Serum Vitamin D and Proinflammatory Markers Correlate Inversely in Overweight Postmenopausal Women with Type-2 Diabetes Mellitus

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Article

Serum Vitamin D and Proinflammatory Markers Correlate Inversely in Overweight Postmenopausal Women with Type-2 Diabetes Mellitus

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Abstract: Background/Objectives: The global burden caused by high body mass index (BMI) particularly in postmenopausal (PMP) women is increasing significantly and is expected to be increasing in geographical and socioeconomic perspectives. Serum vitamin D (vitD) and pro-inflammatory factors influence the development and progression of T2DM particularly in overweight (OW)/obese PMP women. However, since there are controversies about the impact and interactive association of vitD with some of the pro-inflammatory biomarkers (interleukin-6 (IL-6), homocysteine (Hcy) and others) and therapeutic significance of vitD in OW women in PMP with T2DM, we planned to carry out study in NW and OW women with/ without T2DM in PMP. **Methods:** The normal weight (NW, BMI: 18.5 to 24.9 kg/m^2) and OW (n: BMI: 25.0 to29.9 kg/m^2) nondiabetic (ND, n: 199) and T2DM (n: 197) women in PMP (age range: 51-60 years) were consulted. The women subjects (n: 396) were categorized into NW-ND (n: 101), OW-ND (n:98), NW-DM (n:101) and OW-DM (n:96). Fasting blood samples were collected for the determination of serum levels of vitD, IL-6, Hcy, hemoglobin (HB) and hepcidin (Hp). **Results:** The present report in PMP women with and without T2DM showed significant difference of serum vitD for NW-ND vs. OW-ND, NW-DM vs. OW-DM, and NW-ND vs. NW-DM, Hcy for OW-ND vs. OW-DM and NW-DM vs. OW-DM and IL-6 for OW-ND vs. OW-DM and NW-DM vs. OW-DM. Among-groups comparison showed significant variation of serum vitD, Hcy and IL-6. A significant negative linear correlation of BMI was obtained against vitD for OW-ND and OW-DM. A significant positive linear correlation of BMI was found against Hcy as well as IL-6 for OW-ND and OW-DM. A significant inverse linear correlation of vitD was obtained against Hcy for OW-DM, and against IL-6 for NW-ND, NW-DM and OW-DM. Hcy plot with IL-6 levels showed significant positive linear correlation for NW-DM, OW-ND and OW-DM. **Conclusions:** The results obtained for the alterations noted for serum vitD, proinflammatory biomarkers (Hcy and IL-6) and other variables in the present investigation in PMP women (NW and OW) with T2DM, and associations among these factors are quite convincing to introduce a novel therapeutic approach based on vitD and proinflammatory markers for T2DM patients of high BMI levels.

Keywords: Vitamin D; interleukin-6; homocysteine; type-2 diabetes mellitus; overweight postmenopausal women

1. Introduction

Diabetes mellitus type 2 (T2DM) is one of the oldest chronic diseases and a multifactorial disorder that distorts the functions of multiple physiological systems and hence, cannot be associated to a single factor, mechanism or pathway leading to a certain heterogeneity among the patients with T2DM [1]. Some of the factors that influence T2DM are, age [2,3], body mass index (BMI) [4,5], vitamin D (vitD) [6–8], anti-inflammatory markers (homocysteine (Hcy) [4,9,10], and interleukin-6 (IL-6) [11], and some of the other factors related to inflammation (hepcidin (Hp) [12] and haemoglobin (HB) [13]. One of the main factors is the high BMI [4,5]. Overweight (OW) status and obesity influence a number

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of diseases. The T2DM- quite a common non-communicable metabolic disease is one of those diseases [14–16] and requires tomanage the BMI status [17–19].

The progression of prediabetes and diabetes mellitus (DM) was revealed as associated to BMI and influenced by physical inactivity, OW/ obesity, and other related conditions [5]. The global burden caused by high BMI particularly in postmenopausal (PMP) women is increasing significantly and is expected to keep increasing in geographical and socioeconomic perspectives [18].

Studies in women for understanding the role of BMI / BMI related alterations are quite small [20,21]and a controversy exists for the association among BMI with pro-inflammatory factors (Hcy, IL-6, and other biomarkers) in OW/ obesity and T2DM subjects [22], and their interactive activity along with vitamin D (vitD) [7,8]

It was revealed that a gradual elevation appears in the levels of Hcy in BMI-matched quartile study [23]. Middle-aged women manifested positive association of Hcy with the abdominal obesity [20]. Diet-induced effects on the levels of Hcy in obese and T2DM patients [24] and elevated total homocysteine (tHcy) with BMI ≥ 30.7 compared to BMI< 30.7 verified the association existing between them [22]. However, it is not clearly known whether Hcy increases, decreases or shows no change in subjects with diabetes [25]. Increased Hcy associated with T2DM in women without any significant correlation of BMI with Hcy was also investigated [10]. Another study revealed that T2DM subjects do not show elevated Hcy levels except if the T2DM accompanies other complications [26]. No considerable change in total homocysteine (tHcy) under diet-induced weight loss in obese or diabetic patients [24], and no correlation of BMI with Hcy were investigated [10,27].

It has been suggested that pre and early stages in T2DM present preclinical chronic inflammation resulting from elevated concentrations of serum pro-inflammatory cytokines, e.g., IL-6 and other pro-inflammatory biomarkers e.g., Hcy [28]. A positive association between low-grade bio-inflammatory condition and T2DM patients without cardiovascular complications was revealed [29]. Furthermore, the pro-inflammatory status may lead to certain other complications in T2DM patients [30]. Pomegranate peel polyphenols have a proven anti-inflammatory effect though there is a lack of clinical study for confirming its anti-inflammatory efficacy in T2DM patients [31]. A report investigated a favorable effects of pomegranate peel extract (PoPEx) therapy on serum levels of IL-6 and other inflammatory biomarkers [31]] and the beneficial effects of dietary plant protein for women's health initiative (WHI) mediated via IL-6 and other inflammatory cytokines for the T2DM risk by obesity related inflammation [32]. Furthermore, diet-induced effects, weight loss programs and effect of therapeutic products suggested the role of of IL-6 and other related factors in OW/ obesity and T2DM subjects [11,32,33].

Vitamin D is important for good health and sufficient vitD intake is necessary for PMP women [34]. Increased serum Hcy has been noted to be a risk factor for T2DM and characterized as having a relationship with vitD in T2DM patients [35]. It has been suggested that vitD may influence the insulin activity, that can predispose to the development and progression of T2DM.

There is strong evidence of the reduction in low-grade inflammation in T2DM patients by the influence of vitD supplementation that suggests the anti-inflammatory property of vitD [6]. Hence, vitD is beneficial for the management of inflammation in patients with T2DM [8]. Evidence has been obtained from preclinical studies of a correlation between the vitD deficiency and development of T2DM but the randomized controlled trials (RCTs) presented inconclusive results to prove the efficacy of the supplementation of vitD [8].

The anti-T2DM property of vitD provides the novel therapeutic approaches [7]. It was revealed that vitD increased the ameliorative effect of metformin on T2DM-induced dysfunction [1]. The long-term use of metformin for T2DM was found causing progressive oxidative pancreatic β cell damage and inflammatory complications as T2DM progresses, that suggests introducing an alternative therapy by incorporating the use of vitD.

Studies in women for understanding the precise role of BMI / BMI related alterations in PMP women are quite small [20,21] and controversy exists for the association among BMI with pro-inflammatory factors (Hcy, IL-6, and other biomarkers) in OW/ obesity and T2DM subjects [22], and

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their interactive activity along with vitamin D (vitD) [7,8]. Since there are controversies as well for therapeutic significance of vitD with and without interactive involvement of some of the pro-inflammatory biomarkers in OW women in PMP with T2DM, we planned to carry out study in normal weight (NW) and OW nondiabetic (ND) women and women with T2DM in PMP.

2. Materials and Methods

2.1. Study Design and Subjects

Research work for the present case-control and descriptive study was planned and the application for the approval of proposal for collecting the data and carrying out the proposed study was submitted to the bioethical committee of the Faculty of Medicine, Umm Al-Qura University (UQU), Makkah. The application for the submitted proposal was accepted (Ethical Approval No: (HAPO-02-K-012-2022-01-1069) for conducting the proposed research at UQU and hospitals associated with UQU. The research work was done from June 2022 to September 2024 following the ethical standards for human studies set by UQU and the Helsinki Declaration. Before starting the research work, the sample size (n) required in the current study was estimated following the standard formula, $n = z^2 * p * (1 - p) / e^2$, where z, p and e respectively indicate confidence level (α), proportion ($p = 0.5$) and margin of error or confidence interval [36]. The sample size calculator was also used for verification.

The PMP women were consulted after obtaining consent. The total number of experimental T2DM patients and control women (age range: 51-60 years) consulted in the present work was 396. The PMP women either NW or OW included in the present study were DM and ND subjects. The ND (n:199) and T2DM (n: 197) subjects were categorized into NW-ND (n: 101; BMI: 18.5 to 24.9 kg/m^2), OW-ND (n:98, BMI: 25.0 to29.9 kg/m^2), NW-DM (n:101; BMI: 18.5 to 24.9 kg/m^2) and OW-DM (n:96; BMI: 25.0 to 29.9 kg/m^2).

The NW-ND, OW-ND, NW-DM, and OW-DM women in PMP selected for the present study did not suffer from cardiovascular complications. The anemic, type-1 DM (T1DM) and those women having BMI < 18.5 and > 29.9 were not entertained for the present study. Those women taking folic acid and/ or vitamin B supplements were also not included in the present study. The criteria of the study were as according to the study design. The smoking and obese women, and those suffering from endocrine diseases other than T2DM, and reproductive, chronic inflammatory, chronic respiratory, cardiovascular, renal, stroke, or any serious disease were not included in the present work. The PMP women with/ without T2DM selected for the present study had age range of 51-60 years. The PMP women with or without T2DM but with NW were in BMI range of 18.5 to 24.9 kg/m^2. Whereas the PMP women with or without T2DM but with OW status were in BMI range of 25.0 to 29.9 kg/m^2.

2.2. Methods and Measurements

Clinical history of the patients and general features of the T2DM and ND women were recorded in a Questionnaire. The BMI (kg/m^2) was evaluated by dividing body weight (BW; kilograms) by values of the square of body-height (meters) [37–40]. For the Saudi BMI levels, a Saudi study [39] was followed. Diabetes was defined based on a fasting blood glucose (FBG) [41,42], and fasting was considered once ≥ 10 hours passed since the last meal done [43].

Blood samples of the women subjects were taken in the fasting condition in the morning. The HB was determined using a hematology analyzer- Sysmex XN 100i (Sysmex Europe SE, Norderstedt, Germany). The determination of serum vitD was carried out by using enzyme-linked immunosorbent assay (ELISA) kits. For the estimation of serum vitD, the ELISA kits were employed following the manufacturer's instructions (Euroimmun, Lubeck, Germany), and serum IL-6 by incorporating ELISA-kit 96T; Catalogue-No. ELH-IL6; RayBio, USA. Serum Hcy and Hp were estimated employing respectively the Human Homocysteine (Hcy) ELISA Kit, and Human Hepcidin ELISA Kit

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(MyBioSource, Inc. San Diego, CA, United States) using ELISA reader according to manufacturer's instructions.

2.3. Data Analysis

Statistical analysis was carried out by applying the general statistical principles [44]. Mean ± standard deviation (SD) of analysed data was used for descriptive statistics. The distribution of quantitative characteristics corresponded to the normal one. Comparisons of two groups were done by using two tailed P-value using unpaired t–test. The p-values for one-way analysis of variance (ANOVA) for the comparison of four groups of PMP women and other statistical analyses were carried out employing the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Results were further verified by Tukey's Kramer post hoc test. GraphPad Prism (version 6.0) software, San Diego, CA, USA, was also used where required. Regression lines were plotted that presented the values of coefficient of determination (R^2) that manifested correlation of two parameters either positive or negative and the values of significance (p). The p value ≤ 0.05 was considered as statistically significant.

3. Results

Various variables and characteristic features among NW and OW groups of PMP women with and without T2DM are given in Table 1. The age of subjects in various groups did not differ significantly. Age and BMI in mean±SD, and age range and BMI range values are given in Table 1. Since the OW females had higher BMI values, they differed significantly for BMI ($p<0.001$) while compared with their counterpart NW both in T2DM (NW-DM vs. OW-DM) and non-T2DM (NW-ND vs. OW-ND) comparisons. However, NW-ND vs. NW-DM and OW-ND vs. OW-DM did not show significant change ($p>0.05$). Among-groups comparison by one-way ANOVA showed significant variation of BMI levels in various groups (Table 2; $p<0.001$).

Serum vitD varied significantly for NW-ND vs. OW-ND ($p<0.001$), NW-DM vs. OW-DM (p:0.004), and NW-ND vs. NW-DM (p:0.03) but the comparison of OW-ND vs. OW-DM did not present significant change (Table 1). Among-groups comparison by one-way ANOVA showed significant variation of vitD levels in various groups (Table 2; $p<0.001$).

Serum levels of Hcy varied significantly for OW-ND vs. OW-DM (p:0.02) and NW-DM vs. OW-DM ($p<0.001$). The NW-ND vs. NW-DM (p:0.91) and NW-ND vs. OW-ND (p:0.12) were found non-significantly different (Table 1). Among-groups comparison by one-way ANOVA showed significant variation of Hcy levels in various groups (Table 2; $p<0.001$).

Table 1. Variables and characteristic features among groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus											
	NW-ND vs. NW-DM			OW-ND vs. OW-DM			NW-ND vs. OW-ND			NW-DM vs. OW-DM		
	NW-ND	NW-DM	P-value	OW-ND	OW-DM	P-value	NW-ND	OW-ND	P-value	NW-DM	OW-DM	P-value
Number of subjects (n)	101	101	-	98	96	-	101	98	-	101	96	-
Sex (female)	101	101	-	98	96	-	101	98	-	101	96	-
Age (years)	55.34 ± 2.85	55.34 ± 2.74	1.00	55.34 ± 2.77	55.33 ± 2.78	0.98	55.34 ± 2.85	55.34 ± 2.77	1.00	55.34 ± 2.85	55.34 ±2.77	1.00
Age range (years)	51-60	51-60	-	51-60	51-60	-	51-60	51-60	-	51-60	51-60	-
BMI (kg/m²)	21.56±2.05	21.58±2.02	0.95	27.52±1.50	27.47±1.50	0.81	21.56±2.05	27.52±1.50	<0.001	21.58±2.02	27.47±1.50	<0.001
BMI range (kg/m²)	18.5-24.9	18.5-24.9	-	25.0-29.9	25.0-29.9	-	18.5-24.9	25.0-29.9	-	18.5-24.9	25.0-29.9	-
25(OH)D (ng/mL)	33.83±5.74	31.85±7.06	0.03	30.12±6.62	29.02±6.78	0.26	33.83±5.74	30.12±6.62	<0.001	31.85±7.06	29.02±6.78	0.004
Hcy (µmol/L)	5.42± 1.50	5.44± 1.53	0.91	5.90± 2.66	6.95±3.66	0.02	5.42± 1.50	5.90± 2.66	0.12	5.44± 1.53	6.95± 3.66	<0.001
HB (g/dL)	13.72± 1.26	13.69± 1.41	0.90	13.65± 1.47	13.62± 1.75	0.90	13.72± 1.26	13.65± 1.47	0.75	13.69± 1.41	13.62±1.75	0.77
IL-6 (pg/ml)	4.74±4.13	4.78± 4.12	0.95	5.42± 4.23	6.76± 5.10	0.05	4.74± 4.13	5.42± 4.23	0.26	4.78± 4.12	6.76± 5.10	0.003
Hp (ng/mL)	8.58± 3.95	8.37± 4.02	0.99	8.44± 4.33	8.62± 4.57	0.77	8.58± 3.95	8.44± 4.33	0.80	8.37± 4.02	8.62± 4.57	0.94

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NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; values are: mean± standard deviation (SD); two tailed P-value was obtained by using unpaired two samples t–test.

The HB concentration (Table 1) indicated non-significant changes for all comparisons for NW and OW women with DM and ND groups (p>0.05). Among-groups comparison by one-way ANOVA for HB levels showed non-significant change in various groups (Table 2; p>0.05). The Hp levels indicated non-significant variations for all comparisons for NW and OW women with DM and ND groups ((Table 1); p>0.05). Among-groups comparison by one-way ANOVA for Hp levels showed non-significant changes in various groups (Table 2; p>0.05).

Serum IL-6 presented significant difference for OW-ND vs. OW-DM (p;0.05) and NW-DM vs. OW-DM (p:0.003). The NW-ND vs. NW-DM (p:0.95) and NW-ND vs. OW-ND (p:0.26) were obtained as non-significantly different (Table 1). Among-groups comparison by one-way ANOVA showed significant variation of Hcy levels in various groups (Table 2; p:0.004).

Table 2. Analysis of the characteristic features and variables among normal weight and overweight groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus				Significance (P-value)
	NW-ND	NW-DM	OW-ND	OW-DM	
Number of subjects (n)	101	101	98	96	-
Sex (female)	101	101	98	96	-
Age	55.34 ± 2.85	55.34 ± 2.74	55.34 ± 2.77	55.33 ± 2.78	0.99
BMI (kg/m2)	21.56±2.05	21.58±2.02	27.52±1.50	27.47±1.50	<0.001
BMI range (kg/m2)	18.5-24.9	18.5-24.9	25.0-29.9	25.0-29.9	-
25(OH)D (ng/mL)	33.83±5.74	31.85±7.06	30.11±6.62	29.02±6.78	<0.001
Hcy (µmol/L)	5.42± 1.50	5.44± 1.53	5.90± 2.66	6.95±3.66	<0.001
HB (g/dL)	13.72± 1.26	13.69± 1.41	13.65± 1.47	13.62± 1.75	0.974
IL-6 ((pg/ml)	4.74± 4.13	4.78± 4.12	5.42± 4.23	6.76± 5.10	0.004
Hp (ng/mL)	8.58± 3.95	8.37± 4.02	8.44± 4.33	8.62± 4.57	0.99

NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; values are: mean± standard deviation (SD); P-values for one-way ANOVA (analysis of variance); statistical analysis was done by applying the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows.

The plot of the BMI with serum vitD levels (Table 3) in NW and OW groups in PMP women with and without T2DM presented significant negative linear correlation for OW-ND (R^2:0.40; p<0.001) and OW-DM (R^2:0.41; p<0.001). The remaining groups (NW-ND & NW-DM) did not show significant correlations (p>0.05).

Plotting the BMI with serum Hcy levels (Table 3) in NW and OW groups in PMP women with and without type-2 diabetes mellitus presented a significant positive linear correlation for OW-ND (R^2:0.06; p:0.01) and OW-DM (R^2:0.30; p<0.001). The remaining groups (NW-ND & NW-DM) did not show significant correlations (p>0.05).

A plot was drawn for BMI against HB concentration (Table 3). However, no subject group presented a significant correlation (p>0.05). Plot was drawn for BMI against Hp levels (Table 3). However, no subject group presented a significant correlation (p>0.05). The graph of BMI against serum IL-6 levels (Table 3) in NW and OW groups in PMP women with and without type-2 diabetes mellitus presented a significant positive linear correlation for OW-ND (R^2:0.04; p:0.05) and OW-DM (R^2:0.24; p<0.001). Remaining groups (NW-ND & NW-DM) did not present significant correlations (p>0.05).

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Table 3. Association of BMI with other variables in normal weight and overweight groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Association of BMI with other variables in normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus				
	R^2 & P	Normal weight		Overweight	
		NW-ND	NW-DM	OW-ND	OW-DM
25(OH)D (ng/mL)	R^2	0.02	0.02	0.40	0.41
	P	0.16	0.19	<0.001	<0.001
Hcy (µmol/L)	R^2	0.01	0.01	0.06	0.30
	P	0.26	0.24	0.01	<0.001
HB (g/dL)	R^2	0.01	0.00	0.00	0.00
	P	0.31	0.85	0.96	0.58
IL-6 (pg/ml)	R^2	0.00	0.00	0.04	0.24
	P	0.57	0.53	0.05	<0.001
Hp (ng/mL)	R^2	0.01	0.01	0.01	0.02
	P	0.36	0.42	0.40	0.17

NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; regression lines were plotted for obtaining the values of R^2 (coefficient of determination) and the values of significance (P); statistical analysis was done by applying the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows.

Plotting vitD against serum BMI levels (Table 4) gave significant negative linear correlation for OW-ND (R^2:0.40; p<0.001) and OW-DM (R^2:0.41; p<0.001). Other groups (NW-ND & NW-DM) did not show significant associations (p>0.05).

Plotting the vitD with Hcy levels (Table 4) showed significant positive linear association for OW-DM (R^2:0.11; p:0.001). The remaining groups (NW-ND, NW-DM & OW-NM) presented non-significant correlations (p>0.05).

The vitD plotted against HB (Table 4) did not give significant correlation in all groups (p>0.05). The plot drawn for vitD against Hp levels (Table 4) presented non-significant correlation for all groups (p>0.05). The vitD plot with IL-6 levels (Table 4) presented significant negative linear association for NW-ND (R^2:0.04; p:0.05), NW-DM (R^2:0.17; p<0.001) and OW-DM (R^2:0.22; p<0.001). The OW-ND did not show significant correlation (p>0.05).

Table 4. Association of vitD with other variables in normal weight and overweight groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Association of vitD with other variables in normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus				
	R^2 & P	Normal weight		Overweight	
		NW-ND	NW-DM	OW-ND	OW-DM
BMI (kg/m^2)	R^2	0.02	0.02	0.4	0.41
	P	0.16	0.19	<0.001	<0.001
Hcy (µmol/L)	R^2	0.01	0.04	0.00	0.11
	P	0.33	0.06	0.59	0.001
HB (g/dL)	R^2	0.01	0.00	0.01	0.01
	P	0.40	0.92	0.27	0.47
IL-6 (pg/ml)	R^2	0.04	0.17	0.00	0.22
	P	0.05	<0.001	0.89	<0.001
Hp (ng/mL)	R^2	0.00	0.01	0.01	0.01
	P	0.67	0.48	0.45	0.49

NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; regression lines were plotted for obtaining the values of R^2 (coefficient of determination) and the values of significance (P); statistical analysis was done by applying the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows.

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Plot of Hcy against BMI levels (Table 5) provided significant positive linear correlation for OW-ND (R^2:0.06; p:0.01) and OW-DM (R^2:0.30; p<0.001). Other groups (NW-ND & NW-DM) did not show significant correlations (p>0.05).

Plotting Hcy against vitD levels (Table 5) presented significant inverse linear correlation for OW-DM (R^2:0.06; p:0.001). The remaining groups (NW-ND, NW-DM & OW-ND) presented non-significant correlations (p>0.05).

Hcy plotted against HB (Table 5) gave non-significant correlation in all groups (p>0.05). The Hcy against Hp (Table 5) presented significant positive linear correlation for OW-DM (R^2:0.10; p:0.002). All remaining groups (NW-ND, NW-DM and OW-ND) showed non-significant correlation (p>0.05). Hcy plot with IL-6 levels (Table 5) showed significant positive linear correlation for NW-DM (R^2:0.06; p:0.02), OW-ND (R^2:0.06; p:0.02) and OW-DM (R^2:0.18; p<0.001). The NW-ND did not present significant association (p>0.05).

Table 5. Association of homocysteine with other variables in normal weight and overweight groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Association of homocysteine with other variables in normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus				
	R^2 & P	Normal weight		Overweight	
		NW-ND	NW-DM	OW-ND	OW-DM
BMI (kg/m^2)	R^2	0.01	0.01	0.06	0.30
	P	0.26	1024	0.01	<0.001
25(OH)D (ng/mL)	R^2	0.01	0.04	0.00	0.11
	P	0.33	0.06	0.59	0.001
HB (g/dL)	R^2	0.00	0.04	0.03	0.01
	P	0.53	0.06	0.09	0.40
IL-6 (pg/ml)	R^2	0.01	0.06	0.06	0.18
	P	0.46	0.02	0.02	<0.001
Hp (ng/mL)	R^2	0.00	0.00	0.01	0.10
	P	0.76	0.77	0.44	0.002

NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; regression lines were plotted for obtaining the values of R^2 (coefficient of determination) and the values of significance (P); statistical analysis was done by applying the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows.

Serum IL-6 plotted against BMI (Table 6) showed significant positive linear correlation for OW-ND (R^2:0.04; p:0.05) and OW-DM (R^2:0.24; p<0.001). Other groups (NW-ND & NW-DM) did not present significant correlations (p>0.05).

IL-6 against vitD levels (Table 6) presented significant inverse linear correlation for NW-ND (R^2:0.04; p:0.05), NW-DM (R^2:0.17; p<0.001) and OW-DM (R^2:0.22; p<0.001). The OW-ND presented non-significant correlation (p>0.05).

IL-6 plotted against HB (Table 6) gave non-significant correlation in all groups (p>0.05). The IL-6 against Hp (Table 6) showed non-significant correlation for all groups (p>0.05). IL-6 plot with Hcy levels (Table 6) showed significant positive linear correlation for NW-DM (R^2:0.06; p:0.02), OW-ND (R^2:0.06; p:0.02) and OW-DM (R^2:0.18; p<0.001). The NW-ND did not present significant association (p>0.05).

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Table 6. Association of IL-6 with other variables in normal weight and overweight groups in postmenopausal women with and without type-2 diabetes mellitus.

Variables	Association of IL-6 with other variables in normal weight and overweight postmenopausal women with and without type-2 diabetes mellitus				
	R^2 & P	Normal weight		Overweight	
		NW-ND	NW-DM	OW-ND	OW-DM
BMI (kg/m²)	R^2	0.00	0.00	0.04	0.24
	P	0.57	0.53	0.05	<0.001
25(OH)D (ng/mL)	R^2	0.04	0.17	0.00	0.22
	P	0.05	<0.001	0.89	<0.001
Hcy (µmol/L)	R^2	0.01	0.06	0.06	0.18
	P	0.46	0.02	0.02	<0.001
HB (g/dL)	R^2	0.01	0.01	0.04	0.03
	P	0.32	0.48	0.06	0.09
Hp (ng/mL)	R^2	0.00	0.00	0.00	0.00
	P	0.98	0.82	0.65	0.70

NW-ND: Normal weight nondiabetic, NW-DM: normal weight diabetes mellitus, OW-ND: overweight nondiabetic, OW-DM: overweight diabetes mellitus, BMI: body mass index; 25(OH)D: vitamin D; Hcy: homocysteine, HB: haemoglobin; Hp: hepcidin; IL-6: interleukin-6; regression lines were plotted for obtaining the values of R^2 (coefficient of determination) and the values of significance (P); statistical analysis was done by applying the Statistical Package for Social Sciences (SPSS), version 24.0 for Windows.

4. Discussion

In the present study, the age of various groups did not differ significantly. The BMI of NW and OW subjects in their PMP were studied for various variables in ND and T2DM. The findings in the present report showed that T2DM may develop in OW subjects and NW subjects as well. These observations resemble to those obtained in a previous report [45]. There are other studies that document their findings that the lower BMI cut-off values in women subjects presented association with diabetes [46–48]. The effect of BMI in OW or obese subjects has significant involvement in T2DM [49,50]. It was proposed that the T2DM often associated with high BMI levels [51] and develops more often in subjects with high BMI/ obesity status [49]]. The present report is restricted for BMI not exceeding 24.9 kg/m² and 29.9 kg/m² for NW and OW women subjects respectively.

Our results show that the serum vitD decreased significantly for NW-ND vs. OW-ND, NW-DM vs. OW-DM, and NW-ND vs. NW-DM in PMP women with T2DM. Among-groups comparison showed significant variation of vitD levels in various groups. Various reports provide evidence for our results [52–54]. Prevalence of hypovitaminosis D is quite high in PMP women with T2DM but not supplemented with vitD than in non-diabetic controls, and the body weight status specially obesity is a major risk factor [55]. The T2DM had a high prevalence of VitD insufficiency and deficiency [56]. The PMP women revealed hypovitaminosis D, besides a high BMI showing heightened risk of having T2DM [57]). VitD deficiency and even hypovitaminosis D was found prevalent in PMP women [58]. Significantly decreased levels of vitD in PMP women with T2DM were obtained [53].

However, there are studies where the results obtained differed from the present study to some extent [59] or the results obtained were quite different [60,61]. Women with vitamin D deficiency but without DM associated with obesity and BMI correlated negatively with vitD levels [59]. The T2DM women gave low levels of vitD compared to that in control group and hypovitaminosis D associated with obesity but not with the risk of having T2DM [60]. No difference of the levels of vitamin D was also investigated in PMP groups with and without DM [61].The plot of the BMI with serum vitD levels in NW and OW groups in PMP women with and without T2DM in the present report presented significant negative linear correlation for OW-ND and OW-DM. BMI showed significant inverse function to vitD levels in PMP women [52]. The BMI levels associated with vitD levels in PMP women with T2DM [54]. Women with vitamin D deficiency but without DM associated with obesity and BMI correlated negatively with vitD levels [59]. Furthermore, the T2DM women gave low levels of vitD

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compared to that in control group and hypovitaminosis D associated with obesity but not with the risk of having T2DM [60].

We noted that serum levels of Hcy varied significantly for OW-ND vs. OW-DM and NW-DM vs. OW-DM women in PMP. Among-groups comparison showed significant variation of Hcy levels in various groups. Higher Hcy levels occurring in the subjects with higher BMI compared to those with lower BMI are like other related studies [20,22]. However, further studies are needed to be carried out for better explanation. Our data varies from various other studies as the BMI of the population in the present study differed from various other reports and the criteria for clearly defining the NW and OW women varied from the data of several other populations [17,19,47,62,63]. It was convincing to note that the non-obese subjects pertained lower levels of Hcy and other related variables than those in the obese patients [50].

The present report presents significantly elevated Hcy in OW women with T2DM in PMP that varied from another study [25] primarily due to differences in the wide difference of age groups of subjects, different methods and procedures employed, studies in different populations, and BMI for NW and OW subjects categorized in different ways. Our results are further supported by other reports [4,9,21,64,65].

We found that PMP women with and without type-2 diabetes mellitus showed a significant positive linear correlation of Hcy with BMI for OW-ND and OW-DM. The investigation of significant positive association of Hcy with BMI in OW women subjects with wider age range of 18 to 75 years [21] and age range of 40 to 60 age (mean age of 51.45 years) [27], association for elevated Hcy levels and the development/ occurrence of T2DM [9,65] elevated Hcy levels in T2DM than nondiabetic women subjects and elevated levels of Hcy in older women subjects [64] are quite like our present results.

Plotting Hcy against vitD levels in the current study gave significant inverse linear correlation for OW-DM. Hcy plot with IL-6 levels in the present work showed significant positive linear correlation for NW-DM, OW-ND and OW-DM. The NW-ND did not present significant association. It was found in the present report that the serum levels of IL-6 presented significant difference for OW-ND vs. OW-DM and NW-DM vs. OW-DM, and among-groups comparison showed significant variation of IL-6 levels in various groups. We further found that the NW and OW groups in PMP women with and without type-2 diabetes mellitus presented a significant positive linear correlation in IL-6 against BMI for OW-ND and OW-DM. The Hcy levels were found higher in PMP women with T2DM [66]. A previous investigation verifies the increased levels of serum IL-6, Hcy and various other pro-inflammatory markers in youth [4]. Indirect evidence provided the role of mentioned markers in diabetes. Effect of the administration of pomegranate peel extract (PoPEx) revealed a decrease in IL-6 and Hcy levels in T2DM subjects. The beneficial effects of exercise and saffron in T2DM women patients [67] and the beneficial effect of negative pressure suction with platelet rich gel in subjects with diabetic foot ulcer indicated reduction in IL-6 and Hcy levels [31]. These investigations [4,31,67] verify the potential value of these anti-inflammatory biomarkers in diabetic subjects.

Our data for plotting the IL-6 against vitD levels presented significant inverse linear correlation for NW-ND, NW-DM and OW-DM. The HB and Hp concentrations in the present study indicated non-significant changes for all comparisons for NW, OW, ND and DM groups in T2DM women in PMP. Among-groups comparison for HB and Hp levels showed non-significant changes in various groups. The plot drawn for BMI against HB as well as against Hp presented non-significant correlations. The Hcy plotted against Hp presented significant positive linear correlation for OW-DM, and IL-6 plotted against Hp showed significant positive linear correlation for NW-DM, OW-ND and OW-DM. Our results for serum HB are like another report [13]. The nonsignificant difference for Hp in OW compared to NW individuals [12] verifies our results for OW-ND and NW-ND subjects. Another study [68] suggested the age-based as well as gender-based positive correlation of Hp with obesity related BMI levels, but not with non-obesity related BMI levels. This investigation [68] confirms our results obtained for BMI-based Hp in T2DM women in PMP.

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The association of vitD with Hcy levels in the present study showed a significant negative linear correlation for OW-DM. Our study is verified by various reports [33,69–72]. It was suggested that vitD influences the Hcy association as a risk factor involved in developing T2DM [72]. For finding association between diabetes control and various variables including serum homocysteine and vitamin D levels in T2DM patients, it was found that it is mainly the vitD that decreases significantly and influences a variety of other variables including Hcy [71]. VitD causing decrease in Hcy levels was found beneficial for the proinflammatory effects of Hcy in patients with T2DM [70]. Reducing the Hcy and oxidative stress was found helpful for improving the dysfunctions in T2DM and vitD status. The supplementation of vitD to T2DM patients reduced significantly the Hcy levels [69]. The patients with uncontrolled T2DM revealed decrease in the Hcy Levels under the effect of airborne low intensity multi-frequency ultrasound (ALIMFUS) as a therapy [33]. However, no significant change in Hcy levels was found in a report [73]. The T2DM patients with vitD supplementation did not present a significant decrease in Hcy levels [73]. But that was an indirect and experimental approach. Hence that might have been related to their own circumstances for the supplementation of vitD.

Negative linear association of vitD with IL-6 levels was noticed in the present study in NW-ND, NW-DM and OW-DM. Our these results relate to several previous reports [74–78]and other studies for the effect of supplementation of vitD on inflammatory status via IL-6 [30,78]. Reduced levels of vitD associated negatively with IL-6 in patients with T2DM (Murugiah et al., 2024). The IL-6 was significantly higher in T2DM patients with Vit D insufficiency and deficiency [76].

Interaction between VitD and IL-6 as pro-inflammatory cytokines were suggested in T2DM patients [75]. The vitD improved the anti-inflammatory activity and antioxidant effects by significantly reducing T2DM-induced increase in IL-6 [78]. VitD status is inversely associated to T2DM and in view of attenuation of IL-6 levels, it was suggested that the ‘vitD level-T2DM’ association may be mediated partially by subclinical level of inflammation [74]. Association of vitD deficiency with proinflammatory markers in T2DM patients showed the significant impact of vitD in preventing inflammation in T2DM patients [1]. The T2DM with severe deficiency and sufficiency revealed significant difference for IL-6, whereas in moderate deficiency T2DM patients did not show significant variations for IL-6. However, a negative correlation of vitD with IL-6 [79]is quite potential investigation.

VitD is considered as having anti-inflammatory properties. It was verified that vitD supplementation reduced chronic low-grade inflammation in T2DM patients [6]. However, no significant variation in interleukin 6 levels could be obtained, and influence of age, sex, or BMI was not found [6]. T2DM induction was reversed by vitD supplementation combined with other approaches in Wister rats [78]. Effect of vitD supplementation for six months in T2DM patients with vitD deficiency showed improvement in IL-6 and attenuation of inflammation, and when the serum vitD reached to normal level, it corresponded with significant decrease in serum IL-6 [30].

The results obtained for the alterations noted for serum vitD, proinflammatory biomarkers (mainly Hcy and IL-6) and other variables in the present investigation in PMP women (NW and OW) with T2DM, and inter-associations among these factors are quite convincing to suggest for the establishment of a novel therapeutic approach based on vitD and proinflammatory markers for T2DM patients of high BMI levels.

5. Limitations

Present work was carried out in postmenopausal women with and without T2DM. It seems necessary to carry out studies in premenopausal and childbearing age, and in young, middle-aged and elderly men. Age, gender and BMI of wider range including that for obese subjects as well may provide better idea of the impact of levels and correlations among serum vitD, Hcy, IL-6 and related variables in PMP women with T2DM. It is expected that existing and future studies will be carried out for understanding the comparative impact of a wide range of age groups and BMI groups in men and women. Another limitation in the present study was that owing to the limited available facilities,

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influence of lipid profile, other important biomarkers particularly the anti-inflammatory cytokines/ markers and various other variables could not be investigated in PMP women with T2DM. T2DM accompanies a variety of other disorders/ complications that could not be studied in the present study.

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Informed Consent Statement: Informed consent was obtained from subjects involved in the study.

Data Availability Statement: Data are contained within the article.

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