Male Subclinical Hypogonadism: Mechanisms with Interplay of Reproductive Hormones, Undercarboxylated Osteocalcin and Endothelial Dysfunction

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Research

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Abstract

Background

Pathogenesis and endothelial function in subclinical hypogonadism (SCH) are unknown. Undercarboxylated osteocalcin (ucOC) participate in atherosclerosis and reproduction. We studied interplay of endothelial function, ucOC and reproductive hormones with SCH.

Methods

among SCH, late onset hypogonadism (LOH), and healthy eugonadal male (HC) groups, we measured sex hormones and ucOC, calculated luteinizing hormone/testosterone (LH/T), LH.T product and estradiol/T (E/T) as indicators of impaired leydig cell, androgen sensitivity index (ASI) & aromatase activity respectively and regulators for LH set point. We assessed flow mediated dilation of brachial artery (FMD%), carotid- intima media thickness (CIMT) and aortic stiffness index (AS) as markers of subclinical atherosclerosis.

Results

Contrary to LOH, SCH had higher ASI, lower E/T ratio & similar T, follicle stimulating hormone and sex hormone binding globulin (SHBG) compared to HC, LH/ T was significant higher in LOH and lower in HC than SCH. Similar to LOH, SCH had significant lower FMD% and higher CIMT, AS, unOC & inflammatory marker and atherogenic lipid profile than HC. LH, LH/T & ucOC negatively while T positively FMD% meanwhile. LH, LH/T & ucOC positively while testosterone negatively correlated with CIMT. LH and LH/T positively while estradiol and E/T negatively related to AS. ucOC positively correlated to LH, LH/T, E SHBG & negatively correlated with T. Independent predictors were LH for FMD% & AS meanwhile LH and LH/T for CIMT.

Conclusions

SCH as not impaired testicular function state is characterized by androgen insensitivity, impaired aromatase activity, compensatory elevated ucOC and atherogenic role of LH in endothelial dysfunction.

Highlights

- Subclinical hypogonadism (SCH) is distinct clinical entity and not androgen deficiency state
- It is characterized by pituitary androgen insensitivity and impaired aromatase activity with equivocal minimal role of impaired leydig cell.
- SCH is associated with impaired both the haemodynamic and morphological endothelial function, dyslipidemia and pro-inflammatory state.
- Luteinizing hormone and luteinizing hormone /Testosterone ratio are independent predictors to subclinical atherosclerosis.
- Elevated undercarboxylated osteocalcin as compensatory mechanism to androgen insensitivity in SCH, represent a link between reproductive hormone, lipid, inflammation endothelial function and atherosclerosis.

Introduction

According to European Male Aging Study (EMAS), late onset hypogonadism (LOH) is a clinical and biochemical syndrome characterized by gradual decreased serum testosterone (T) level as an aspect of age correlated reproductive and sexual decay across life span and its diagnosis depends on presence of at least three sexual symptoms and either repeated (at least twice) total T level <8 nmol/l or serum total T of 8–11 nmol/l and free T <220 pmol /l. The concept of subclinical hypogonadism (SCH) or compensated hypogonadism has begun recognized as clinical entity affects 9.5% of men. Similar to other subclinical endocrine disorders, it is identified with normal T level with elevated luteinizing hormone (LH) level (1). The hypothalamic–pituitary–testicular (HPT) axis is highly regulated. Gonadotropin releasing hormone pulse generator controls gonadotropins release namely LH and follicle stimulating hormone (FSH) from the anterior pituitary gland which bind to testicular receptors on the Leydig cell for T production and to Sertoli cells for spermatogenesis respectively. Aromatase enzyme can convert T to estradiol (E) in gonads and extragondal target tissues. Both T and E mediate negative feedback on HPT axis via binding to their receptors and regulate of HPT set point for LH (2). Impaired leydig cell function, altered androgen receptor sensitivity and aromatization capacity can consequently affect the LH set point and can be estimated by LH/T ratio, androgen sensitivity index (ASI) and E/T ratio respectively (3-5).
SCH has been significantly associated with physical symptoms and identical increased cardiovascular risk and ~10-fold increased risk of cardiovascular mortality compared to overt hypogonadism (6,7). Moreover, an independent associations of elevated LH level with both decreased muscle strength and increased cardiovascular disease regardless of T level were reported (8,9).

Osteocalcin (OC) is a polypeptide protein specifically expressed in osteoblasts and is the most abundant non-collagenous bone protein. Undercarboxylated Oc(ucOC) as the active form acts as a link for bone-pancrease –reproductive axis, It binds to its receptor in target tissue to play regulatory role in glucose metabolism, T synthesis and muscle mass (10). Either OC or ucOC showed conflict relations with different atherosclerotic markers in a meta-analysis study (11). Moreover, in-vitro and vivo studies showed either protective or no role of ucOC in atherogenesis (12,13).

Up till now, little data exists about underlying mechanisms of SCH and debates whether it's a para physiological condition as a barometer of poor health in ageing men, distinct clinical entity or precursor of overt hypogonadism (2,6,7,14). Our study aimed to explore role of impaired leydig cell response, androgen insensitivity and aromatase activity parameters as HPT set point regulatory factors in pathogenesis of SCH; secondly, to address dual role of ucOC as a bridge from bone to gondal state and atherosclerosis. Thirdly, to study association of SCH with cardiovascular risk factors and cardiovascular function via studying morphological and hemodynamic functional parameters of endotheliem and defining role of sex hormones and studied HPT regulatory factors.

Methods

Study Population

The present cross case-control study was conducted at Endocrinology and diabetec unit, Internal medicine department, Minia University Hospital from March 2018 to July 2019. It included men with primary LOH group (n=60), SCH group (n=76) aged ≥ 50 years and age matched eugonadal apparently healthy men (HE, n=50) served as control group. Patients groups were selected from general population who were asked for andropausal symptoms according to Androgen Deficiency in Aging Males (ADAMS) Quationaire (15). Subjects with one or more positive sexual symptoms were underwent further laboratory investigations for diagnosis. Diagnosis SCH depended on presence of normal total testosterone level with elevated LH (T ≥10.5 nmol/L and LH ≥ 9.4 IU/L). Diagnosis of primary LOH depend on essentially three sexual symptoms (lessened sexual thoughts, weakened morning erections and erectile dysfunction), and either repeated (at least twice) total T level <8 nmol/l. Those with hypogoadotrphic hypogonadism were excluded from our study and the term of LOH means primary type in our study (1).

The study protocol was approved by the institutional ethics committee and carried out according to ethical guidelines of the declarerion of Helsinki and international conference of harmonization guidelines for good clinical practice. Prior to inclusion, all participants gave informed written consent before enrolling this study.

All enrolled men underwent thorough history taking including marital state, fertility, smoking and ADAM questionnaire. Clinical examination and anthropometric measurements were done

Exclusion criteria: Subjects with one of the following criteria were excluded: age <50 years, hypogonadism since young or hypogonadotrophic hypogonadism, alcohol intake, any endocrine or systemic disorders, obesity>30kg/m², malignancy, medical or hormonal therapy within three months of the study, surgical casteration or varicoceles.

Biochemical and Hormonal Assay:

At two separate occasions, fasting (8:00-10:00 AM), drawing venous samples to assess serum total testosterone and LH to establish the diagnosis. Routine biochemical investigations including haemogram, blood glucose level, renal and liver function, and complete lipogram were carried out according to standardized laboratory methods. Serum blood samples were stored at -20c for measurement of sex hormone binding globulin (SHBG), estradiol, FSH, high sensitive C-reactive protein (hsCRP) and undercarboxylated osteocalcin levels. Serum Testosterone, estradiol, LH and FSH were measured using enzyme linked immune sorbant assay (ELISA) (Chemux bioscience, USA). hsCRP was also assessed by ELISA method by commerrial kits from (Elbascience, USA). The free androgen index (FAI) was calculated by multiplying values for TT(nmol/L) by 100/SHBG(nmol/L) (16). ASI is the multiplication product of LH and T values. Elevated ASI has been suggested as an indication of androgen insensitivity because the impaired feedback regulatory mechanism of HPT axis leads to an elevation of LH and T. The estradiol-to-testosterone (E/T) ratio was calculated to assess aromatase activity (3,4,17). Serum undercarboxylated Osteocalcin(ucOC) was measured using commercial available ELISA kit that supplied by Takara Bio Inc., Japan.

Assessment of Endothelial Function:

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Using high resolution color-Duplex ultrasonography (TOSHIBA, Aplio 500, superficial prob., Japan) using a 8-12-MHz linear array for

Measurement of common carotid artery intimal media thickness (CIMT) following the recommendations of the Manheim CIMT Consensus (18)

Calculated flow mediated dilation (FMD)% of brachial artery: (post occlusion diameter – baseline diameter) × 100/ baseline diameter according to method described by Celermajer et al., 1992 (19)

Using M-mode transthoracic echocardiograph (ACUSON SC2000 PRIME Ultrasound system, Germany)

According to the last ASE/AHA guidelines of the aortic root: calculation of aortic stiffness index (AS) = 100× [natural systolic blood pressure (SBP)/diastolic blood pressure (DBP)] / [(aortic maximum systolic diameter - aortic end diastolic diameter) / aortic end diastolic diameter](20)

Testicular Ultrasound and Duplex Scan:

They were performed to exclude testicular abnormalities such as varicocele or others

Statistical Analyses

All statistical analyses used SPSS version 22.0. Parametric quantitative data is expressed as mean + SD and were compared by using One-way ANOVA test for comparison between the three groups followed by post hoc Tukey analysis between each two groups. Qualitative data was expressed as a percentage and compared using Chi square test. Non-parametric quantitative data was expressed as median (25%-75% inter-quartile) and compared using Kruskal Wallis test between the three groups followed by Mann Whitney test between each two groups. Spearman's or Pearson's correlation as bivariate analysis was used to determine the relations. Multiple linear regression analysis was done to determinate the independent predictors for endothelial function parameters. Significant level was at P value < 0.05.

Table (1): Clinical, laboratory and Endothelial Function Parameters among Studied Groups.
| Variables                  | Late onset hypogonadism (LOH, n=60) | Subclinical hypogonadism (SCH, n=76) | Healthy euogonadism (HE, n=50) | P value | All groups ANOVA | LOH versus SCH | LOH versus HE | SCH versus HE |
|----------------------------|--------------------------------------|--------------------------------------|--------------------------------|---------|------------------|----------------|--------------|--------------|
| Age(years)                | 57.1±4                               | 56.7±4.6                             | 57.8±5.1                       | 0.48    | 0.59             | 0.76           | 0.25         |
| Smoker n(%)               | 25 (83.3%)                           | 24(63.2%)                            | 16(64%)                        | 0.16    | 0.10             | 0.10           | 0.9          |
| BMI (kg/m2)               | 23.4±3.2                             | 23.4±3.2                             | 23.4±3.1                       | 0.98    | 0.92             | 0.86           | 0.86         |
| SBP (mmHg)                | 123.6±14.3                           | 123±12.6                             | 114±9.7                        | 0.95    | 0.978            | 0.09           | 0.09         |
| DBP (mmHg)                | 84.4±7.8                             | 81.8±8                               | 75.8±4.9                       | 0.22    | 0.371            | 0.06           | 0.08         |
| FBS (mg/dl)               | 96.5±13.1                            | 89.4±12.8                            | 89.9±5.7                       | 0.001   | 0.002            | 0.003          | 0.81         |
| PPBS (mg/dl)              | 152.2±7.1                            | 154.5±15.1                           | 137.2±13.9                     | 0.47    | 0.13             | 0.23           | 0.14         |
| Cholesterol (mg/dl)       | 192.3±17.4                           | 195.7±18.5                           | 166.7±18.6                     | <0.001  | 0.27             | <0.001         | <0.001       |
| Triglyceride (mg/dl)      | 205.9±11.9                           | 205.6±12.3                           | 145.7±34.6                     | <0.001  | 0.89             | <0.001         | <0.001       |
| LDL-c (mg/dl)             | 131.4±17.5                           | 131.4±15.4                           | 114.7±14.5                     | 0.003   | 0.97             | <0.001         | <0.001       |
| HDL-c (mg/dl)             | 43.2±4.1                             | 43.2±4.6                             | 48.3±6                         | <0.001  | 0.98             | <0.001         | <0.001       |
| LH (IU/L)                 | 14.1(11.9-16.6)                      | 20.6(13.4-24.6)                      | 7.1(5.8-8.9)                   | <0.001  | <0.001           | <0.001         | <0.001       |
| FSH (nmol/L)              | 15.4(11.2-17.7)                      | 9.7(4.4-11.1)                        | 7.6(4.2-10.4)                  | <0.001  | <0.001           | <0.001         | 0.88         |
| Testosterone (nmol/L)     | 4.9(3.8-5.5)                         | 23.5(16.5-25.9)                      | 21.7(15.8-24.8)                | <0.001  | <0.001           | <0.001         | 0.22         |
| Estradiol (nmol/L)        | 2.8(0.9-6.6)                         | 3.1(2.6-4.7)                         | 3.8(1.7-8.2)                   | 0.009   | 0.008            | 0.83           | 0.01         |
| SHBG (nmol/L)             | 247(215-281)                         | 199.5(171-220)                       | 203.5(136-244)                 | <0.001  | <0.001           | <0.001         | 0.78         |
| Free Androgen index       | 1.9(1.4-2.5)                         | 11.3(86-16.8)                        | 11.7(9.7-15.9)                 | <0.001  | <0.001           | <0.001         | 0.92         |
| LH / T ratio              | 3.5(2.2-3.8)                         | 0.9(0.6-1.3)                         | 0.3(0.2-0.5)                   | <0.001  | <0.001           | <0.001         | <0.001       |
| ASI (LH × T product)      | 66(50-74)                            | 456(246-590)                         | 133(101-187)                   | <0.001  | <0.001           | <0.001         | <0.001       |
| E/ T ratio                | 0.74(0.27-2)                         | 0.08(0.04-0.21)                      | 0.17(0.07-0.39)                | <0.001  | <0.001           | <0.001         | 0.005        |
| ucOC (nmol/L)             | 249(181-453)                         | 292(212-403)                         | 212(184.3-260)                 | 0.001   | 0.46             | 0.03           | 0.001        |
| hsCRP (mg/l)              | 4.5(2.1-8.4)                         | 4.8(1.7-9.3)                         | 1.2(0.44-3)                    | <0.001  | 0.82             | <0.001         | <0.001       |
| FMD %                     | 5.7(5.6-6.1)                         | 5.6(2.7-5.9)                         | 11.6(10-12.8)                  | <0.001  | 0.08             | <0.001         | <0.001       |
| CIMT (mm)                 | 0.9±0.1                              | 0.9±0.1                              | 0.65±0.1                       | <0.001  | 0.90             | <0.001         | <0.001       |
| Aortic stiffness index    | 18±1.5                               | 18.5±1.7                             | 12.7±1.6                       | <0.001  | 0.397            | <0.001         | <0.001       |

1=Parametric quantitative data is expressed as mean ± SD and are compared by using One-way ANOVA test for comparison between the three groups followed by post hoc Tukey analysis between each two groups. 2= qualitative data is expressed as a percentage and compared using Chi square test. 3= non-parametric quantitative data is expressed as median (25%-75% interquartile) and compared using Kruskal Wallis test between the three groups followed by Mann Whitney test between each two groups. Significant level at P value < 0.05
Results

1- Clinical and Laboratory Data:

The three studied groups were comparable as regard age, smoker %, blood pressure, and BMI. In comparison to both LOH and HE groups, SCH group had significant higher LH and ASI and lower E/T ratio. In addition, SCH group had significant higher TT, FAI and estradiol and lower serum FSH, SHBG and LH/T than LOH and both groups were comparable as regard atherogenic lipid profile, ucOC and hs-CRP. SCH group had significant higher atherogenic lipid profile, unOC and hsCRP and significant lower estradiol than HE group and both groups were comparable TT, FAI, FSH and SHBG levels. LOH had significant higher fasting blood sugar than other groups (table, 1, figure 1).

Box Plot of Undercarboxylated Osteocalcin among healthy eugonadal , subclinical hypogonadism and late onset hypogonadism groups. Each box indicates the 25th and 75th percentiles, the median is represented by the horizontal line within the box, the extreme measured values are indicated by whiskers. Dots and stars represent the outliers.

2- Endothelial Function Parameters:

LOH and SCH groups were comparable as regard FMD %, CIMT, AS meanwhile both groups had significant lower FMD %, higher CIMT and higher AS than HE subjects (p<0.001 for all).

3- Correlations in All Studied Cases (Table, 2).

a. Correlation of studied hormonal parameters with cardiovascular risk factors:

LH was related to dyslipidemia being positively correlated with total cholesterol, triglyceride and hsCRP and negatively with HDL-c (p<0.001 for all except 0.01 for HDL-c). Total testosterone was negatively correlated with DBP, fasting blood sugar, and total cholesterol and positively with HDL-c (p <0.001,0.003, 0.04, 0.03 respectively) meanwhile FAI were negatively correlated with DBP, FBS, total cholesterol, triglyceride and hsCRP (p= <0.001, <0.001, 0.02, 0.003, 0.006 respectively ). LH/T as marker of impaired leydig cell function was positively correlated to many cardiovascular risk factor as DBP , FBS, cholesterol, triglyceride, hsCRP and negatively with HDL-c (p = 0.005, <0.001, <.001, <0.001, 0.01, <0.001 respectively). Correlation with FBS revealed that FSH, E/T ratio were positively while LH×T product was negatively correlated (p=0.005, 0.002, 0.005 respectively). Estradiol positively correlate to BMI (p=0.02)

b. Correlation of ucOC with Hormonal Parameters:

ucOC was positively correlated with LH, LH/T, estradiol and SHBG and negatively with FAI (p<0.001, 0.003, 0.02, <0.001, 0.02 respectively), with cardiovascular risk factors: DBP, total cholesterol, hsCRP(p=0.04, <0.001, <0.001 respectively);and endothelial function parameters: it was negatively correlated to FMD% and positively with CIMT(p=0.01, 0.02 respectively).

c. Relation of Hormonal Parameters with Endothelial Function:

FMD% was negatively correlated with LH and LH/T and positively correlated with TT and FAI ( p<0.001, <0.001, 0.01, 0.002 respectively). Carotid IMT was positively correlated with LH, FSH, LH/T and was negatively correlated TT and FAI (p=0.001, 0.008, <0.001, 0.03, 0.02 respectively). AS was positively correlated LH and LH/T and negatively with estradiol and E/T (p<0.001, <0.001, <0.001, 0.02 respectively).

Table (2): Correlation of Reproductive Hormone Level, Hypothalamo-Pituitary Testicular Axis Regulatory Factors and Undercarboxylated Osteocalcin with Studied Cardiovascular Risk Factors and Endothelial Function Parameters in All Cases.
|                      | LH (IU/l) | FSH (nmol/L) | T (nmol/l) | E (nmol/L) | SHBG (nmol/L) | FAI | LH/T ratio | LH×T product | E/T ratio | ucOC (nmol/L) |
|----------------------|-----------|--------------|------------|------------|---------------|-----|------------|--------------|------------|---------------|
| Age(years)           | 0.01      | -0.15        | 0.001      | -0.9       | 0.06          | -0.03| -0.003     | -0.01        | -0.1       | -0.11         |
| BMI (kg/m2)          | 0.09      | -0.08        | -0.02      | 0.16*      | -0.05         | 0.01 | 0.06       | 0.005        | 0.11       | 0.02          |
| SBP (mmHg)           | 0.08      | -0.02        | -0.04      | -0.01      | 0.05          | -0.07| -0.03      | -0.08        | 0.004      | 0.09          |
| DBP(mmHg)            | 0.01      | 0.09         | -0.26***   | 0.01       | 0.13          | -0.3***| 0.21**     | -0.11        | -0.03      | 0.15*         |
| FBS(mg/dl)           | -0.11     | 0.23**       | -0.22**    | 0.12       | 0.2**         | -0.28***| 0.28***    | -0.22**      | 0.23**     | 0.03          |
| PPBS (mg/dl)         | 0.06      | -0.11        | 0.14       | -0.06      | 0.05          | 0.14 | -0.09      | 0.13         | 0.12       | -0.02         |
| Cholesterol(mg/dl)   | 0.26***   | 0.09         | -0.15*     | -0.01      | 0.09          | -0.16*| 0.28***    | 0.03         | 0.07       | 0.31***       |
| Triglyceride(mg/dl)  | 0.33***   | 0.07         | -0.13      | -0.09      | 0.08          | -0.22**| 0.26***    | 0.06         | 0.03       | 0.12          |
| LDL-c ((mg/dl)       | 0.05      | -0.08        | -0.12      | -0.11      | 0.06          | 0.08 | 0.1        | 0.02         | 0.01       | 0.13          |
| HDL-c(mg/dl)         | -0.18*    | -0.12        | 0.16*      | -0.04      | 0.04          | 0.09 | -0.24**    | -0.01        | -0.13      | -0.12         |
| hsCRP(mg/l)          | 0.33***   | 0.07         | -0.07      | -0.04      | 0.27***       | -0.2**| 0.28***    | 0.09         | 0.08       | 0.22***       |
| ucOC(nmol/L)         | 0.31***   | 0.14         | 0.12       | 0.16*      | 0.38***       | -0.18*| 0.22**     | 0.14         | 0.13       | NA            |
| FMD %                | -0.55***  | -0.10        | 0.18*      | 0.1        | -0.12         | 0.23**| -0.43***   | -0.13        | -0.003     | -0.18*        |
| CIMT(mm)             | 0.55***   | 0.19**       | -0.16*     | -0.01      | 0.01          | -0.16*| 0.45***    | 0.13         | 0.06       | 0.16*         |
| Aortic stiffness index| 0.43***   | 0.01         | -0.02      | -0.26***   | 0.01          | -0.13| 0.35***    | 0.14         | -0.17*     | 0.1           |

Correlation coefficients are written Significant correlation coefficients are given in bold, ***=p < 0.001; **=p < 0.01; *=p < 0.05.

BMI= body mass index; SBP= systolic blood pressure; DBP=diastolic blood pressure; FBS=fasting blood sugar, PPBS:2hours postprandial blood sugar; LDL-c: low density lipoprotein-cholesterol ;HDL-c: high density lipoprotein-cholesterol ; LH: leutinizing hormone; FSH: follicle stimulating hormone; T= Testosterone ; E= Estradiol ; SHBG: sex hormone binding globulin ;FAI= Free Androgen index ; ucOC= Undercarboxylated osteocalcin; hsCRP: highly sensitive C- reactive protein; ; FMD%= brachial artery flow mediated dilation percentage CIMT=carotid artery intimal medial thickness.

**3-Multiple linear Regression (table, 3):**

LH was an independent predictor for FMD% and AS (p <0.001 for both) (adjusted R 2= 0.24, 0.23 respectively, p<0.001 for both). LH and LH/T (p <0.001, 0.02) were independent predictors of CIMT (adjusted R 2= 0.32, p < 0.001).

**Table (3): Multiple Linear Regression Analysis with Endothelial Function Parameters As Dependent Predictors**
### Table 1: Regression Analysis Results

| Dependent predictors | Unstandardized Coefficients | Standardized Coefficients |
|----------------------|----------------------------|--------------------------|
|                      | B(Std.Error) | Beta | T | p-value |
| **a-Flow mediated dilation% R=0.51, R²=0.26** |
| Constant             | 10.4(0.79)   | 13.1 | <0.001 |
| Luteinizing hormone (IU/L) | -0.19(0.03) | -0.42 | -5.9 | <0.001 |
| Luteinizing hormone / testosterone | -0.22(0.17) | -0.11 | -0.13 | 0.21 |
| Free androgen index | 0.04(0.03) | 0.11 | 1.34 | 0.18 |
| Undercarboxylated osteocalcin (nmol/l) | -0.001(0.002) | -0.06 | 0.87 | 0.38 |
| **b-Carotid intima media thickness R=0.58, R²=0.34** |
| (Constant)           | 0.62(0.02)   | 20.9 | <0.001 |
| Luteinizing hormone (IU/L) | 0.007(0.001) | 0.43 | 6.4 | <0.001 |
| Follicle stimulating hormone(nmol/l) | 0.001(0.002) | 0.05 | 0.78 | 0.43 |
| Luteinizing hormone / testosterone | 0.01(0.006) | 0.21 | 2.37 | 0.02 |
| Free androgen index | 0.000(0.001) | 0.02 | 0.29 | 0.76 |
| Undercarboxylated osteocalcin (nmol/l) | 0.001(0.000) | 0.103 | 1.57 | 0.11 |
| **c-Aortic stiffness index R= 0.49, R²=0.24** |
| Constant             | 14(0.44)      | 31.47 | <0.001 |
| Luteinizing hormone (IU/L) | 0.18(0.02) | 0.47 | 6.8 | <0.001 |
| Luteinizing hormone /testosterone | 0.11(0.11) | 0.06 | 0.99 | 0.32 |
| Estradiol (nmol/l) | -0.00(0.008) | -0.001 | -0.02 | 0.97 |

### Discussion

Up till now, the underlying mechanisms of subclinical hypogonadism is unclear, whether it is a precursor of hypogonadism or marker of poor general health. We suggested that SCH is a distinct clinical entity that greatly differs from LOH in pathophysiology but both may be represent cardiovascular risk factors as both were associated with dyslipidemia, pro-inflammatory state, hemodynamic and morphological endothelial dysfunction and elevated ucOC levels. LH was independent predictor to hemodynamic change of peripheral muscular and central elastic arteries meanwhile LH and LH/T were independent predictors to CIMT. ucOC may be a link connects bone to inflammation, dyslipidemia , reproductive and endothelial functions among SCH. These issues were not mentioned before in literature.

We suggested that SCH is a diverse clinical state and not typical feature of hypogonadism. Main underling mechanisms are impaired both androgen sensitivity and aromatase activity with equivocal minimal contributing role of compensated impaired leydig cell function as evidence by high ASI, decreased E/T and mild elevated LH/T ratio with high normal testosterone respectively. Similar to Corona et al., 2014, we reported elevated LH with high- normal testosterone among SCH in range even that was insignificant higher than healthy eogondal subjects. In light of following knowledge and our results, we suggested SCH is not linked to leydig cell dysfunction but is attributed to hormone-resistant state: lack of feedback inhibition of androgen and estradiol on the pituitary that caused by insensitive pituitary androgen receptor and impaired aromatization respectively with reset new HPT set point to a higher level. Androgen receptor polymorphism alters LH/T ratio rather than being hall mark of primary leydig cell dysfunction. Both androgen receptor and aromatase genes mutation polymorphism are associated with impaired feedback inhibition with relative elevated testosterone and different individual HPT set point. In our study, SCH group had normal SHBG level and FSH level which is predominantly regulated by inhibins produced in testicular cells. This supported normal testicular function in SCH (2, 21, 22).
Corona et al 2014 suggested that SCH is not a novel clinical state but denotes the response of HPT axis to somatic illness with more psychiatric not sexual symptoms than overt hypogonadism. Recent follow-up study suggested that SCH is reversible process especially at young age and is not typical androgen deficiency but rather reflects worsening health with vicious circle exists as factors related to accelerated functional aging promote CVD (diabetes, chronic pain, physical inactivity) predispose to SCH occurrence which in turn, promotes further functional decline (decreased hemoglobin and impaired cognitive functions) along with CVD development (14). In contrary, other suggested it is precursor for overt hypogonadism with compensatory increased LH to stimulate the Leydig cell reserve to maintain normal T level similar to other subclinical endocrine disorders (6).

On the other hand, LOH was associated with marked impaired uncompensated leydig cell function (marked reduced TT and FAI and the highest ratio of LH/T), enhanced both androgen sensitivity and aromatase activity (low ASI and high E/T ratio respectively). It may be associated with impaired spermatogenesis with high FSH as well as high SHBG in our study. This was matched to previous described pathophysiology mechanisms of LOH (23, 24).

Undercarboxylated osteocalcin (ucOC) is an osteocalcin with deficient -carboxylation at one or more sites produced by osteoblast and represents the active metabolic form and majority of circulating OC. It stimulates both insulin secretion and sensitivity. Also, evidences in human and mice studies (murine model not in rate or mouse) suggested that ucOC may stimulate testosterone production by leydig cell in similar manner to LH hormone via binding to its receptor G-protein coupled receptor (GPRC6A) either in a bone-testicular axis independent on HPT axis or as enhancer to testosterone synthesis upon gonadotropin stimulation. Testosterone in turn stimulates osteoblasts proliferation and differentiation. So, testosterone and unOC are engaged in bidirectional relationship (10, 25).

We are pioneering in reporting elevated ucOC in primary LOH and SCH men, its positive correlation with LH, LH/T, estradiol and SHBG and negative relation with FAI. According to previous reported elevated OC in male LH b-/- mice, idiopathic hypogonadotrophic hypogonadism men and among elderly men with testosterone (25-27), we hypothesized that elevated unOC may be a compensatory mechanism to androgen deficiency due to underlying impaired leydig cell function to increase T level or androgen insensitivity among LOH and SCH respectively. This suggestion may explain its positive correlation with impaired leydig cell function (LH/T) and LH level in our study. However, one study reported low OC in low testosterone males presenting to clinic for evaluation of hypogonadism and not mentioned as primary or secondary type (28).

Relation of ucOC with SHBG can be explained by similar molecular moieties between SHBG and ucOC and their competition to bind to common receptor GPRC6 with displacement of the SHBG by ucOC when co-incubated with high concentration (29). Inconsistent results exist regarding relation of testosterone (total and free) with OC or unOC in clinical and therapeutic male studies: positive correlation was observed mainly in secondary hypogonadism as among obese, type 2 diabetic, hypothyroid or among men presenting to clinic for evaluation of hypogonadism while no association was detected among eugonadal men or in young adults male from infertile couples (27,28,30-33). The different underlying pathophysiological mechanism and methodology might be accountable for such disparities. Our study was in line with Nah et al., 2017 as no and negative correlation with FAI were observed among subjects with normal (healthy and SCH groups) and low testosterone level (LOH group) respectively (data not shown). ucOC was correlated positively with SHBG not with testosterone and inversely with estradiol a large cohort of old men (34). On the other direction, increasing either endogenous testosterone or exogenous testosterone intake did not affect OC level in animal study while increased osteocalcin after testosterone replacement in hypo gonadal men in small, non-randomized nor placebo-controlled trial showed debates (28,35,36).

We recognized positive association of estradiol with ucOC. Neither estradiol production in leydig cell nor aromatase gene expression was affected by osteocalcin in experimental studies (26). However, decreased estradiol and increased testosterone following administration of aromatase inhibitors in elderly men were associated decreased OC in eugonadal but not in hypogonadal groups. Importantly, extra-glandular aromatization of circulating androgen precursors is the major source of estrogen in men. So, we supposed that elevated estradiol may regulate ucOC level (37-39).

Our study is the first to reported established markers of subclinical atherosclerotic among SCH with hemodynamic and morphological endothelial dysfunction using different standard measures: impaired FMD% of brachial artery, increased CIMT as well as AS. The use of these imaging to detect subclinical atherosclerosis has the potential to predict the risk of future cardiovascular events (40). Interestingly these findings of our results were supported by previous two studies. One study reported identical increased cardiovascular risk and a nearly 10-fold increased risk of cardiovascular mortality among SCH compared to overt hypogonadism while other reported exciting sharing common contributing factors and viscous circle (7, 14).

Our study suggested greatly association of elevated LH, LH/T with atherosclerosis as LH was independent predictor to impaired endothelium function and morphology (FMD%, CIMT, AS) and LH/ T was predictor to increase CIMT.
In accordance to our findings, LH “with a normal T” was related to CIMT among andropausal middle-aged men; and increased ischemic heart disease events was reported among either elderly men with elevated LH or those with primary hypogonadism than secondary hypogonadism (9,41). LH/T were associated with CVD mortality among elderly men in a prospective study (42). Presence of extra-gonadal LH receptor expression in vascular and smooth muscle cell and in addition to association of increased its expression with endothelial proliferation may theoretically illuminate its suggested atherosclerotic role in our study. This was similar to atherosclerotic role of endothelial expressed TSH receptor in subclinical hypothyroidism (43-45).

Estradiol level and E/ T were negatively correlated to AS but not contributors to it. Estradiol enhances vasodilation in men via several mechanisms. Estradiol receptors and aromatase are expressed in endothelium and vascular smooth muscle cell. Although endogenous estradiol in men exerts anti-atherosclerotic effect via limiting their proliferation and migration, estradiol exposure increased atherosclerosis in coronary arteries harvested from men. Aromatase inhibition also decreases FMD% in men (46). Higher estradiol was associated with lower CIMT among diabetic men but other reported lack of association or positive association (47-49) This may be attributed to the fact that estradiol level in adult men do not exactly indicate tissue activity owing to extra-gonadal aromatization activity and partly estradiol elimination in situ.

In our study, FSH was significant correlated to CIMT but not contributor to it, FSH receptors are expresses endothelium with enhancement of through stimulating vascular endothelial growth factor expression (50). In prostate cancer, also administration of androgen deprivation therapy agonist which leads to elevated FSH level showed increased cardiovascular risk than androgen deprivation antagonist therapy (51).

Overall, the evidence is conflicting regarding effect ucOC on vascular function: both an anti-atherosclerotic effect with improved endothelial function and unrecognized impact on endothelial function and hemostasis were reported in vivo and vitro studies (12, 13). In our study, ucOC was positively correlated to CIMT and negatively correlate to FMD% in bivariate analysis but these findings were not confirmed in multivariate analysis. Also ucOC was positively correlated to hsCRP, cholesterol and DBP. In this context, ucOC positively associated with CIMT in offspring with positive family of metabolic syndrome but negatively among chronic kidney disease (52, 53). Meta-analysis of global human studies showed that no clear relation could be built between measured total OC or ucOC and extent of calcification or atherosclerosis as positive and negative relation, as well as no association were reported among these mostly cross-sectional observational studies that are limited in their clarification as a cause–effect relationship (11). One longitudinal study showed that baseline total OC was correlated positively baseline carotid plaque score but delta change of OC was negatively correlated with final score changes. They hypothesized that atherosclerotic plaques may initially enhance OC secretion as protective mechanism to suppress atherosclerosis or calcification progression (54). However, all studies measuring OC positive cells or histological staining of OC showed a positive relationship with calcification may be due to its role in calcification rather than atherosclerosis by mediating abnormal vascular repair via activation of osteogenic genes with further mineralization (11, 55).

**Perspectives and significance**

We concluded that SCH is distinct clinical entity and not androgen deficiency state: pituitary androgen insensitivity and impaired aromatase activity with equivocal minimal role of impaired Leydig cell with normal SHBG and FSH while late onset hypogonadism is a state of impaired Leydig cell and Sertoli cell, enhanced both aromatase and androgen sensitivity with increased SHBG. Similar to late onset hypogonadism, SCH is associated with impaired both haemodynamic and morphological endothelial function of peripheral and central elastic artery. Also both conditions are associated with elevated ucOC as compensatory mechanism to impaired Leydig cell or androgen insensitivity respectively, atherogenic lipid profile and inflammation. ucOC correlated to reproductive hormone, lipid, inflammation endothelial function and atherosclerosis

Luteinizing hormone is independent predictor for FMD% and aortic stiffness index while LH and LH/ T are independent predictor for CIMT.

Limitation of our studies: the present study is cross section study. So, causal relationship could not be established. We did not involve secondary hypogonadism in our study. Further studies to address relation of SCH with coronary artery disease, stroke, peripheral arterial disease are recommended

**Declarations**

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investigation of the study.

Authors’ contributions

Dr. Matta provided the ideas and the design of the study and wrote the protocol. She shared in conducting the literature review and recruited the participants, did statistical analyses and wrote the draft of the manuscript. Mr. Abdelrahman shared in conducting the literature review recruited the participants and collected data. Dr. Farrage did the Echcardiographic and shared in duplex studies. Dr. Saedii did the laboratory work of this study and the authors approved the final draft. The author(s) read and approved the manuscript.

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Availability of data and materials

The datasets generated and analyzed during the present study are not Publicly accessible due to concerns of participants confidentiality but are offered by the corresponding author on realistic request.

Ethics approval and consent to participate

All procedures performed in our clinical study including human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration. All individual participants gave informed consent to be included in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**Figures**

**Figure 1**

Undercarboxylated Osteocalcin Levels in The Studied Group. Box Plot of Undercarboxylated Osteocalcin among healthy eugonadal, subclinical hypogonadism and late onset hypogonadism groups. Each box indicates the 25th and 75th percentiles, the median is represented by the horizontal line within the box, the extreme measured values are indicated by whiskers. Dots and stars represent the outliers.