Screening over weight and diabetic women from serum 25- hydroxy vitamin D and serum thyroid-stimulating hormone levels with Age, Body Mass Index and Fast Plasma Glucose

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Accepted 15 June, 2015

Background: It has been reported that serum 25- hydroxyl vitamin D (25(OH)D) level may affect glucose homeostasis and is inversely correlated with serum thyrotropin TSH concentrations. We proposed some classes of levels of serum 25(OH)D and TSH, we wondered whether fast plasma glucose concentrations or less costly criterion as age or BMI could be used to predict classification of each woman of our cohort in classes of serum 25(OH)D/TSH concentrations. Methods: Measurements of serum 25(OH)D and TSH were administrated among 165 adult African migrants women living in Paris. ROC analysis was used to identify serum 25-hydroxyl vitamin D and TSH thresholds. Machine-learning tools were performed for prediction. Results: A threshold of serum 25(OH)D of 48±5 nmol/L and TSH of 1.44±0.17 UI/mL level was found with a sensitivity of 86%, a specificity of 83%. We identified 3% of the sample as a particular class of fifties overweight and diabetic women with high levels of serum 25(OH)D and high serum TSH concentrations suspected of sub-hypothyroidism disease. We found 38% of mid-aged women, obese with high risk for developing hypothyroidism disease. Conclusion: Estimation with Fast Capillary Glucose measurement instead of Fast Plasma Glucose, Age or BMI could be a less costly method to screen glucose, thyroid and vitamin D status among migrant women.

Keywords: 25-hydroxyvitamin D - Thyroid-stimulating hormone - Fast glucose plasma - Data mining.

INTRODUCTION

Vitamin D is required for efficient absorption of dietary calcium and for good health. It is obtained from exposure to sun and diet, and is converted in the liver to hydroxyl cholecalciferol (25(OH)D), the primary storage form of the vitamin. Subsequently 25(OH)D is converted into its biologically active form, 1,25 dihydroxy vitamin D (1,25(OH)\textsubscript{2}D); in human kidneys, vitamin D and its analogs are necessary for healthy bones. However, other implications of vitamin D metabolism has been mentioned; a low vitamin D status increases the prevalence of obesity...
and for individual differences, its onset and severity is associated with thyroid diseases (Foss 2009). Recently, effects of vitamin D on glucose homeostasis were reported where an inverse relationship between 25(OH)D and Fast Plasma Glucose (FPG) was postulated (Need et al., 2005). TSH is the most sensitive marker of peripheral tissue levels of the thyroid. It is known that TSH is inversely correlated with diabetes (Islam et al., 2008), aging (Mariotti et al., 1993), obesity (Donders et al., 1985), and numerous other conditions (Peeters et al., 2005). In most developed societies including France, ethnic differences presenting the prevalence of obesity, is particularly noticeable in migrant women (Darmon and Khlat 2006). Of note, thyroid dysfunction, obesity, low vitamin D status, are common endocrine disorders encountered within the African population. Our purpose was to determine classes of serum 25(OH)D/TSH levels with the use of 25(OH)D threshold below which TSH concentrations increase. Then, in our sample, we wondered if FPG measurement or a less costly criterion as age or BMI could be used for each African migrant women; for the prediction of her class of serum 25(OH)D/TSH levels.

METHODS

Patients

The study was conducted in a Community Clinic located in eastern Paris at latitude 48.5N. The inclusion and the criteria were adult African migrant women. Patients were excluded if younger than 18 years, if they were pregnant, had a body mass index (BMI) >30, or if their medical history might influence their serum TSH concentration (medical treatment: corticosteroids, known as thyroid disease or pregnant women). In France, a migrant is defined as someone who was born in a foreign country as a non-French citizen. One hundred sixty-five migrant adult women with a mean age of 38.6 ± 9.9 years were included in the study between February 2008 and November 2009. This study was approved by the Independent Ethics Committee of Paris IV and all participants provided written informed consent.

Calcium intake

We used a food-frequency type self-assessment questionnaire to estimate the daily calcium intake of each patient (Fardellone et al., 1991).

Measurements

Serum 25(OH)D was measured using chemiluminescence methodology (Diasorin, LIAISON®) (Horst and Hollis 1999) by the Pasteur Cerba laboratory. The test interval measure was between 10 and 350 nmol/L. For all seasons of the year and for both genders, 25(OH)D norms (Holick 2006), were as follows: Recommended levels: 75 – 200 nmol/L (30 – 80µg/L), Insufficiency: 25 – 75 nmol/L (10 – 30µg/L), Deficiency: < 25 nmol/L (< 10µg/L). Functional sensitivity was 17.5 nmol/L with inter assay coefficients of variations (CVs) of 12.9%. Samples were collected in dry tubes for serum 25(OH)D. FPG was measured with hexokinase technic -Cobas c501 norms were 4.11- 5.89 mmol/L. Hba1c was measured with high pressure chromatography- D10 Biorad norms were <6. Serum TSH and serum free Thyroxine (T4) were measured by CMIA technic- Architect Abbott system, serum TSH norms were 0.35-4.94 mU/L and serum Free T4 norms were 9-19 pmol/L. After an overnight fast, blood samples were drawn from 8.00 to 10.00 a.m. for measurement of 25(OH) D.

Statistical analysis

Regression analysis

We used non-parametric kernel regression method implemented in the ‘npreg’ function of the Hayfield et al. (np) package (Efron 1979), for the R software version 2.10.0.

ROC analysis

The diagnostic performance of a test, or the accuracy of a test to discriminate diseased cases from normal cases is evaluated using ROC curve analysis (Hand and Till 2001). In practice the AUC performs very well and is often used when a general measure of predictability is desired (Provost and Fawcett 2001). In this study, we tested all serum 25 (OH)D values in the range of class 1 levels, 38-60 nmol/L, previously estimated with Gaussian mixture model fitted for serum 25(OH)D distributions. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. We took the best threshold point (Hanley and McNeil 1982).

Machine learning (Moon et al., 2007).

We used Adaboost (Schapire 2013), to estimate classes of 25 (OH)D/TSH. Boosting is an approach to machine learning, based on the idea of creating a highly accurate prediction rule by combining many relatively weak and inaccurate rules. Adaboost algorithm is particular because it needs no prior knowledge of the accuracies of the weak hypotheses. It adapts to these accuracies and generates a weighted majority hypothesis in which the weight of each weak hypothesis is a function of its accuracy. Based on 20 repetitions of 10-fold cross validation (CV), Adaboost algorithm was used to predict from data observed classification of patients in different classes of serum 25(OH)D levels and serum TSH levels defined with our 25(OH)D/TSH threshold as below. We choose three strong predictor variables selected as FPG, BMI and age. We used Adaboost algorithm implemented in R software version 2.10.0.
Table 1: Basic statistics

<table>
<thead>
<tr>
<th></th>
<th>(n=165)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African migrants women</td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>38.6 ± 9.9</td>
</tr>
<tr>
<td>Clearance creatinine(ml/min)</td>
<td>119.32 ± 36</td>
</tr>
<tr>
<td>Daily Calcium Intakes (mg/day)</td>
<td>800.59 ± 303.16</td>
</tr>
<tr>
<td>Baseline calcium intakes</td>
<td></td>
</tr>
<tr>
<td>Milk products</td>
<td>50%</td>
</tr>
<tr>
<td>Mineral waters</td>
<td>14%</td>
</tr>
<tr>
<td>Others</td>
<td>36%</td>
</tr>
</tbody>
</table>

RESULTS

Basic statistics (Table 1).
Regression analysis found serum 25(OH) D threshold of 50 ± 5nmol/L and of TSH 1.48± 0.21 UI/mL.

ROC analysis

In the whole sample, ROC analysis found a decision threshold point of serum 25(OH)D of 48 ± 5nmol/L and TSH of 1.44 ± 0.17 UI/mL with a sensitivity of 90.91%, a specificity of 91.11%, a positive predictive value of 53.2 % and a negative predictive value of 98.9 %.

Classes of 25(OH)D/TSH levels (Tables 2-3).
We found four classes of serum 25(OH)D and serum TSH concentrations. We noticed that there were two classes of levels with a weak negative relationship between serum 25(OH)D and TSH levels as class 4 (serum 25(OH)D levels ≥48 ± 5nmol/L, serum TSH concentrations ≥ 1.44 ± 0.17 UI/mL) and class 2 (serum 25(OH)D levels ≤ 48 ± 5nmol/L serum TSH concentrations <1.44 ± 0.17 UI/mL). And two classes of levels with a strong relationship between serum 25(OH)D and serum TSH concentrations as class 1 (serum 25(OH)D levels ≤ 48 ± 5nmol/L and serum TSH concentrations≥ 1.44 ± 0.17 UI/mL) and class 3 (serum 25(OH)D concentrations >48 ± 5nmol/L , serum TSH levels <1.44 ± 0.17 UI/mL).

Correlations (Table 4)
We found that between FPG and age a significant positive correlation of 0.45 (p<0.001). We found a significant negative correlation of -0.24 (p = 0.08) between FPG and serum 25(OH)D with serum 25(OH)D <48 ± 5nmol/L. We found a non significant correlation between serum 25(OH)D ≥ 48 ± 5nmol/L and FPG of -0.08.

In our sample, we found 3% of women with serum TSH ≥ 4.5 mUI/L

Adaboost algorithm (Table 5)

We found age, BMI and FPG as stronger criteria to discriminate each patient of our cohort. Classes of 25(OH)D/TSH levels were predicted with an error rate of less than 1%.

DISCUSSION

The determination of the prevalence of thyroidism in a population may produce different results, according to the subject groups or diagnostic criteria chosen. In the present study, we found an average prevalence of thyroid dys function of African migrant women of 5%. Baghi et al found in a urban population of American African (mean age 65 ± 1.4 years ) a prevalence of 5.8% of thyroid disorders (Bagchi et al., 1990). Martin et al. in a study on American population found Seventy percent of older patients with serum TSH levels greater than 4.5 mUI/L were within their age-specific reference range (Surks and Hollowell 2007).

In our study we found that women with increased TSH above 4.5 mUI/L were mainly in our oldest classes. This suggested that our method was more reliable than those used in previous studies and it provided for each patient in our cohort, significant information on body size, glucose and vitamin D status. Thyroid hormones are known to play an important role in controlling gene so associated with obesity disease(Koritschoner et al., 2001). TSH is the most sensitive marker of peripheral tissue levels of the thyroid and it is inversely correlated with diabetes (Islam et al., 2008), aging, obesity, and numerous other conditions (Mariotti et al., 1993). In most developed societies including France, ethnic differences in the prevalence of obesity is particularly noticeable in migrant women (Darmon and Khlat 2006). Then, we choose to examine African migrant population because obesity, low vitamin D status and glucose disorders are common endocrine disorders encountered in African population. Thus,
### Table 2: Means of 25(OH)D/TSH level classes

<table>
<thead>
<tr>
<th>Class</th>
<th>25(OH)D (nmol/L)</th>
<th>Calcium intakes (mg/d)</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>FGP (mg/dL)</th>
<th>HbA1C (%)</th>
<th>Serum Free T4 (pmol/L)</th>
<th>Serum TSH (mUI/L)</th>
<th>Serum PTH (ng/L)</th>
<th>Serum 25(OH)D (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (19%)</td>
<td>35.55 ± 14.50</td>
<td>635.42 ± 24.13</td>
<td>43 ± 5</td>
<td>35.45 ± 6.33</td>
<td>980 ± 168</td>
<td>6.20 ± 0.95</td>
<td>1.07 ± 0.17</td>
<td>2.55 ± 0.60</td>
<td>62.07 ± 34.19</td>
<td>35.55 ± 14.50</td>
</tr>
<tr>
<td>2 (41%)</td>
<td>482.5 ± 28.99</td>
<td>482.5 ± 28.99</td>
<td>26 ± 7</td>
<td>21.5 ± 5.4</td>
<td>886 ± 96</td>
<td>5.66 ± 0.31</td>
<td>1.29 ± 0.30</td>
<td>0.90 ± 0.29</td>
<td>48.43 ± 2.32</td>
<td>32.51 ± 12.47</td>
</tr>
<tr>
<td>3 (19%)</td>
<td>1137.47 ± 31.34</td>
<td>1137.47 ± 31.34</td>
<td>37 ± 6</td>
<td>23.71 ± 0.66</td>
<td>919 ± 120</td>
<td>5.75 ± 0.66</td>
<td>1.11 ± 0.12</td>
<td>0.88 ± 0.28</td>
<td>52.31 ± 23.14</td>
<td>78.76 ± 16.15</td>
</tr>
<tr>
<td>4 (22%)</td>
<td>1983.28 ± 31.16</td>
<td>1983.28 ± 31.16</td>
<td>51 ± 7</td>
<td>28.48 ± 2.54</td>
<td>1270 ± 111</td>
<td>7.07 ± 2.19</td>
<td>1.01 ± 0.10</td>
<td>2.37 ± 0.75</td>
<td>49.93 ± 22.82</td>
<td>91.21 ± 34.22</td>
</tr>
</tbody>
</table>

### Table 3: Comparing class means: Wilcoxon tests with p-values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Class 1 vs Class 2</th>
<th>Class 1 vs Class 3</th>
<th>Class 1 vs Class 4</th>
<th>Class 2 vs Class 3</th>
<th>Class 2 vs Class 4</th>
<th>Class 3 vs Class 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium intakes</td>
<td>m₁&gt;m₂ (0.01)</td>
<td>m₁&lt;m₃ (0.01)</td>
<td>m₁&lt;m₄ (0.01)</td>
<td>m₂&lt;m₃ (0.01)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>m₁&gt;m₂ (0.03)</td>
<td>m₁&gt;m₃ (&lt;0.01)</td>
<td>m₁&lt;m₄ (0.01)</td>
<td>m₂&lt;m₃ (0.04)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>m₁&gt;m₂ (&lt;0.01)</td>
<td>m₁&gt;m₃ (0.01)</td>
<td>m₁&lt;m₄ (0.03)</td>
<td>m₂&lt;m₃ (0.01)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
<tr>
<td>FGP (mg/dL)</td>
<td>m₁&gt;m₂ (&lt;0.01)</td>
<td>m₁&gt;m₃ (&lt;0.01)</td>
<td>m₁&lt;m₄ (0.08)</td>
<td>m₂&lt;m₃ (0.01)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>m₁&gt;m₂ (0.01)</td>
<td>m₁&lt;m₃ (0.076)</td>
<td>m₁&lt;m₄ (0.065)</td>
<td>m₂&lt;m₃ (0.04)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
<tr>
<td>Serum Free T4L</td>
<td>m₁&lt;m₃ (0.15)</td>
<td>m₁&gt;m₃ (0.16)</td>
<td>m₁&gt;m₄ (0.03)</td>
<td>m₂&gt;m₃ (0.20)</td>
<td>m₂&lt;m₄ (&lt;0.01)</td>
<td>m₃&gt;m₄ (&lt;0.01)</td>
</tr>
<tr>
<td>Serum TSH</td>
<td>m₁&gt;m₂ (&lt;0.01)</td>
<td>m₁&gt;m₃ (0.08)</td>
<td>m₁&gt;m₄ (0.03)</td>
<td>m₂&gt;m₃ (0.23)</td>
<td>m₂&lt;m₄ (&lt;0.01)</td>
<td>m₃&gt;m₄ (&lt;0.01)</td>
</tr>
<tr>
<td>Serum PTH (ng/L)</td>
<td>m₁&gt;m₃ (&lt;0.01)</td>
<td>m₁&gt;m₄ (&lt;0.01)</td>
<td>m₁&gt;m₄ (0.03)</td>
<td>m₂&gt;m₃ (0.03)</td>
<td>m₂&lt;m₄ (&lt;0.01)</td>
<td>m₃&lt;m₄ (&lt;0.01)</td>
</tr>
<tr>
<td>Serum 25(OH)D</td>
<td>m₁&gt;m₃ (&lt;0.01)</td>
<td>m₁&lt;m₄ (0.01)</td>
<td>m₁&lt;m₄ (0.01)</td>
<td>m₂&lt;m₃ (0.01)</td>
<td>m₂&lt;m₄ (0.01)</td>
<td>m₃&lt;m₄ (0.01)</td>
</tr>
</tbody>
</table>
identifying a serum 25(OH)D/TSH threshold below which TSH concentrations are elevated might be of use for determining classes of 25(OH)D/TSH levels and health outcomes risk due to inadequate serum TSH or 25(OH)D concentrations.

Calcium sufficiency as adequate iodine intake

In our study, the daily calcium intake of African women, estimated with 20% precision (Fardellone et al., 1991), was higher than 400 mg/day. This estimate was higher than the 300 mg/day reported by Prentice et al. among African women living in Africa (Yan et al., 2009), and close to the 900 mg/day recommended for French women younger than 55 years old (Martin 2001). However, Heaney et al. estimated that African American women require 300 mg less calcium per day compared to white women (Heaney 2002), which suggests that the average calcium daily intake of African women in our sample was sufficient. Milk and dairy products are important determinants of iodine intake in Europe. Iodine content of milk is of 259 µg/L (79-490 µg/L) (Federico et al., 2011), with average calcium content of 120mg/mL in France (Martin 2001). With a recommended iodine intake of 150 µg/d for adults, a daily intake of 0.4 L milk meets the requirement with 25% during the summer and more than 60% during the winter season (Dahl et al., 2003). In our study, done in winter period, subjects had average calcium intakes of 1050 mg/d. This suggested that African migrant women were iodine sufficient.

25(OH)D-TSH cut-offs.

According to the third National Health and Nutrition Examination Survey (NHANES III) the reference range for TSH is 0.45–4.12 mU/L that varied with age, sex, and ethnic background, although these differences were small (Hollowell et al., 1988-1994). Some authors reported that the mean concentration is 1.4 mU/L consistent with a skewed distribution (Biondi et al., 2005). It was a similar value of our TSH threshold. According to Andersen et al. the individual reference range for serum TSH is approximately half the width of laboratory reference range and was close to our results. We found a similar serum 25(OH) D cut-off in a previous study (Emilion and Emilion 2011).

Classes of 25(OH) D.

According to Mackawy et al. low status of serum 25(OH)D3 is significantly associated with low TSH levels (Mackawy et al., 2013). The authors found a significant negative correlation between serum 25(OH) D and TSH (r = -0.589, P < 0.05), with non-significant correlation with T4 (r = 0.045, P > 0.05). It was similar to our results. Hollowell et al. showed that North American women had a mean serum TSH of 1.57 (1.52–1.62) mIU/L. We noticed that it was similar to the mean serum TSH of our mid-aged women classes. According to Surks et al. patients with subclinical thyroid disease have few or no symptoms or signs of thyroid dysfunction and thus by its very nature subclinical thyroid disease is a laboratory diagnosis. Subclinical hypothyroidism is defined as a serum TSH above the defined upper limit of the reference range, with a serum-free T4 within the reference range. Some experts stated that the range of 0.45-4.5 mU/L should be adopted [28]. In our study women with serum TSH above 4.5 mU/L were in class1. According to Biondi et al. the definition of subclinical hyperthyroidism is based only on laboratory but not clinical criteria. Subclinical hyperthyroidism definition includes a low or undetectable concentration of serum TSH with and free thyroxine (FT4) levels within laboratory reference ranges. However, experts defined the lower limit of the normal range for TSH being 2.1% with a TSH cut-off of 0.3mU/L (Canaris et al., 2000). In our study there were no subjects with such low serum TSH levels. Vanderpump et al; showed that increasing values of serum TSH above 2mU/L at first survey, increased the probability of

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### Table 4: Correlations between serum 25(OH)D and serum TSH levels

<table>
<thead>
<tr>
<th>Serum 25(OH)D</th>
<th>Who FPG values</th>
<th>FPG &lt; 110 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 48 ± 5 nmol/L</td>
<td>-0.20 (0.942)</td>
<td>-0.33 (0.068)</td>
</tr>
<tr>
<td>&lt;48 ± 5 nmol/L</td>
<td>-0.105 (0.842)</td>
<td>-0.18 (0.073)</td>
</tr>
</tbody>
</table>

### Table 5: Performance of Adaboost algorithm for 25(OH)D/TSH classes data based on 20 Repetitions of 10-fold CV (S.D. in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive value</th>
<th>Negative Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.998 (.002)</td>
<td>1.00 (.002)</td>
<td>.994 (.003)</td>
<td>.993 (.001)</td>
<td>.998 (.007)</td>
</tr>
</tbody>
</table>
developing hypothyroidism (Vanderpump et al., 1995). In our study, when we took into account BMI (Pinto-Sietsma et al., 2003) and definition of glucose disorders based on HbA1c classification (American Diabetes Association (ADA) 2010), we determined class of low serum 25(OH)D concentrations and high serum TSH levels, as class 1 of oldest forties women, obese and pre-diabetic with a high risk for developing hypothyroidism. Class of high serum 25(OH)D levels and low serum TSH concentrations determined as class 4 of fifties diabetic women with risk for developing of hypothyroidism could not be excluded. We classified younger forties and thirties subjects as classes of slender women with low serum TSH concentrations and with a high risk for developing glucose disorders in the future. Chailurkit et al. showed that high vitamin D status in younger individuals is associated with low circulating TSH. It was in agreement with our class 3 (Chailurkit et al., 2013).

Discriminant factors of classes of levels and machine learning

In our study, we used three criteria that classified whole sample, as FPG, BMI and age. From women Age or BMI measurements and using boosting data method as Adaboost we estimated classes of serum 25(OH) D and serum TSH levels. Each patient was classified in one of the four classes defined. Whether routine screening of older in particular with serum TSH analysis are to be recommended remains controversial, particular when the added costs are considered. In our study estimations were done with criteria quick, easy to obtain, acceptable to patients in clinical settings and less costly. Note that there are non-calcemic effects of vitamin D in other biological metabolisms which likely need higher 25(OH)D concentrations than those required to maintain TSH secretion found in our study (Grau et al., 2003). Therefore, 25(OH)D threshold of 48 ± 5nmol/L should not be interpreted as optimum vitamin D status. There is lacking for identifying the exact serum TSH levels within the normal range that may be detrimental. Then, serum TSH cut-off of 1.44 ± 0.17 UI/mL should not be interpreted as absolute TSH cut-off.

CONCLUSION

From our sample of 165 calcium-sufficient African migrant women living in Paris, ROC analysis found a 25(OH)D threshold of 48 ± 5nmol/L and TSH of 1.44 ± 0.17 UI/mL in the whole sample. We identified 4% of the sample as a particular class of overweight and diabetic women with high levels of serum 25(OH) D and low serum TSH concentrations suspected of sub-hypothyroidism disease. We found 38% of mid-aged women, obese with high risk for developing hypothyroidism disease. In our sample, 3% of women had sub-hypothyroidism disease. With our model of machine learning From Age, Body Mass Index and Fast Plasma Glucose we classified each patient of our sample in different classes of 25(OH)D and serum TSH levels with a sensitivity of 99%, a specificity of 99%, a true positive value of 98% and a true negative value of 98%. Estimation with Fast Capillary Glucose measurement instead of Fast Plasma Glucose could be a less costly method to screen thyroid and vitamin D status among African migrants women. Estimating vitamin status and thyroid status in others samples of population might be of interest.

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