60	11.40	3.80	19.00
FSH (IU/ml)	8.30±2.60	8.30	5.70	10.90
Testosterone (ng/dl)	NaN	NaN	NaN	NaN
E2 (pg/ml)	67.90±16.90	67.90	51.00	84.80
Ca+2 (mg/dl)	9.12±0.38	9.10	8.56	9.70
P+4 (mg/dl)	3.81±0.74	3.75	2.80	4.70

Table 1. *Characteristics of patients and controls (Legend: 25OHD: 25-Hydroxy-Vitamin D,* ***PTH:*** *Parathyroid Hormone,* ***Ht:*** *Hematocrit,* ***FT4:*** *Free Thyroxine,* ***TSH:*** *Thyroid-Stimulating Hormone,* ***LH:*** *Luteinizing Hormone,* ***FSH:*** *Follicle Stimulating Hormone,* ***E2:*** *Estradiol,* ***SD:*** *Standard Deviation).*

Ethics Statement

All experiments were conducted in compliance with the international biomedical studies stipulations, with reference to the Declaration of Helsinki of the World Medical Association. All participants gave their written informed consent after a detailed description of the study protocol. This study was approved by the ethical committee of the Medical School of the National and Kapodistrian University of Athens.

Results

The demographic, clinical and biochemical characteristics are summarized in **Table 1**. Further on, a very good regression result was obtained for the frequency of transfusion days and with respect to the levels of testosterone (R^2=0.77, p<0.05) (*rho*=0.88, p<0.01) (**Fig. 1a**) as well as to the levels of P+4 (R^2=0.77, p<0.05) (*rho*=0.68, p<0.01) (**Fig. 1b**). In addition, it has been found that ferritin levels correlated significantly with Ca^{+2} levels (R^2=0.51, p<0.05) (*rho*=0.47, p<0.05) (**Fig. 2**). Further on, we have found that the Ca^{+2} levels corre-

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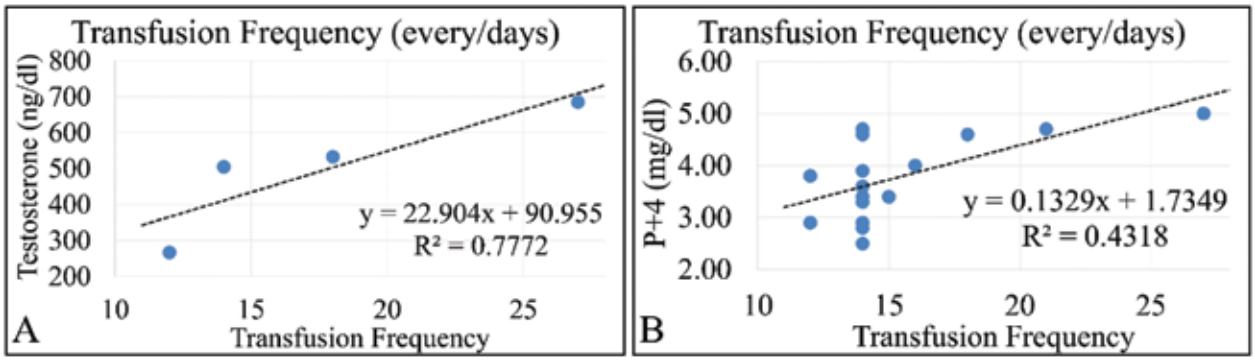


Fig. 1 *Regressions of the transfusion frequency (transfusion every certain number of days) vs. testosterone levels (A) and P^{+4} (B) in the total population of TM patients.*

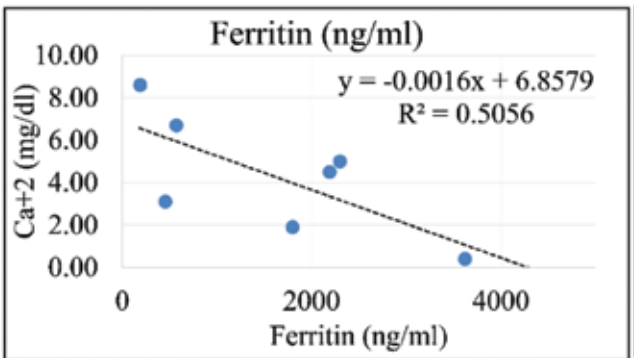


Fig. 2 *Regressions of Ferritin vs. Ca^{+2} in the total population of TM patients.*

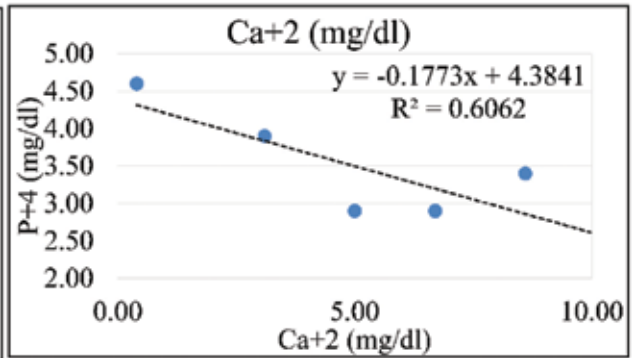
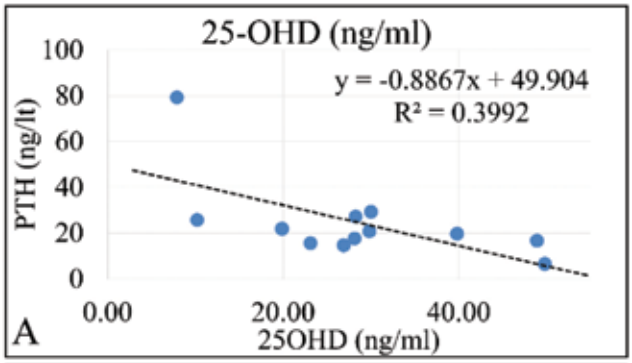
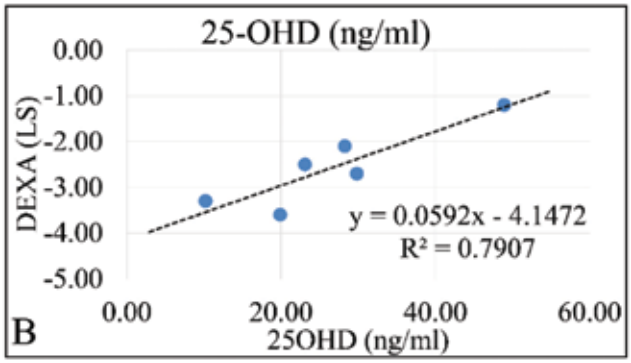


Fig. 3 *Regressions of Ca^{+2} vs. P^{+4} in the total population of TM patients.*





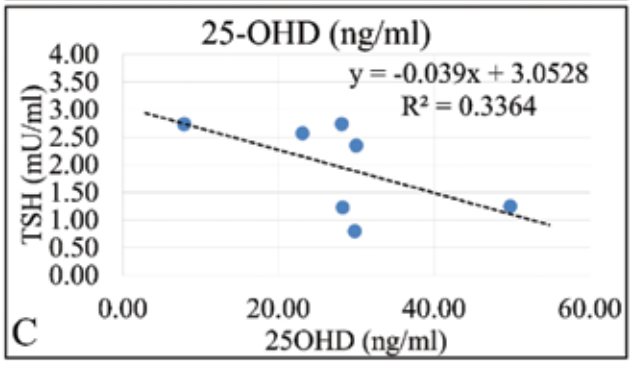


Fig. 4 *Regressions of the 25-OHD vs. PTH (A), DEXA (LS) (B) and TSH (C) in the total population of TM patients (Legend: 25-OHD: 25-Hydroxy Vitamin D, PTH: Parathormone, LS: Lumbar Spine, TSH: Thyroid Stimulating Hormone).*

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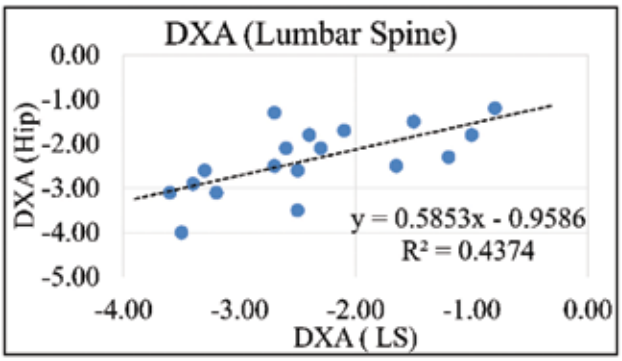

Fig. 5 *Regressions of DEXA (LS) vs. DEXA (Hip) in the total population of TM patients.*

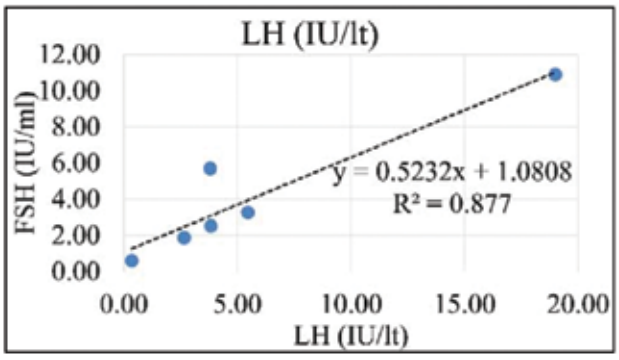

Fig. 6 *Regressions of LH vs. FSH in the total population of TM patients.*

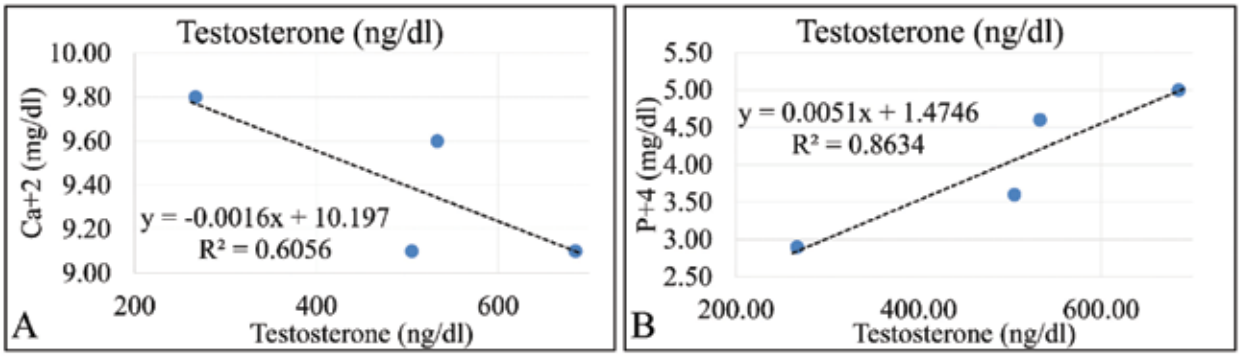

Fig. 7 *Regressions of testosterone vs. Ca^{+2} (A) and P^{+4} (B) in the total population of TM patients.*

lated with the P+4 levels in the total population of TM patients (R^2=0.61, p<0.05) (*rho*=0.28, p<0.05) (**Fig. 3**).

Another interesting result was obtained for 25-Hydroxy-Vitamin D, which included the significant correlation with Parathormone (PTH) (R^2=0.4, p<0.05) (*rho*=-0.63, p<0.01) (**Fig. 4a**), T-Score of the Lumbar Spine (LS) (R^2=0.79, p<0.05) (*rho*=0.89, p<0.01) (**Fig. 4b**) and Thyroid Stimulating Hormone (TSH) (R^2=0.34, p<0.05) (*rho*=-0.58, p<0.01) (**Fig. 4c**). Similarly, we have found that the T-score for LS significantly correlated to the T-score for hip (R^2=0.43, p<0.05) (*rho*=-0.66, p<0.01) (**Fig. 5**). In addition, when examining the role of hormones we have found that there was significant correlation between Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) (R^2=0.877, p<0.05) (*rho*=-0.94, p<0.01) (**Fig. 6**). Further on, we have found that testosterone levels significantly correlated to the levels of Ca^{+2} (R^2=0.61, p<0.05) (*rho*=-0.77, p<0.01) (**Fig. 7a**) as well as the levels of P^{+4} (R^2=0.86, p<0.05) (*rho*=-0.92, p<0.01) (**Fig. 7b**).

Discussion

As life expectancy of TM patients increases, osteoporosis is becoming a prominent problem. Prevention and early diagnosis of osteoporosis are as important as the treatment of the established disease [20]. In this study we examined the correlations of biochemical factors in TM patients with osteoporosis. Our study revealed several statistically significant relations among these parameters. Bone disease may be without symptoms for a long time and the first manifestation is fractures either in femoral neck or vertebral ones. Patients sometimes present without pain, only with loss of height and bone deformities. Treatment initiation should always be considered when there is evidence of osteoporosis as defined by WHO criteria, or there is a fracture.

One of our first results showed that testosterone levels were positively linearly correlated with the transfusion frequency. A possible reason for this is that hypogonadism is connected to TM patients, therefore transfusion helps to testosterone levels elevation, thus

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functioning as a sort of supplementation. Yet, hormonal supplementation has been shown that it does increase bone mineral density [21]. This explanation is in agreement with the next finding that transfusion frequency is related to phosphorus levels. Previous reports demonstrated that TM patients had unbalanced bone turnover with an increased resorption phase. However, it is possible that various and partially unknown pathogenetic factors could be involved in this bone remodeling imbalance. Factors such as an excessive iron accumulation in the cells of the bone marrow that may have altered or provoked defective bone remodeling and osteoblastic activity, desferrioxamine itself, which inhibits DNA synthesis, and low levels of the insulin-like growth factor 1 (IGF-1); a very important anabolic factor for bone, may account for the observed differences among patients [22]. Another interesting finding was that ferritin was negatively correlated to calcium. There are no previous reports on this finding, yet an older report has shown that calcium and phosphorus were inversely correlated to ferritin levels in TM patients [23].

The levels of PTH and 25-OHD are lower in TM patients as in control subjects [24]. The finding that PTH and 25-OHD reversely correlate was in agreement with previous studies in osteoporotic patients [25, 26]. Similarly, our finding that TSH was negatively correlated to 25-OHD is in agreement with recent studies where it has been reported that TSH and BMD as well as 25-OHD are negatively correlated [27].

In order to prevent osteoporosis and osteoporotic fractures in TM patients they should be evaluated often with clinical assessment (height, BMI, age, smoking habits), routine screening for causes of secondary pathologies, imaging procedures with DXA and X-ray of lumbar and thoracic spine to identify early deformities. Our approach attempted to identify correlations between biochemical factors in TM patients. This approach potentially leads to the identification of patterns in such a way that a possible prediction would be feasible from such data. For example, regression analysis gives the possibility to predict bone metabolic factors from biochemical factors.

A limitation of the current study is that the total number of patients included is small, as well as missing measurements in patient cohort that could improve the presented measurements. Another limitation is that few TM patients were already receiving treatment for osteoporosis. In addition, no conclusions can be made of how we can reduce fracture incidence [28].

Conclusions

To conclude, the current study showed that patients with TM manifest correlations among biochemical factors and in particular it is probable that bone metabolism could be predicted from bone turn-over biochemical factors. These factors could be used for routine diagnosis and prognosis of bone metabolism and osteoporosis. The success of such an approach could lead to the prevention of fractures, which would decrease morbidity mortality and the health system's economic burden.

Declarations

Ethics approval and consent to participate:
Not Applicable.

Consent for publication: *Not applicable.*

Availability of data and material: The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Please also refer to the "Materials and Methods" section.

Competing interests: *Nothing to declare.*

Conflict of Interest: *Nothing to declare.*

Funding: *None*

Authors' contributions: ANT: *Data collection, review of the literature, primary reviewer, statistical analysis, drafted the manuscript,* ***GIL:*** *Review of the literature, secondary reviewer, statistical analysis, drafted the manuscript,* ***AS:*** *Data collection, review of the literature, reviewer* ***EV:*** *Data collection, review of the literature, reviewer,* ***IKT:*** *Data collection, review of the literature, third reviewer.* ***AS:*** *Data collection, review of the literature,* ***CK:*** *Review of the literature, critical reading,* ***GPC:*** *Review of the literature, critical reading, proof-edited the manuscript, gave final permission for submission,* ***AT:*** *Critical reading*

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