

Urinary Neopterin and Biopterin in Community-Dwelling Older Adults: A Cross-Sectional Study

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Abstract

Objective: Immune activation and oxidative stress are key biological features of aging, and urinary pteridines such as neopterin and biopterin may provide non-invasive insight into these processes. In this cross-sectional study, we characterized urinary neopterin and biopterin levels in community-dwelling older adults and examined their associations with cardiometabolic parameters and dietary patterns.

Materials and Methods: We recruited 50 adults aged ≥ 65 years. Urinary neopterin and biopterin were measured using high-performance liquid chromatography, normalized to creatinine, and evaluated in relation to blood pressure and glucose. Dietary intake over the previous 7 days was assessed using a structured recall and categorized into four dietary patterns: traditional Mediterranean, vegetarian, protein-focused, and light eaters.

Results: Mean urinary concentrations were 264.9 ± 110.4 $\mu\text{mol/mol}$ creatinine for neopterin and 487.4 ± 407.7 $\mu\text{mol/mol}$ creatinine for biopterin, indicating inter-individual variability. A weak but statistically significant correlation was observed between the two biomarkers ($r = 0.289$, $p = 0.041$). Neither biomarker was significantly associated with age, blood pressure, or glucose levels. No statistically significant differences were observed across dietary pattern groups; however, these findings should be interpreted cautiously given the qualitative nature of the dietary assessment and the relatively small subgroup sizes.

Conclusion: Overall, urinary neopterin and biopterin levels in older adults were within the general adult reference range but may nonetheless reflect underlying immune activation and oxidative stress associated with aging. Given the exploratory design of the study, further investigations in larger and more diverse populations are warranted to clarify the clinical and epidemiological relevance of urinary pteridines.

Keywords: Neopterin, pteridines, biopterin, elderly, diet

Introduction

Numerous physiological alterations are associated with aging, many of which affect immunological and metabolic processes, contributing to chronic low-grade inflammation (“inflammaging”) and increased vulnerability to age-related noncommunicable diseases (1-3). Biomarkers help detect these modifications and provide insight into how the body responds to disease and stress, supporting earlier risk stratification and monitoring in older populations (3). Early identification of key biomarkers can aid in the prognosis and management of chronic illnesses, including diabetes, neurological disorders, and cardiovascular diseases in

older adults (1,3). In this context, neopterin and biopterin are two clinically relevant pteridines linked to immune activation, redox balance, and pathways implicated in age-related morbidity (4,5).

Neopterin, a pteridine derivative, is a biochemical marker of cell-mediated immune activation. It is produced predominantly by activated monocytes/macrophages following stimulation by interferon- γ (IFN- γ), and neopterin concentrations rise across a wide range of inflammatory and infectious conditions, reflecting immune activation (6,7). Neopterin is well-suited for clinical and research monitoring because it is chemically stable, present in body fluids, and can be quantified conveniently in urine (4,8,9).

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In addition, neopterin levels have been shown to increase with advancing age, consistent with heightened basal immune activation in aging (10,11). Biopterin—commonly discussed in the context of tetrahydrobiopterin (BH4) metabolism—is central to pathways regulating nitric oxide (NO) production and the synthesis of monoamine neurotransmitters. BH4 acts as an essential cofactor for aromatic amino acid hydroxylases involved in dopamine and serotonin biosynthesis and for NO synthases, thereby linking biopterin/BH4 status to vascular function, oxidative stress balance, and neurobiological processes (5,12). Perturbations in the biopterin/BH4 redox system can therefore reflect or contribute to oxidative stress and dysregulated signaling pathways implicated in aging-related disorders, including conditions with neurodegenerative and cardiometabolic components (12-14).

Despite their biological relevance, studies simultaneously evaluating urinary neopterin and biopterin in very old, community-dwelling populations remain limited, and their combined behavior in relation to simple clinical and lifestyle parameters is not well characterized.

Monitoring pteridine metabolites such as neopterin and biopterin may provide insight into immune activation, oxidative balance, and related metabolic processes in aging. However, their interpretation in older populations is complex, as these biomarkers are influenced by multiple physiological systems and may not correspond directly to isolated clinical measurements.

Therefore, the present cross-sectional study aimed to characterize urinary neopterin and biopterin levels in community-dwelling older adults and to explore their relationships with simple cardiometabolic indicators and broad dietary pattern categories. In contrast to many previous studies that focused on single biomarkers or specific disease groups, this study simultaneously evaluates both pteridines in a relatively underexplored population of very old, community-dwelling individuals. Given the limited availability of data in this context, the analyses were designed to be exploratory, aiming to provide baseline insights into urinary pteridine variability and its potential clinical relevance in aging populations.

Materials and Methods

Study Population and Ethical Considerations

A sensitivity power analysis was performed using the final sample size of 50 participants. For a two-tailed Pearson correlation analysis with $\alpha = 0.05$ and 80% power, this sample size was sufficient to detect an effect size of approximately $r = 0.39$. Therefore, the study was powered only to detect moderate-to-large bivariate correlations. This estimate does not apply to the dietary pattern subgroup comparisons because the subgroup sizes were small and unequal. Accordingly, the

subgroup analyses were considered exploratory and may have been underpowered to detect modest differences. This cross-sectional study was conducted in collaboration with Famagusta Municipality. It included older adults who resided in their homes or in care facilities regularly serviced by the municipality. Fifty participants were included in this study. Ethical approval was obtained from the Eastern Mediterranean University Ethics Committee (approval no: 2019/04-05, date: 04.02.2019). Consent was obtained from all the participants or their legal guardians.

Data and Sample Collection

In collaboration with the municipality, researchers visited participants' homes to collect urine samples for measuring neopterin and biopterin concentrations. All demographic information, including age, blood pressure, and blood glucose level, was recorded. Blood pressure was measured three times using an Omron M3 Comfort HEM-7155-E Intellisense (Omron Healthcare, Kyoto, Japan) while the participant was seated and at rest. Blood glucose levels were measured by finger prick using a Contour Plus (Ascensia Diabetes Care). Dietary information from the previous week was collected using questionnaires. All urine samples were immediately refrigerated at 4 °C and transported to the laboratory for analysis. Information on renal function and comorbidities was not systematically collected. Data on medication use were partially available but not sufficiently complete or standardized for inclusion in the analysis.

Dietary Assessment and Classification of Nutritional Patterns

Dietary intake over the previous 7 days was assessed during home visits using structured, interviewer-administered forms developed for use in the study and in the field among this older population. Each day, participants (or caregivers, when applicable) reported the types and approximate frequencies of main meals and snacks, including bread and cereals, fruits and vegetables, legumes, dairy products, meat and processed meat, fish, eggs, sweets, and beverages. Based on reported weekly intake of these food groups, participants were assigned to one of four predominant dietary patterns:

Traditional Mediterranean diet: Frequent consumption of vegetables, fruits, legumes, olive oil, and whole grains, with regular but moderate intake of fish and limited red or processed meat.

Vegetarian: Avoidance of meat and fish, with the main protein sources being legumes, eggs, and dairy products.

Protein-focused: High intake of meat and/or fish and other rich protein foods, with comparatively lower consumption of fruits, vegetables, and whole grains.

Light eaters: Generally low reported intake across most food groups, characterized by small portion sizes and/or skipping main meals.

The investigators classified the data based on the overall pattern of reported intake over seven days. Because of the modest sample size and the qualitative nature of the dietary assessment, these groups were primarily used for exploratory comparisons of urinary neopterin and biopterin levels. The dietary assessment tool was not intended as a formally validated quantitative dietary instrument. Therefore, the resulting dietary pattern classification should be interpreted as qualitative and exploratory, and recall bias cannot be excluded, particularly in this older population.

Determination of Neopterin and Biopterin Levels

(HPLC; Agilent 1100) was used to quantify neopterin, biopterin, and creatinine in urine specimens. Reversed-phase chromatography was conducted at a flow rate of 1 mL per minute, using a mobile phase comprising a 15 mM phosphate buffer (pH 7) and 2.5% (v/v) methanol. A fluorescence detector with excitation at 353 nm and emission at 438 nm was used to quantify the concentrations of neopterin and biopterin. A UV detector (HP Agilent 1100) was used to quantify urinary creatinine at 235 nm. Calibration standards for neopterin and biopterin were prepared in aqueous solution and injected into each analytical run along with the urine samples. The concentrations were calculated from external calibration curves using peak areas. The chromatographic conditions were adapted from previously validated methods that demonstrated adequate linearity, precision, accuracy, and sensitivity for quantifying urinary neopterin and biopterin in our laboratory. The analytical performance of the method, including limits of detection (LOD) and quantification (LOQ), has been described in detail in these prior validated studies (15). The LOD and LOQ were determined from ten calibration-curve replicates using the standard deviation (SD) method. The LOD/LOQ values were 0.00699/0.02308 $\mu\text{mol/mL}$ for neopterin and 0.01728/0.05704 $\mu\text{mol/mL}$ for biopterin, respectively.

Statistical Analysis

Descriptive statistics were calculated for all continuous variables and are presented as the mean $\pm$ SD. Differences in urinary neopterin and biopterin concentrations among the four dietary pattern groups were analyzed using the Kruskal-Wallis test, given the modest group sizes and variability in pteridine concentrations. Accordingly, non-parametric tests were preferred for group comparisons. Sex differences in urinary neopterin levels (women vs. men) were evaluated using an independent samples t-test. Linear associations of urinary neopterin and biopterin with clinical variables (systolic and diastolic blood pressure, blood glucose, and age) were examined using Pearson correlation coefficients. All tests were two-tailed, and p-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). Given the relatively small sample size and the number of comparisons performed, all analyses were considered exploratory and hypothesis-generating. No

formal correction for multiple testing was applied; therefore, the results should be interpreted with caution.

Results

Fifty elderly volunteers (25 women and 25 men) were enrolled in this study. The demographic and clinical characteristics of the participants are shown in Table 1. The mean age was 80.3 ± 7.3 years (range, 67-93 years). The mean systolic and diastolic blood pressures were 123 ± 21 and 71 ± 11 mmHg, respectively, and the mean blood glucose level was 106.6 ± 21.5 mg/dL.

The mean urinary neopterin concentration in the entire group was 264.9 ± 110.4 $\mu\text{mol/mol}$ creatinine. The mean urinary biopterin concentration was 487.4 ± 407.7 $\mu\text{mol/mol}$ creatinine. The values are listed in Table 1. The boxplot in Figure 1 illustrates the distribution of neopterin concentrations, showing several low and high outliers that indicate inter-individual variability.

The correlation matrix is presented in Table 2. Urinary neopterin and biopterin showed a weak but statistically significant

Table 1. Demographic and clinical characteristics of the study population (n = 50).	
Variable	Mean $\pm$ SD
Age (years)	80.3 $\pm$ 7.3
Systolic blood pressure (mmHg)	123 $\pm$ 21
Diastolic blood pressure (mmHg)	71 $\pm$ 11
Blood glucose (mg/dL)	106.6 $\pm$ 21.5
Neopterin ($\mu\text{mol/mol}$ creatinine)	264.9 $\pm$ 110.4
Biopterin ($\mu\text{mol/mol}$ creatinine)	487.4 $\pm$ 407.7
SD: Standard deviation.	

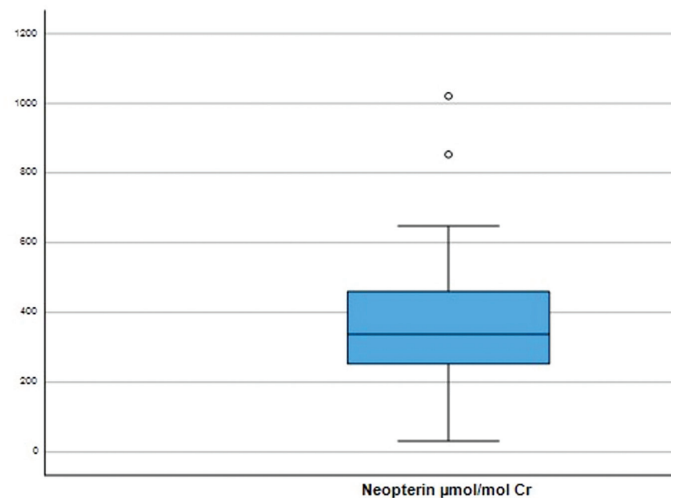


Figure 1. Box-and-whisker plot of urinary neopterin concentrations ($\mu\text{mol/mol}$ Cr) in the study population (n = 50). The box represents the interquartile range; the horizontal line is the median; the whiskers show the minimum and maximum values within $1.5 \times$ IQR, and the circles indicate outliers. IQR: Interquartile range.

correlation ($r = 0.289$, $p = 0.041$). Systolic and diastolic blood pressures were moderately positively correlated ($r = 0.470$, $p < 0.001$). In addition, systolic blood pressure was moderately correlated with blood glucose levels ($r = 0.452$, $p < 0.001$). No significant correlations were observed between either neopterin or biopterin and blood pressure, glucose, or age (all $p > 0.05$). Age was not significantly associated with blood glucose or blood pressure.

Mean urinary neopterin levels were similar in women and men (259.6 ± 108.5 vs. 270.2 ± 114.3 $\mu\text{mol/mol}$ creatinine). The independent samples t-test showed no significant sex difference in urinary neopterin levels [$t(48) = -0.334$, $p = 0.740$]. Biopterin concentrations did not differ significantly between women and men ($p > 0.05$; data not shown).

Participants were classified into four dietary pattern groups based on their 7-day dietary records: traditional Mediterranean ($n = 14$), vegetarian ($n = 10$), protein-focused ($n = 18$), and light-eaters ($n = 8$). The mean neopterin and biopterin concentrations in each group are shown in Table 3. Kruskal-Wallis tests showed no statistically significant differences in neopterin [$H(3) = 1.704$, $p = 0.636$] or biopterin [$H(3) = 2.897$, $p = 0.408$] levels among the dietary patterns. These findings should be interpreted cautiously, given the qualitative nature of the dietary assessment and the relatively small subgroup sizes.

Discussion

In our cohort of community-dwelling elderly individuals, mean urinary neopterin and biopterin concentrations were 264.9 ± 110.4 and 487.4 ± 407.7 $\mu\text{mol/mol}$ creatinine, respectively.

Compared with classical age-stratified reference values for urinary neopterin (Table 4), the mean concentration in our cohort was slightly above the upper limit reported for individuals older than 75 years [$70\text{--}230$ $\mu\text{mol/mol}$ creatinine; (16,17)], suggesting that at least some participants exhibited increased cell-mediated immune activation. In contrast, after converting the adult reference intervals reported by Nardecchia et al. (18) to the same unit, our mean neopterin and biopterin values fell within the reported ranges for healthy subjects aged 18–74 years: $220\text{--}920$ $\mu\text{mol/mol}$ creatinine for neopterin and $240\text{--}820$ $\mu\text{mol/mol}$ creatinine for biopterin. This indicates that overall pteridine excretion remained broadly within the normal adult range despite advanced age.

Our findings can also be placed alongside studies that specifically examine urinary neopterin and biopterin levels among older adults and individuals with neurodegenerative diseases. Blair et al. (19) reported significantly higher urinary neopterin and biopterin levels in patients with Alzheimer's disease and Down's syndrome than in standard elderly controls, with particularly marked elevations in Alzheimer's disease. When expressed in the same units, neopterin in our sample appears to be intermediate between the low values observed in healthy elderly controls and the higher concentrations reported in patients with Alzheimer's disease. In contrast, the mean biopterin level was closer to the upper end of the adult reference range and exceeded the values reported in some Alzheimer's disease cohorts (19). These comparisons suggest that, in very old, predominantly community-dwelling individuals, urinary pteridines may reflect a background of "inflammaging" and oxidative stress that is more

Table 2. Pearson correlation coefficients (r) between urinary biomarkers and clinical variables (n = 50).

	Neopterin	Biopterin	Systolic BP	Diastolic BP	Glucose	Age
Neopterin	1.000	0.289*	-0.109	0.125	0.000	-0.275
Biopterin		1.000	-0.245	0.124	-0.067	-0.143
Systolic BP			1.000	0.470**	0.452**	0.253
Diastolic BP				1.000	0.233	-0.084
Glucose					1.000	0.123
Age						1.000

* $p < 0.05$; ** $p < 0.01$. Only the upper triangle of the table is shown.
BP: Blood pressure.

Table 3. Urinary neopterin and biopterin concentrations across dietary pattern groups (n = 50).

Dietary pattern group	n	Neopterin	Biopterin
Traditional mediterranean	14	264.07 ± 136.82	655.07 ± 686.31
Vegetarian	10	309.50 ± 114.62	489.70 ± 215.30
Protein-focused	18	247.72 ± 99.26	419.83 ± 212.64
Light eaters	8	249.25 ± 76.56	343.25 ± 166.66
Total	50	264.90 ± 110.41	487.42 ± 407.72

Values are presented as mean $\pm$ standard deviation and expressed as $\mu\text{mol/mol}$ creatinine. The Kruskal-Wallis test for neopterin yielded $H(3) = 1.704$, $p = 0.636$; for biopterin, $H(3) = 2.897$, $p = 0.408$.

Table 4. Age-stratified reference intervals for serum and urinary neopterin in healthy individuals (16,17).		
Neopterin		
Age (years)	Serum (nmol/L)	Urinary (μmol/mol creatinine)
	0 - 15	50 - 250
1-18	3.5 - 13.5	40 - 310
19-75	2.6 - 8.7	55 - 220
>75	3.7 - 19.0	70 - 230

pronounced than in younger or exceptionally healthy elderly individuals, yet does not reach the levels typically associated with manifest neurodegenerative disease. This interpretation is consistent with population-based data from Werner et al. (20), who showed that neopterin concentrations are higher in subjects younger than 18 and older than 75 years than in middle-aged adults, with no significant sex differences.

Beyond neurodegeneration, several studies in geriatric and chronically ill populations have highlighted urinary neopterin as a marker of systemic inflammation and adverse health status. In hospitalized patients aged >80 years, Hrniciarikova et al. (21) observed significant inverse correlations between urinary neopterin (and C-reactive protein) and serum lipids, supporting neopterin’s role as a biomarker of inflammation and malnutrition in frail elderly individuals. Mangoni and Zinellu (22) similarly reported that circulating pteridines are altered in mild cognitive impairment and Alzheimer’s disease, with effect sizes that depend on age and study design, underscoring the complexity of interpreting pteridine changes in older populations. Taken together, our data support the view that urinary neopterin and biopterin in elderly individuals may function as integrative markers of immune activation and redox status, and are influenced by age and overall health rather than by any single metabolic parameter.

Macrophages produce neopterin upon IFN-γ stimulation (6,7), and its oxidation product is considered an indirect marker of both cellular immune activation and reactive oxygen species (ROS) generation (23). Several studies in older adults have shown that circulating or urinary neopterin levels increase with advancing age and are associated with frailty, morbidity, and mortality, fitting the broader concept of “inflammaging”, a state of chronic, low-grade immune activation characteristic of aging populations (11,24-26). In our sample, the correlation between neopterin and age did not reach statistical significance, possibly because participants were drawn from a relatively narrow, older age range, and heterogeneity in comorbidities and functional status may have been more influential than chronological age. In this context, the weak but significant correlation between neopterin and biopterin suggests that both analytes capture

overlapping aspects of age-related immune and oxidative stress. However, they are not interchangeable and may respond to different pathways. The modest strength of this association may reflect that neopterin and biopterin capture partially overlapping yet biologically distinct processes, including IFN-γ-mediated immune activation and BH4-related redox metabolism, which may vary independently across individuals in this heterogeneous elderly population.

Biopterin is the fully oxidized form of BH4, an essential cofactor for NO synthases and aromatic amino acid hydroxylases. Disturbances in BH4 metabolism have been implicated in endothelial dysfunction, neurodegeneration, and other age-related conditions (12,27). Under oxidative stress, BH4 is readily oxidized to dihydrobiopterin and biopterin, which lack cofactor activity and can promote NO synthase uncoupling, thereby further enhancing ROS production (28). The substantial inter-individual variability in urinary biopterin levels in our cohort likely reflects differences in these redox processes, superimposed on chronic low-grade inflammation. Together with the moderate correlation we observed between systolic blood pressure and glucose and the absence of clear associations between pteridines and these metabolic markers, our data support the notion that urinary neopterin and biopterin may integrate multiple age-related stressors (immune, vascular, and metabolic) rather than mirroring single clinical measurements obtained at one time point.

The lack of significant differences in neopterin and biopterin levels among the four dietary pattern groups in our study should be interpreted with caution. It does not necessarily rule out the role of nutrition in modulating pteridine metabolism. Experimental and clinical evidence indicate that diets rich in fruits, vegetables, whole grains, and omega-3 fatty acids can attenuate systemic inflammation and oxidative stress, whereas Western-type dietary patterns tend to have the opposite effect (29,30). However, studies directly linking diet and pteridines remain scarce. A longitudinal study that manipulated dietary macronutrient composition (normal, carbohydrate-rich, or fat-rich) in healthy adults found no significant effect on urinary neopterin levels, even when combined with different antioxidant supplementation and exercise loads, suggesting that neopterin is a robust marker of immune activation that is largely unaffected by short-term macronutrient changes (31). A randomized clinical trial in overweight and obese adults showed that a high intake of cod (lean fish) over 8 weeks significantly reduced serum neopterin concentrations, whereas a high intake of salmon (fatty fish) did not, suggesting that specific dietary factors may influence neopterin metabolism (32). Animal studies have also indicated that plant-based nutritional supplementation attenuates inflammation-induced increases in urinary neopterin levels (33). Simultaneously, comprehensive metabolomics studies of urinary markers of healthy dietary

habits rarely highlight pteridines as the primary indicators of dietary adherence (34). Overall, these observations suggest that short-term or moderate changes in dietary patterns may have a limited impact on urinary neopterin levels under relatively stable conditions. In contrast, specific foods or long-term dietary habits may exert subtle cumulative effects. Importantly, the dietary assessment approach used in this study was not designed to support quantitative or mechanistic conclusions; therefore, the observed lack of association should be considered exploratory and context-dependent.

In our cross-sectional study, dietary patterns were derived from a 7-day qualitative food record, and group sizes were modest; this may have limited our ability to detect subtle diet–pteridine relationships. It is plausible that long-term dietary habits, specific nutrient deficiencies or excesses, and the balance between pro- and anti-inflammatory components of the diet exert more gradual, cumulative influences on neopterin and biopterin than those captured within a short observational window. Moreover, urinary pteridines are likely shaped by the interplay of nutritional, inflammatory, metabolic, and genetic factors. Longitudinal studies that combine detailed dietary assessments, repeated pteridine measurements, and comprehensive inflammatory and metabolic profiling are better suited to clarify how nutrition interacts with age-related immune activation and BH4/biopterin redox cycling in older adults. In addition, the absence of renal function data, particularly the estimated glomerular filtration rate (eGFR), limits the interpretation of creatinine-normalized urinary neopterin and biopterin values. Although creatinine normalization helps reduce variation related to urine dilution, urinary creatinine correction may introduce bias when creatinine excretion varies, and this issue is particularly relevant in very old adults because muscle mass and renal function may differ substantially between individuals (35,36). Therefore, the measured pteridine/creatinine ratios should be interpreted with caution, and future studies should include eGFR or other markers of renal function.

In this cohort of community-dwelling elderly individuals, urinary neopterin and biopterin concentrations were, on average, within the adult reference intervals, but exhibited considerable inter-individual variability and a weak yet significant correlation between the two biomarkers. Along with the absence of strong associations with single measurements of blood pressure, glucose, or short-term dietary patterns, these findings support the possibility that urinary pteridines may act as integrative markers of age-related immune activation and redox imbalance, rather than as simple reflections of isolated metabolic parameters. Although exploratory, our results suggest that neopterin and biopterin may help characterize heterogeneity in “inflammaging” among older adults and could, in future studies, be combined with more detailed clinical, nutritional,

and inflammatory profiling to improve risk stratification. Larger longitudinal studies with comprehensive phenotyping and formal analytical validation are warranted to clarify the prognostic value of urinary pteridines and their potential role in the early identification of vulnerable older adults.

Study limitations

This study had several strengths, including its focus on community-dwelling older adults, use of home visits to facilitate participation among frail individuals, and combined assessment of neopterin and biopterin using high-performance liquid chromatography. However, this study has some limitations. The sample size was modest and drawn from a single municipality, which may limit generalizability. Urinary pteridines, blood pressure, and glucose levels were measured at a single time point, and detailed information on comorbidities, medication use, and inflammatory markers was unavailable. Dietary patterns were derived from a 7-day qualitative record, and the group sizes were small, reducing the statistical power to detect subtle nutrition-related differences. Finally, the cross-sectional design precludes any causal inference regarding the relationship between urinary pteridine levels and clinical variables.

Conclusion

In this cohort of community-dwelling elderly individuals, urinary neopterin and biopterin concentrations were, on average, within the adult reference intervals but showed considerable inter-individual variability and a weak yet significant correlation between them. Together with the absence of strong associations with single measurements of blood pressure, glucose, or short-term dietary patterns, these findings support the possibility that urinary pteridines may act as integrative markers of age-related immune activation and redox imbalance, rather than simple reflections of isolated metabolic parameters. Although exploratory, our results suggest that neopterin and biopterin may help characterize heterogeneity in “inflammaging” among older adults and could, in future studies, be combined with more detailed clinical, nutritional, and inflammatory profiling to improve risk stratification. Larger longitudinal studies with comprehensive phenotyping and formal analytical validation are warranted to clarify the prognostic value of urinary pteridines and their potential role in the early identification of vulnerable older adults.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Eastern Mediterranean University Ethics Committee (approval no: 2019/04-05, date: 04.02.2019).

Informed Consent: Consent was obtained from all the participants or their legal guardians.

Footnotes

Authorship Contributions

Concept: G.Ş., Design: G.Ş., Data Collection or Processing: S.S., H.K., Ş.A., Analysis or Interpretation: S.S., Literature Search: S.S., H.K., G.Ş., Writing: S.S., H.K., G.Ş.

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