STUDY PROTOCOL

Title: Metformin and Its Impact on the Substances Associated With NO Production in Prediabetes Patients. NCT03398356

Main Researcher: Dr Sutkowska Edyta

Sponsor: Wroclaw Medical University

Document Date: Jun 13,2017

Aim:

The study evaluated the effect of different doses of metformin on the intermediates of NO (nitric oxide) biosynthesis in people with pre-DM (pre-diabetes).

Material and Methods:

Population and groups

The treatment-naïve patients (regarding to hypoglycemic drugs) with new-diagnosed pre-DM were recruited from Diabetes Centre between October 2017 and December 2018.

Inclusion criteria for the patients:

- Pre-diabetes based on FG (fasting glucose) and / or OGTT (oral glucose tolerance test) according to the local (similar to EASD- European Association for the Study of Diabetes) criteria
- Age 40-65 yo
- Any gender, profession, place of residence
- Negative history for diabetes
- No hypoglycemic drugs in the past
- Negative history for cardio-vascular diseases
- Written consent to participate in the study

Criteria for exclusion for the patients:

- Age <40 or >65 years
- History of diabetes –from patients, patients family and/or medical records if available
- FG>125 mg% (6.9mmol/L) and or glucose after 2 hours in OGTT >199mg% (11mmol/L)
- History of treatment with any hypoglycemic drug
- History of cardio-vascular disease
- Contraindications to the use metformin according to the Product Characteristics
- Known gastro-intestinal disease which could be potentially responsible for metformin intolerance (eg IBD- irritable bowel disease, GERD- gastro-esophageal reflux disease)

- Known potential difficulties in collaboration with patients (dementia, mental disorders, distance from patients residence if considered as potentially problematic)
- No consent to participate in the study
- Patient participation in another study

Inclusion criteria for * healthy volunteers:

- Age 40-65 yo
- Any gender, profession, place of residence
- Negative history for diabetes, pre-diabetes and FG<100 mg% (5.6 mmol/L)
- No hypoglycemic drugs in the past
- Negative history for cardio-vascular diseases
- Written consent to participate in the study

Methods: definitions, information collected, lab-tests performed, treatment scheme, lab-tests technique, statistical analysis

1. Pre-diabetes diagnosis definitions:

IFG (impaired fasting glycaemia): FG (fasting glucose): 100-125 mg% (5.6- 6.9 mmol/L);

IGT (impaired glucose tolerance): glucose 140-199 mg% (7.8- 11.0 mmol/L) after 2 hours of 75 glucose solution taken

2. Negative history for cardio-vascular diseases definitions:

Patients without ischemic heart disease and/or stroke and/or PAOD (peripheral arterial occlusive disease) in a history

3. Demographic information collected: age, sex

4. Baseline parameters collected: weight, height, BMI (body mass index), information about fatty liver (based on the available ultrasonography organs' description), family history for DM (YES/NO), hypertension (YES/NO), active nicotinism (YES/NO); and biochemical: FPG, lipids profile, creatinine, AlaT (alanine transaminase), creatinine, HbA1c (glycated hemoglobin) if available.

5. Before the first dose of the metformin and after: 6, 12, 15 weeks of the treatment, the blood samples were collected for the assessment of the:

a. concentration of the metformin

b. concentration of the compounds related to the production of the NO: L-arginine, L-citrulline, ADMA – asymmetric dimethylarginine, DMA – dimethylamine, SDMA – symmetric dimethylarginine, ornithine

* For healthy volunteers only once concentration for the compounds related to the production of the NO was assessed and no blood samples for metformin concentration were taken.

The admissible delay in taking blood for lab-testing was set at 4 days. The blood samples were taking randomly (no hours or gaps between metformin taking were defined).

6. Patients with pre-DM were treated 3 weeks with an increasing dose of the immediate-release (regular form) metformin to 3 x 500 mg, and then randomly assigned to:

group A – continuation of a dose of 3 x 500 mg or

group B – increase dose to: 3 x 1000 mg (also by titration); after 12 weeks this group returned to the dose 3 x 500 mg for the next 3 weeks

7. If potential side effects appeared in group B, the patients could take a lower dose (3 x 500 mg if well-tolerated) and thus change to group A.

8. If the smallest dosage (3 x 500mg) was not tolerated the patient was excluded from the study.

9. During titration period (by phone) and blood collection visits (personally), patients were asked about the compliance and drug's tolerance. The patients were also encouraged to contact in any case of the problem with metformin tolerance.

10. The technique used to assess concentration of the metformin in serum and the wider panel of L-arginine/NO pathway metabolites: LC-MS/MS (liquid chromatography-mass spectrometry).

11. Safety outcomes: Adverse events were reported at each clinic visit and revised via telephone contact during treatment period.

12. SAP- Statistical Analysis Plan

- to prove the homogeneity of the compared groups, analysis of the baseline parameters was done

- to show the potential differences between people with pre-DM and healthy people the comparison of the baseline parameters was done

- to follow the metformin intake by the patients, the metformin concentration with its change during the therapy was done

- for the analysis of the impact of the metformin dosage on the concentration of the compounds related to the production of the NO the difference of these compounds between two groups was done

- as an extra analysis, the correlations of the serum drug concentration with BMI and body mass, after 6 and 15 weeks of the treatment was done, while research was ongoing

The Statistica 13 program was used with cut-off point for statistical significance (P) at 0.05.

The normality of the distribution of the examined quantitative variables was checked using the Shapiro-Wilk test. In order to determine the significance, statistical tests were used in accordance with the distribution of variables and the nature of the data (Student t test, Welch test, Mann-Whitney test, chi – square test, the Wilcoxon signed-rank test, Kruskal – Wallis test, Friedman test, F test). To determine the correlation the Spearman's rank-order correlation and the Pearson correlation was used.

The flow chart of the management of the patients

Met "Inclusion criteria": YES; Written consent: YES

Initial visit: blood samples for: arginine, citrulline, ADMA, DMA, SDMA, ornithine

Metformin start (1 x 500 mg)

3 weeks titration to the dose: 1500mg/day

Good dose tolerance: YES

3 weeks continuation of the dose 1500mg/day

Control visit: blood samples for: arginine, citrulline, ADMA, DMA, SDMA, ornithine and metformin

Randomization

Group B

3 weeks titration to the dose: 3000mg/day

Group A

6 weeks continuation of the dose 1500/day

Good dose tolerance: YES NO

3 weeks continuation of the dose 3000mg/day

Control visit: blood samples for: arginine, citrulline, ADMA, DMA, SDMA, ornithine and metformin

3 weeks continuation of the dose 1500mg/day

Final visit: blood samples for: arginine, citrulline, ADMA, DMA, SDMA, ornithine and metformin

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OŚWIADCZENIE BADANEGO (see below for English version)

– zgoda na udział w badaniach

Nazwisko i imię osoby badanej.....

.....Lat.....

Adres:.....

.....

Temat badań: Ocena wpływu metforminy i jej stężenia w surowicy na stężenie substancji związanych z produkcją tlenku azotu u pacjentów z zaburzeniami gospodarki cukrowej.

Niniejszym oświadczam, że zostałem/am szczegółowo poinformowany/na o sposobie przeprowadzenia badań i moim w nich udziale (Informacja dla Pacjenta). Rozumiem, na czym polegają badania i do czego potrzebna jest moja zgoda. Zostałem poinformowany/na, że mogę zadawać pytania dotyczące projektu w trakcie jego trwania oraz odmówić uczestnictwa w badaniach na każdym ich etapie bez podawania powodu.
Wszystkie informacje jakie zostaną przekazane na cel badania będą poufne (nie będzie możliwości identyfikacji mojej osoby).

Wyrażam świadomą i dobrowolną zgodę na uczestnictwo w powyższym badaniu.

....

podpis badanego podpis badacza

miejscowość..... data .....

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STATEMENT OF THE PARTICIPANT
- consent to participate in the research

Surname and first name of the participant .....
.... Years .....

Address:..... .....
....

Research topic: Metformin and its impact on the substances associated with nitric oxide (NO) production in pre-diabetes patients.

I hereby declare that I have been informed in detail about the way of conducting the study. I understand what the research is for and what my consent is needed for. The purpose and nature of the study have been explained to me in writing and I have been able to ask questions about the study. I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.
I understand that all information I provide for this study will be treated confidentiall (no identification of my personal data).

I express my informed and voluntary consent to participate in the above research.

....
signature of researcher signature of participant

place……, date ....

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OŚWIADCZENIE BADANEGO (see below for English version)

- zgoda na przetwarzanie danych

Nazwisko i imię osoby badanej.....

.....Lat.....

Adres:.....

.....

Temat badań: Ocena wpływu metforminy i jej stężenia w surowicy na stężenie substancji związanych z produkcją tlenku azotu (NO) u pacjentów z zaburzeniami gospodarki cukrowej (stan przedcukrzycowy)

Niniejszym oświadczam, że wyrażam zgodę na przetwarzanie moich danych osobowych w zakresie niezbędnym do realizacji projektu badawczego, w którym biorę udział.

....
podpis badacza podpis badanego

miejscowość..... data .....

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STATEMENT OF THE PARTICIPANT
- data processing agreement

Surname and first name of the participant .....
.... Years ....

Address:..... .....
....

Research topic: Metformin and its impact on the substances associated with nitric oxide (NO) production in pre-diabetes patients.

I hereby declare that I agree to the processing of my personal data to the extent necessary for the implementation of the research project in which I participate.

....
signature of researcher signature of participant

place…… date .....