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# Biomarkers status and their relation with the presence of type 2 diabetes with and without angiopathy

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## Introduction

The knowledge on the status of several biomarkers [1] is a useful tool for disease clinical characterization and treatment of type 2 diabetes. The additive clinical effect of the presence of different biomarkers can be used to evaluate the risk to develop angiopathy and also in disease management.

## Aim

The aim of this study was to evaluate the levels of cardiovascular, oxidative stress and nutritional biomarkers and their relationship with the presence of type 2 diabetes and angiopathy.

## Methods

A population-based case-control study in 150 Portuguese type 2 diabetic patients was performed. Group I - 75 diabetics with angiopathy, group II - 75 diabetics without angiopathy and group III – non-diabetic controls. Plasma levels of homocysteine, cysteine, malondialdehyde (MAD), vitamins $B_6$, C, A and E and carotenoids were measured by HPLC methods. Vitamin $B_{12}$ and folate serum levels were achieved by an electrochemiluminescence method.

## Results

The baseline characteristics of diabetic patients studied are presented in **Table 1**. The results of biomarkers plasma or serum levels evaluation are presented in **Table 2**. The hyperhomocysteinemia prevalence was 20% (group I), 8.7% (group II) and 0.71% (group III). Group I showed the higher prevalence of hypercysteinemia (17%). The MAD serum levels were above the reference value for all groups. The percentage of subjects with ascorbic acid low plasma levels were statistically different in diabetic (I: 55%; II: 47%) compared to non-diabetic subjects (III: 22%). The prevalence of hypovitaminosis $B_6$ deficiency was at least 30% for all groups. Type 2 diabetes predisposes to hypovitaminosis C (OR: 3.10; p = 0.0002) (**Table 3**). In group I, the probability to have hyperhomocysteinemia was around 3 times higher (p = 0.04) in comparison with group II and 35 times (p = 0.0006) with group III (**Table 4**). The combined effect of type 2 diabetes and angiopathy is associated with high MAD (OR: 5.33; p = 0.002) serum levels compared to group III (**Table 5**). The effect of the angiopathy presence was only significant for homocysteine biomarker (**Table 5**).

**Table 1 –** Baseline characteristics of the study population.

| Characteristics | Group I (n = 75) | Group II (n = 75) | Group III (n = 143) |
|---|---|---|---|
| Age (years) | 62.9[a] ± 7.17 | 62.8[a] ± 7.01 | 65.6[b] ± 5.75 |
| Men/Women | 36[a]/39[a] | 31[a]/44[a] | 33[b]/110[b] |
| Diabetes duration (years) | 19.4[a] ± 9.33 | 13.5[b] ± 7.95 | NA |
| Hb (g/dL) | 13.4[a] ± 1.72 | 13.7[a] ± 1.42 | 13.8[a] ± 1.17 |
| HbA1c (%) | 8.70[a] ± 1.42 | 8.27[a] ± 1.39 | NA |
| Hematocrit (%) | 39.3[a] ± 4.74 | 40.3[a] ± 4.03 | 40.7[a] ± 3.30 |
| Erythrocytes (millions/µL) | 4.58[a] ± 0.588 | 4.64[a] ± 0.437 | 4.52[a] ± 0.426 |
| Total Cholesterol (mmol/L) | 5.24[a] ± 1.22 | 5.18[a] ± 0.965 | 5.35[a] ± 0.962 |
| HDL-Cholesterol (mmol/L) | 1.27[a] ± 0.378 | 1.34[a] ± 0.348 | 1.48[b] ± 0.320 |
| LDL-Cholesterol (mmol/L) | 3.34[a] ± 0.964 | 3.27[a] ± 0.847 | 3.59[a] ± 0.902 |
| Triglycerides (mmol/L) | 2.19[a] ± 1.07 | 1.94[a] ± 1.21 | 1.19[b] ± 0.520 |
| Systolic blood pressure (mmHg) | 154[a] ± 20.3 | 146[b] ± 25.9 | 136[c] ± 19.8 |
| Diastolic blood pressure (mmHg) | 79.2[a] ± 13.1 | 82.0[b] ± 12.7 | 76.3[a] ± 11.5 |
| Resting heart rate (beats/min) | 73.2[a] ± 12.0 | 77.6[a] ± 12.9 | 68.8[b] ± 10.8 |

Results are expressed as mean ± standard deviation or as number of individuals. The averages on the same line marked with different letters (a, b, c) are statistically different according to the Bonferroni post hoc test (p <0.05) or according to the t-student test for two independent samples. To compare the proportions of men and women between the groups, the Z test was used to compare proportions (p <0.05). Hb, hemoglobin; HbA1c, glycosylated hemoglobin; NA, not applicable.

**Table 2 –** Evaluation of plasma or serum levels of biomarkers.

| Biomarker | RSV | Reference | Prevalence of inadequacy | | |
|---|---|---|---|---|---|
| | | | Group I (n = 75) | Group II ( n = 75) | Group III (n = 143) |
| Hcy (µM) | ≥15 | [3] | 15 (20)[a**] | 6 (8.7)[b**] | 1 (0.71)[c**] |
| Cys (µM) | >300 | [2] | 13 (17)[a**] | 6 (8.0)[b**] | 0 (0.0)[c**] |
| MAD (µM) | >1.71 | [4] | 71 (95)[a**] | 70 (93)[a**] | 110 (77)[b**] |
| Vitamin A: retinol (µM) | <0.7 | [5] | 0 (0,0) | 0 (0.0) | 0 (0.0) |
| Vitamin E: α-tophoferol (µM) | <12 | [5] | 1 (1.3)[a*] | 1 (1.4)[a*] | 13 (9.1)[b*] |
| Lutein (µM) | <0.6 | [6] | 0 (0.0) | 0 (0.0) | 1 (0.70) |
| Vitamin C: AA (µg/mL) | <4.0 | [7] | 41 (55)[a**] | 35 (47)[b**] | 31 (22)[c**] |
| Vitamin $B_6$: PLF (nM) | ≤30 | [8] | 25 (33)[a**] | 24 (32)[a**] | 51 (38)[a**] |
| Folic acid (ng/mL) | <2.2 | [5] | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Vitamin $B_{12}$ (pg/mL) | <200 | [9] | 1 (1.4)[a] | 2 (2.7)[a] | 3 (2.1)[a] |

The results are expressed as number of individuals and as (percentage). The number of individuals on the same line marked with different letters (a, b, c) are statistically different according to the Chi-square test and the Z test for comparison of proportions (* p <0.05, ** p < 0.001). RSV, recommended blood value; Hcy, homocysteine; Cys, cysteine; MAD, malondialdehyde; AA, ascorbic acid; PLF, pyridoxal 5 phosphate. Prevalence of inadequacy

**Table 3 –** Effect of type 2 diabetes presence on the variation of biomarkers.

| Biomarker | Group II (n = 75) | Group III (n = 143) | OR | CI (95%) | *p* |
|---|---|---|---|---|---|
| | Exposed / Not Exposed | Exposed / Not Exposed | | | |
| Hcy ≥15 µM | 6/69 | 1/141 | 12.3 | 1.45 - 104 | 0.02* |
| Cys >300 µM | 6/69 | 0/143 | NA | NA | NA |
| MAD >1.71 µM | 70/5 | 110/33 | 4.20 | 1.57 - 11.3 | 0.004** |
| AA <4.0 µg/mL | 35/40 | 31/110 | 3.10 | 1.70 - 5.68 | 0.0002** |
| PLF <30 nM | 24/51 | 51/85 | 0.784 | 0.432 - 1.42 | 0.425 |

AA, ascorbic acid; Cys, cysteine; CI, confidence interval; Hcy, homocysteine; MAD, malondialdehyde; NA, not applicable; PLF, pyridoxal-5-phosphate; OR, Odd ratio. Statistically significant (* p <0.05, ** p <0.001).

**Table 4 –** Effect of angiopathy presence on the variation of biomarkers.

| Biomarker | Group I (n = 75) | Group II (n = 75) | OR | CI (95%) | *p* |
|---|---|---|---|---|---|
| | Exposed / Not Exposed | Exposed / Not Exposed | | | |
| Hcy ≥15 µM | 15/60 | 6/69 | 2.88 | 1.05 - 7.88 | 0.04* |
| Cys >300 µM | 13/62 | 6/69 | 2.41 | 0.864 - 6.73 | 0.09 |
| MAD >1.71 µM | 71/4 | 70/5 | 1.27 | 0.327 - 4.92 | 0.73 |
| AA <4.0 µg/mL | 41/34 | 35/40 | 1.38 | 0.725 - 2.62 | 0.33 |
| PLF <30 nM | 25/50 | 24/51 | 1.06 | 0.537 - 2.10 | 0.86 |

AA, ascorbic acid; Cys, cysteine; CI, confidence interval; Hcy, homocysteine; MAD, malondialdehyde; PLF, pyridoxal-5-phosphate; OR, Odd ratio. * Statistically significant (p <0.05).

**Table 5 –** Combined effect of type 2 diabetes and angiopathy presence on the variation of biomarkers.

| Biomarker | Group I (n = 75) | Group III (n = 143) | OR | CI (95%) | *p* |
|---|---|---|---|---|---|
| | Exposed / Not Exposed | Exposed / Not Exposed | | | |
| Hcy ≥15 µM | 15/60 | 1/141 | 35.3 | 4.55 - 273 | 0.0006** |
| Cys >300 µM | 13/62 | 0/143 | NA | NA | NA |
| MAD >1.71 µM | 71/4 | 110/33 | 5.33 | 1.81 - 15.7 | 0.002** |
| AA <4.0 µg/mL | 41/34 | 31/110 | 3.31 | 1.85 - 5.92 | 0.0001** |
| PLF <30 nM | 25/50 | 51/85 | 0.833 | 0.461 - 1.51 | 0.546 |

AA, ascorbic acid; Cys, cysteine; CI, confidence interval; Hcy, homocysteine; MAD, malondialdehyde; NA, not applicable; PLF, pyridoxal-5-phosphate; OR, Odd ratio. Statistically significant (* p <0.05, ** p <0.001).

## Conclusion

The prevalence of hypovitaminosis $B_6$ was relevant in all studied groups. Low levels of vitamin C were more frequent in type 2 diabetics with angiopathy than without or non-diabetic subjects. The presence of type 2 diabetes increases the risk of hyperhomocysteinemia, oxidative stress and hypovitaminosis C. The isolated effect of angiopathy increases the probability to have hyperhomocysteinemia.

**REFERENCES**
[1] Garban Z, Gârban G, Ghibu GD. Biomarkers: theoretical aspects and applicative peculiarities Note II, Nutritional Biomarkers. J Agro Process Technol 2006; 12(2):349-356.
[2] El-Khairy L, Ueland PM, Refsum H, Graham IM, Vollset SE. Plasma total cysteine as a risk factor for vascular disease. Circulation. 2001; 103(21):2544-2549.
[3] Brattstrom L, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect?. Am J Clin Nutr. 2000; 72(2):315-323.
[4] Dixon ZR, Shie FS, Warden BA, Burri BJ, Neidlinger TR. The effect of a low carotenoid diet on malondialdeyde-thiobarbituric acid (MDA-TBA) concentrations in women: a placebo-controlled double-blind study. J Am Coll Nutr. 1998; 17(1):54-58.
[5] Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Laboratory reference values. N Engl J Med. 2004; 351(15):1548-1563.
[6] Granado F, Olmedilla B, Blanco I. Nutritional and clinical relevance of lutein in human health. British J Nutr. 2003; 90(3):487-502.
[7] Iverson C, Christiansen S, Flanagin A, et al. AMA Manual of Style: a guide for authors and editors. 10nd ed. New York: Oxford University Press; 2007.
[8] Leklem JE. Vitamin B-6: A status report. J Nutr. 1990; 120(11):S1503-S1507.
[9] Carmel R, Green R, Rosenblatt DS, Watkins D. Update on Cobalamin, Folate, and Homocysteine. Hematology Am Soc Hematol Educ Program. 2003; 1:62-81.

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