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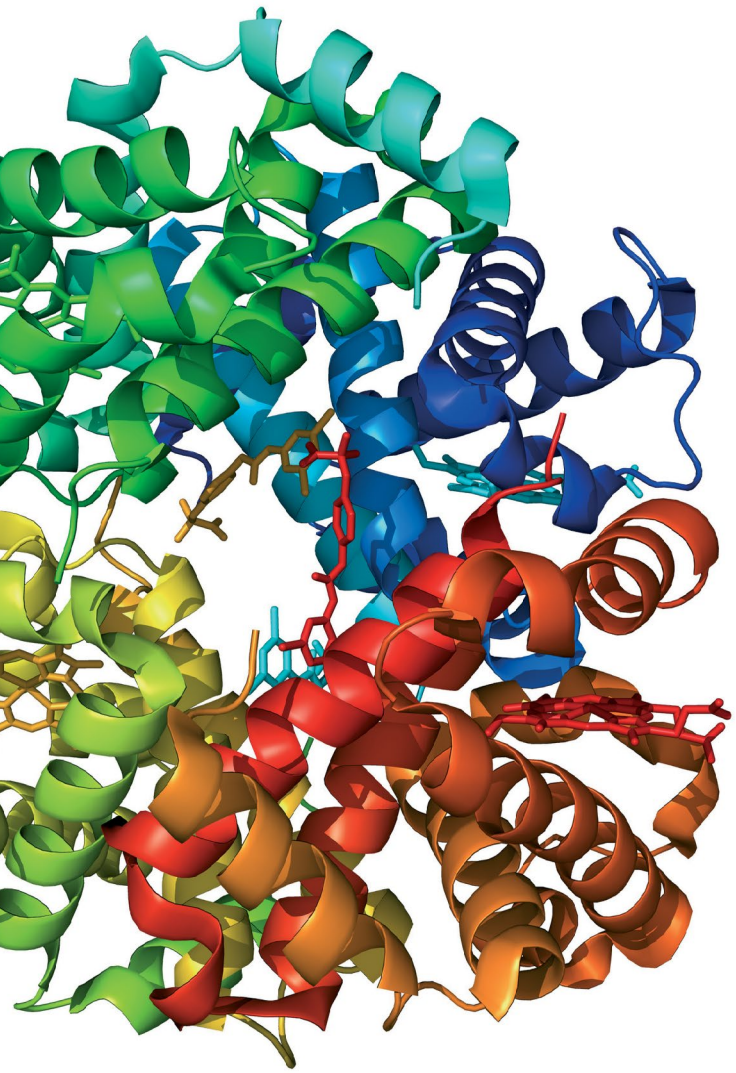

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The effect of linagliptin treatment on gut microbiota in patients with HNF1A-MODY or type 2 diabetes — a preliminary cohort study

ABSTRACT

Introduction. Many studies have evaluated the relationship between diabetes and microbiota. In animal models, the dipeptidyl peptidase-4 inhibitors altered the gut microbiota. We investigated whether linagliptin alters the gastrointestinal flora in humans.

Materials and methods. This prospective cohort study enrolled 24 patients: 5 patients with maturity onset diabetes of the young associated with *HNF1A* mutation and 19 patients with type 2 diabetes mellitus. Stool samples were collected at baseline and 4 weeks after treatment intensification with either linagliptin or a sulphonylurea alongside current treatment. Faecal 16S rRNA was analysed by next-generation sequencing.

Results. Nine patients initiated linagliptin whereas 15 patients initiated or increased the dose of a sulphonylurea. After linagliptin treatment, we did not observe changes in taxa in L2–L7 based on analysis of composition of microbiomes (ANCOM). The same held true for pairwise alpha diversity (Shannon diversity, p = 0.59; Pielou's measure of evenness, p = 0.68; and observed operational taxonomic units [OTUs], p = 0.77) and beta diversity distances (unweighted UniFrac, p = 0.99; weighted UniFrac, p = 0.93; Bray-Curtis, p = 0.98; and Jaccard, p = 0.99). Similarly, after sulphonylurea intensification, we did not observe changes in taxa in L2–L7 in ANCOM, nor were there changes in alpha diversity (Shannon diversity, p = 0.19; Pielou's measure of evenness, p = 0.21; and observed OTUs, p = 0.42) or beta diversity distances (unweighted UniFrac, p = 0.99; weighted UniFrac, p = 0.99; Bray-Curtis, p = 1; and Jaccard, p = 0.99).

Conclusion. We did not observe changes in colonic microbiota 4 weeks after addition of linagliptin to current diabetes treatment. Further studies are required to determine whether linagliptin influences the colonic microbiota in humans. (Clin Diabetol 2019; 8, 6: 263–270)

Key words: diabetes, HNF 1 alpha, linagliptin, microflora, sulfonylurea

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Introduction

Despite extensive research in type 2 diabetes (T2DM), the pathogenesis of the condition and the factors underpinning disease progression and therapeutic response remain incompletely understood [1]. Established risk factors for T2DM include obesity, sedentary lifestyle, older age and strong family history of the disease [2]. Genome-wide association studies have provided valuable insights into the genetic predisposition to various conditions, including T2DM, but have had

limited success in explaining the heritability of complex diseases [3, 4]. To account for this unexplained heritability, a role has been proposed for gene–environment interactions, including gastrointestinal bacterial flora or nutrition/medication-related alterations in gastrointestinal hormone activity [3].

Many recent studies have focused on the relationship between T2DM and gastrointestinal bacterial flora [5]. For example, differences in microbiota have been demonstrated between healthy subjects and patients with monogenic forms of diabetes [6, 7]. Obesity, a risk factor for T2DM, has also been associated with alterations in gut microbiota [8]; however, body mass index-independent differences in bacterial flora have been observed in patients with T2DM compared with healthy subjects [9]. Some changes in microbiota previously shown in patients with T2DM versus healthy individuals have been attributed to metformin use [10].

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a well-established class of medications used widely in the management of T2DM [11]. Of interest, it has been shown that at least three DPP-4 inhibitors (sitagliptin, saxagliptin and vildagliptin) may alter the structure of gastrointestinal flora in animal models [12–16]. Some other antidiabetic medications, including liraglutide and acarbose, are also postulated to modify gut microbiota [12]. However, it remains unclear whether these effects on the microbiota influence therapeutic response.

The DPP-4 inhibitor linagliptin has not been studied in regard to gut microbiota and is the only member of the drug class that is primarily eliminated as the parent compound in faeces (95%) [17]. The aim of this prospective study was to compare the colonic bacterial flora structure before and after the addition of linagliptin to current treatment in patients with T2DM or maturity onset diabetes of the young associated with *HNF1A* mutation (HNF1A-MODY).

Materials and methods

Study setting and eligibility

The cohort study was conducted between 2013 and 2015 at the Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland and the University Hospital, Krakow, Poland, in collaboration with the Center for Medical Genomics (OMICRON) and Department of Microbiology, Jagiellonian University Medical College, Krakow, Poland. Patients treated in our Outpatient Clinic and volunteers took a part in a study. The cohort has been described in detail previously [7]. Briefly, the study enrolled men and women aged 18–65 years with T2DM or HNF1A-MODY receiving metformin alone or metformin plus a sulphonylurea (SU) at or below the submaximal dose, or (for patients with HNF1A-MODY only) insulin therapy. Patients were also required to have poorly controlled glycaemia (glycosylated haemoglobin [HbA_{1c}] > 7% or > 53 mmol/mol) and no advanced, chronic complications of diabetes. Confirmed patient readiness to cooperate with the research centre was required for study participation. All participants declared not using antibiotics 4 weeks before stool sample collection. The exclusion criteria were as follows: lack of consent to participate in the study, withdrawal during the study, taking antibiotics or probiotics up to 30 days before the sample collection, confirmed infection of the gastrointestinal tract, chronic inflammatory bowel disease of unknown etiology, active cancer (especially of the gastrointestinal tract), immunodeficiency, features of liver damage (with the exception of nonalcoholic fatty liver transaminase levels less than three times the upper limit of the normal level). The study received approval from the Jagiellonian University Ethics Committee. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Laboratory investigations

Blood samples were obtained from all patients. HbA_{1c}, triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, aspartate transaminase and alanine transaminase were assessed by standard laboratory techniques.

Treatment and follow-up

Diabetes therapy was intensified in all patients. In the linagliptin group, patients were prescribed linagliptin in addition to current treatment, while in the control group (SU group), the dose of a current SU was increased or a new SU was initiated alongside current treatment. Treatment allocation was not randomised, but was instead based on local guidelines and long-term drug affordability for the patient (given the substantially greater cost of DPP-4 inhibitors than SU in Poland). Patients were asked to avoid changing their dietary habits and level of physical activity during the study.

Stool samples were collected from all patients before and 4 weeks after treatment intensification. In 21 patients, information from blinded continuous glucose monitoring (iPro2, Medtronic, Dublin, Ireland) was obtained from 1 week before and 3 weeks after treatment intensification (i.e., in the fourth week of intensified treatment).

RNA isolation and 16S metagenomic sequencing

Bacterial RNA was isolated using Genomic Mini AX Stool Spin (A&A Biotechnology, Gdynia, Poland),

modified to include enzymatic treatment (lysozyme, lysostaphin and lyticase) and a bead-beating step. Libraries were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol (https://support.illumina.com/content/dam//illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Briefly, universal external primers were used to amplify regions V3 and V4 of the 16S rRNA. After the polymerase chain reaction clean-up, samples were indexed, cleaned and pooled. Finally, 10 pM libraries with 10% PhiX Spike-In were sequenced on Illumina MiSeq (Illumina, Inc., San Diego, CA, US) using the V3 sequencing kit (300 bp paired-end reads).

Sequencing data analysis

Samples were processed and analysed using the Quantitative Insights Into Microbial Ecology 2 (QIIME2, version 2018.11) [18] custom pipeline. Briefly, the quality of demultiplexed paired-end reads from MiSeq (2 × 300 bp) was evaluated and the reads were trimmed to remove primers and poor-quality bases with cutadapt [19]. Trimmed sequences were denoised and joined with DADA2 [20]. Next, closed-reference clustering of features [21] and reference-based chimera filtering were performed using vsearch [22] and the Greengenes database at 99% similarity [23]. Generated operational taxonomic units (OTUs) were assigned to taxonomy using a naive Bayes classifier [24], which was pre-trained on the sequenced target RNA regions. For further analysis, we included only features occurring in at least three samples that had more than 20 total reads. The bacterial composition was analysed at phylum, order, family, genus and species levels. Filtered feature tables were used to generate the trees for phylogenetic diversity analyses. Rarefaction curve analysis was used to estimate the completeness of microbial community sampling. We also computed default alpha and beta diversity metrics and generated principal coordinates analysis (PCoA) plots for each of the beta diversity metrics using Emperor [25]. Group significance between alpha and beta diversity indices were calculated with the QIIME2 longitudinal pairwise-differences plugin using the *t*-test. Correlations with alpha diversity indices were calculated with the QIIME2 plugin using Spearman correlation. Differential abundance between groups at each taxonomic level was tested using analysis of composition of microbiomes (ANCOM) [26].

Statistical analysis

The reported p-values were not corrected for multiple testing. Continuous variables are presented as medians and interquartile ranges (IQRs), whereas categorical variables are expressed as counts with percentages. Groups were compared with the Mann-Whitney *U* test, Fisher's exact test and Wilcoxon test using Statistica 13 software (StatSoft Inc., Tulsa, OK, US). Post-hoc power analysis was performed in package R [27], v.3.5.1. Power was set at 0.8 and the significance level (alpha level) was set at 0.05.

Results

Patient population

A total of 60 patients were initially interviewed, of whom 29 patients with T2DM and 11 patients with HNF1A-MODY agreed to participate and follow the study protocol. Of this group, we enrolled 24 patients who met the eligibility criteria.

Five patients had HNF1A-MODY and 19 patients had T2DM. The median age was 60 years. Median HbA_{1c} was 66 mmol/mol (8.2%), with an IQR of 62–70 mmol/mol (7.8–8.6%). In 5 patients with T2DM and 4 patients with HNF1A-MODY, linagliptin was added to current treatment (linagliptin group). In 1 patient with HNF1A-MODY and 14 patients with T2DM, an SU was initiated or the dose of a current SU was increased according to local guidelines (SU group). There were no differences in patient characteristics between groups at baseline (Table 1).

16S rRNA sequencing

Sequencing analysis of the 48 samples provided a mean 57,145 reads per sample (median, 52,547). The best sample contained 142,914 read pairs, while the worst contained 10,608. OTU picking resulted in 570, with total frequency of 2,742,964 features.

Bacterial profile at baseline

In the linagliptin and SU groups, respectively, baseline bacterial profiles with an abundance of > 1% at the phylum level were *Actinobacteria* (6.23% *vs*. 10.98%), *Bacteroidetes* (1.15% *vs*. 2.02%), *Firmicutes* (88.42% *vs*. 78.83%), *Proteobacteria* (1.83% *vs*. 2.81%) *Verrucomicrobia* (2.13% *vs*. 5.09%) and 'other' (0.24% *vs*. 0.28%; Figure 1A). At the class level, baseline bacterial profiles included *Actinobacteria* (1.27% *vs*. 4.50%), *Coriobacteriia* (4.96% *vs*. 6.47%), *Bacteroidia* (1.15% *vs*. 2.02%), *Bacilli* (2.21% *vs*. 2.39%), *Clostridia* (81.12% *vs*. 71.89%), *Erysipelotrichi* (5.09% *vs*. 4.55%), *Gammaproteobacteria* (1.56% *vs*. 2.75%), *Verrucomicrobiae* (2.13% *vs*. 5.09%) and 'other' (0.50% *vs*. 0.33%; Figure 1B).

Comparing the SU group with the linagliptin group prior to intensification, we observed a single significantly increased abundance at the class level (for *Actinobacteria*; centred log-ratio [clr]= 2.57, W = 5).

Table 1. Patient characteristics at baseline

Variable*	All patients (n = 24)	Linagliptin group (n = 9)	SU group (n = 15)	p-value
Male sex, n (%)	13 (54.2)	5 (55.6)	8 (53.3)	0.42
Age (years)	60 (55.5–62.5)	55 (50–61)	60 (57–63)	0.16
Duration of diabetes (years)	5 (1.9–9)	11 (1.3–23)	5 (2.5–6)	0.24
BMI [kg/m^2]	29.7 (26.8–32.2)	29 (26.8–32)	30 (26.8–32.5)	0.73
Obesity, n (%)	12 (50.0)	3 (33.3)	9 (60.0)	0.99
HbA_{1c} (%)	8.2 (7.8–8.6)	8.4 (8–9.7)	8.2 (7.6–8.4)	0.11
Creatinine [μmol/L]	81.2 (69.6–92)	75 (69.1–82)	85 (76.2–99.2)	0.1
TG [mmol/L]	2.4 (1.4–2.9)	2.9 (1.6–3.1)	1.9 (1.2–2.6)	0.14
TC [mmol/L]	5.4 (4.4–6.1)	5.2 (4.8–6.2)	5.5 (4.2–5.9)	0.77
LDL-C [mmol/L]	3.1 (2.3–3.8)	2.3 (1.9–3.7)	3.2 (2.4–3.8)	0.24
HDL-C [mmol/L]	1.2 (1.1–1.4)	1.2 (1.1–1.3)	1.2 (1–1.4)	0.82
CRP [mg/L]	3.3 (1–6.6)	1.1 (0.7–4)	4.2 (1.5–7.5)	0.06
AST [IU/L]	21.5 (18.5–29)	19 (18–33)	23 (20–26)	0.73
ALT [IU/L]	37 (27–48)	38 (36–45)	34 (25–49)	0.48
Leucocytes [× 10^9/L]	6.9 (6–8.7)	7.2 (6.3–9.9)	6.5 (5.8–8.6)	0.24
Erythrocytes [× 10^9/L]	4.8 (4.6–5.1)	5 (4.9–5.2)	4.6 (4.5–5.1)	0.11
Haemoglobin [g/dL]	14.7 (13.7–15.6)	14.9 (14.7–15.9)	14 (13.5–15)	0.06
Haematocrit (%)	43.7 (41–45.3)	44.1 (43.8–45.9)	42.3 (40.5–44.6)	0.08
Platelets [× 10^9/L]	220.5 (196.5–253)	222 (187–231)	219 (197–274)	0.52

*Continuous variables are summarised as medians (IQRs), qualitative variables are presented as the number (percentages)
ALT — alanine transaminase; AST — aspartate transaminase; BMI — body mass index; CRP — C-reactive protein; DPP-4 — dipeptidyl peptidase-4; HbA_{1c} — glycosylated haemoglobin; HDL-C — high-density lipoprotein cholesterol; IQR — interquartile range; LDL-C — low-density lipoprotein cholesterol; SU — sulfonylurea; TC — total cholesterol; TG — triglycerides

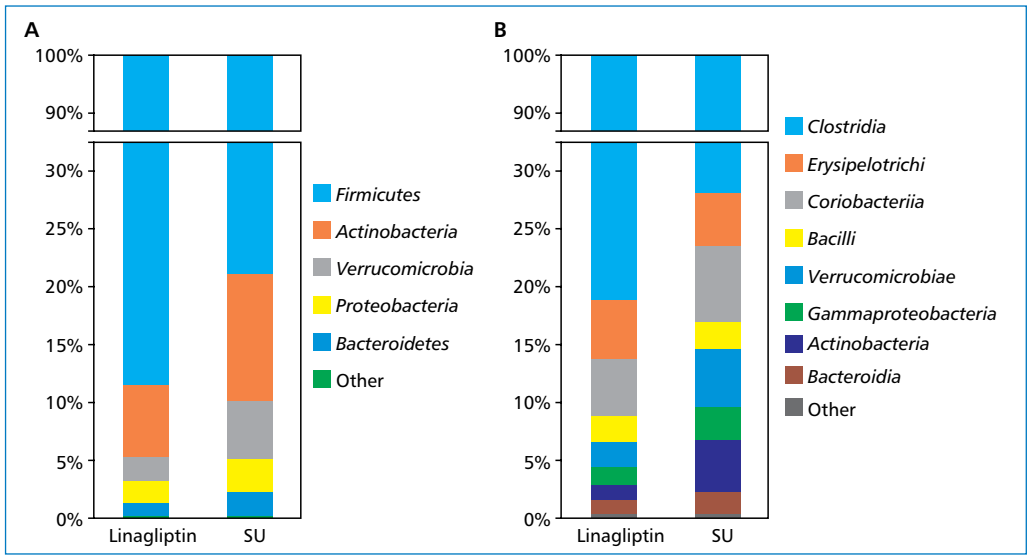

Figure 1. Composition of the bacterial community at the phylum — L2 (**A**) and class — L3 (**B**) levels for linagliptin and SU patients prior to intensification. Taxa with abundance below 1% were merged and represented as 'other'

No significant differences were observed between groups in ANCOM results for phylum, order, family, genus or species at baseline.

No differences in within-sample phylotype richness and evenness (alpha diversity) metrics were detected between groups. Shannon diversity (p = 0.14), Pielou's measure of evenness (p = 0.16), observed OTUs (p = 0.10) and Faith's phylogenetic diversity (p = 0.18) did not differ between linagliptin and SU samples (Figure 2). Linagliptin and SU samples did not show statisti-

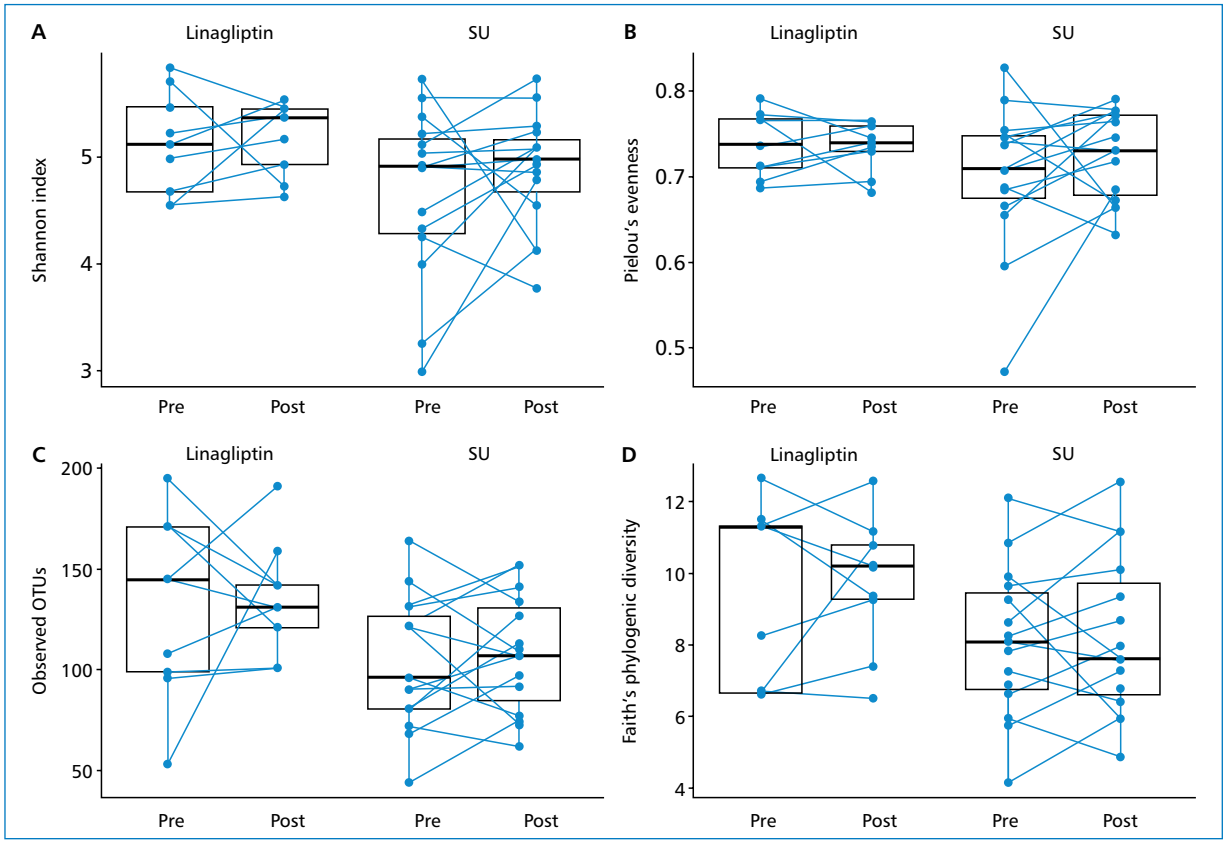

Figure 2. Pairwise alpha diversity analysis of linagliptin and sulfonylurea (SU) groups before and after treatment intensification. Differences in alpha diversity were measured by Shannon index (**A**), Pielou's measure of species evenness (**B**), observed OTUs (**C**) and Faith's phylogenetic diversity (**D**). Student's *t*-test was performed to analyse statistical significance

cally differences in any evaluated distance metrics: unweighted UniFrac (p = 0.39), weighted UniFrac (p = 0.60), Bray-Curtis (p = 0.78) and Jaccard (p = 0.39). Moreover linagliptin and SU patients were not separated or clustered according to PCoA of beta diversity metrics (Figure 3).

The two groups shared 397 OTUs, whereas 77 OTUs were unique to the linagliptin group and 89 were unique to the SU group.

Bacterial profile after optimisation of treatment

After 4 weeks of linagliptin treatment, ANCOM results showed no changes in taxa in L2–L7 compared with baseline. The same held true for pairwise alpha diversity, where differences in Shannon diversity (p = 0.59), Pielou's measure of evenness (p = 0.68), observed OTUs (p = 0.77) and Faith's phylogenetic diversity (p = 0.51) were not statistically significant (Figure 2). Moreover, after treatment intensification, unweighted UniFrac (p = 0.99, weighted UniFrac (p = 0.93), Bray-Curtis (p = 0.98) and Jaccard (p = 0.99) distances were also not significant (Figure 3). After treatment with linagliptin, we observed 43 new OTUs while 58 OTUs present at baseline were lost.

After optimisation of therapy in the SU group, we did not observe changes in taxa in L2–L7, compared with baseline, in the ANCOM analysis. We did not demonstrate changes in alpha diversity metrics (Shannon diversity, p = 0.19; Pielou's measure of evenness, p = 0.21; observed OTUs, p = 0.42; and Faith's phylogenetic diversity, p = 0.65; Figure 2) or beta diversity distances (unweighted UniFrac, p = 0.99; weighted UniFrac, p = 0.99; Bray-Curtis, p = 0.99; and Jaccard, p = 0.99; Figure 3). After treatment, we observed 50 new OTUs while 37 OTUs present at baseline were lost.

Continuous glucose monitoring

Continuous glucose monitoring data were collected before and after optimisation of treatment in 21 patients. The median glucose level in the linagliptin group (n = 9) was 178 (171–190) mg/dL at baseline and 166 (157–174) mg/dL after intensification (p = 0.02). In patients in the SU group with available data (n = 12), the median glucose level was 168 (159.5–190.5) mg/dL at baseline and 144.5 (131.5–154) mg/dL at follow-up (p = 0.015).

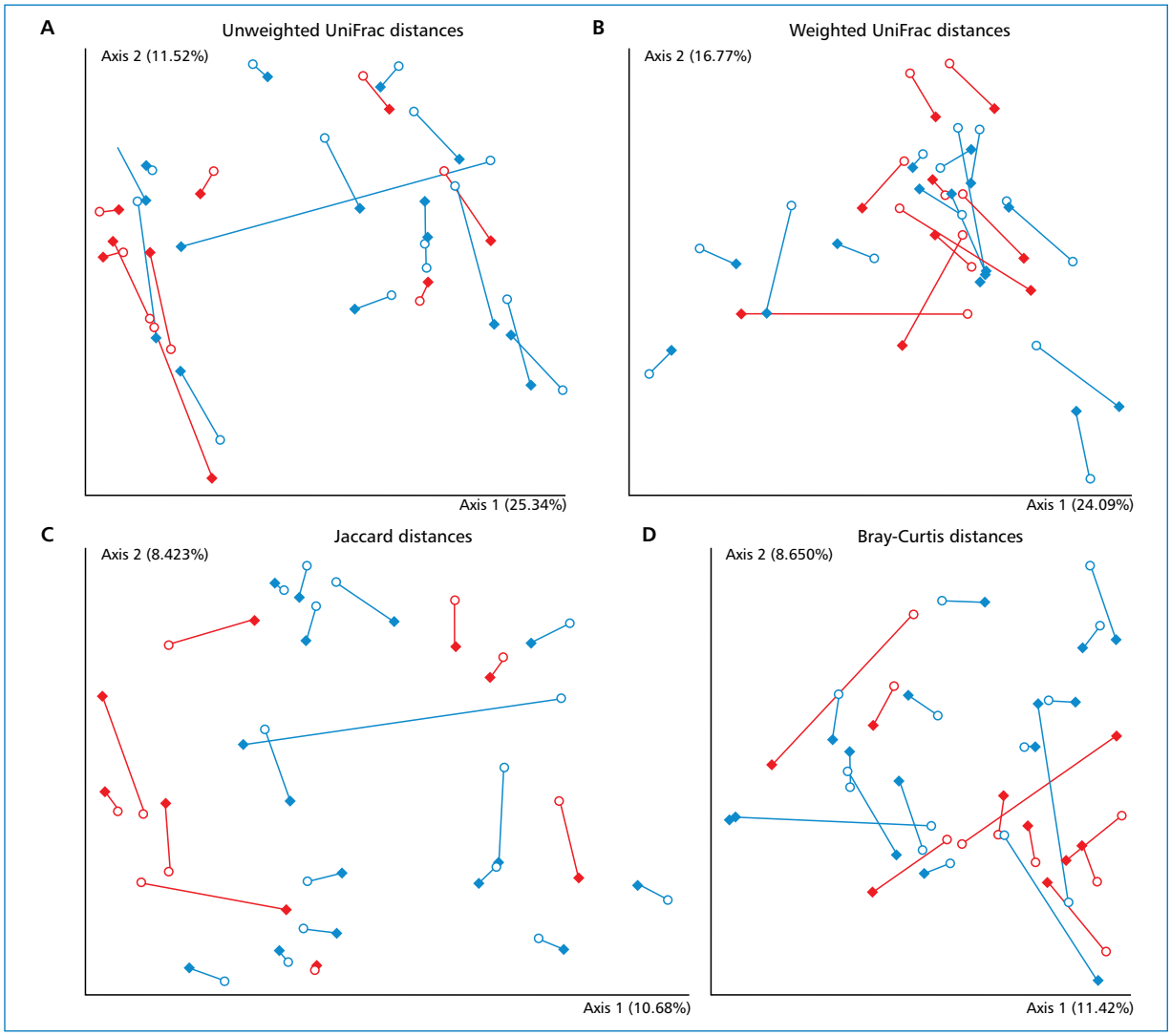

Figure 3. PCoA 2D plots of beta diversity analysis of linagliptin and sulfonylurea (SU) patients before and after treatment intensification. Beta diversity was measured by unweighted UniFrac distances (**A**), weighted UniFrac distances (**B**), Jaccard distances (**C**) and Bray-Curtis distances (**D**). Permutational multivariate analysis of variance (PERMANOVA) was performed to analyse statistical significance. Paired samples are connected with lines. Red lines represent linagliptin group and blue lines represent SU group. Rhombus represent pre-treatment intensification samples and rings represent post-treatment intensification samples

Post-hoc power analysis (sample size calculation)

Based on our results, we determined that comparing the SU group with the linagliptin group before intensification, we need at least 43 samples per group to achieve 0.8 statistical power with the 0.05 significance level. As for pre and post-treatment samples, the necessary sample size to achieve desired power at desired significance level is at least 95 patients for the linagliptin group, and at least 53 patients for SU group.

Discussion

To our knowledge, this is the first study to compare microbiota before and after the addition of linagliptin to current treatment in patients with T2DM or HNF1A--MODY. Although the analysis failed to show differences in microbiotal composition after treatment with either the DPP-4 inhibitor or SU intensification, our study was performed on a very small number of patients, in linagliptin group there were only nine patients.

The study was underpowered. No statistical methods were used to predetermine sample size and the final number of patients was limited. Post-hoc analysis revealed that much larger groups and need to detected differences between group.

The small sample size was at least partially due to the limitations on patients and requirement for stool samples imposed by the study protocol, which may have discouraged patient participation. In addition, due to expected difficulties with longer-term patient compliance, the study was designed to have a relatively

short follow-up period (4 weeks). We cannot exclude that differences in microbiotal composition would be observed during longer follow-up. Further studies should be conducted to examine and confirm this hypothesis-generating study. Of note, previous animal studies using DPP-4 inhibitors reported some changes in colonic microbiota after 8–12 weeks, although the results were equivocal [13–16].

So far, the effects of three DPP-4 inhibitors (vildagliptin, sitagliptin and saxagliptin) on microbiota have been investigated in animal models [13–16], but there are no previous animal or human studies on the effects of alogliptin or linagliptin on gut microbiota. Olivares et al. reported a reduction in genus *Oscillibacter* spp. and unclassified *Ruminococcaceae* (OTU 241) following 8 weeks of vildagliptin use [13]. Zhang et al. also observed changes in bacterial flora after administration of vildagliptin in diabetic rats for 12 weeks [14]. Yan et al. showed changes in microbiota composition in diabetic rats after the administration of sitagliptin for 12 weeks, with an increase in the relative abundance of *Bacteroidetes* and *Proteobacteria*, and a decrease in *Firmicutes* after treatment [15]. Zhang et al. also reported a higher relative abundance of *Bacteroidetes* and lower abundance of *Firmicutes* after treatment with vildagliptin. Both sitagliptin [15] and vidagliptin [14] have been shown to increase the amount of butyrate-producing bacteria. Finally, Wang et al. observed an increase in the relative abundance of *Firmicutes* after administration of saxagliptin for 8 weeks [16], in contrast to the studies of Yan et al. (sitagliptin) [15] and Zhang et al. (vildagliptin) [14].

One cannot exclude that the lack of effect on colonic microbiota in our study was due the use of linagliptin, which has not yet been evaluated for effects on colonic microflora in animals. The observed effect of linagliptin on microbiota across patients in our study was heterogeneous, however, we failed to determine factors associated with this phenomenon, possible due to limited patients number in subgroups. In more numerous subgroups we could notice different trends, for example in HNF1A and T2DM patients. Further studies could reveal potential features or phenotypes responsible for different trends in individual patients.

Furthermore, although patients were encouraged to follow general dietary recommendations for diabetes before the study, our results could also have been influenced by individual variations in diet composition, food preparation methods or quality of ingredients. For instance, one could speculate that individuals who could afford linagliptin (which is not reimbursed) could also afford better quality food. It has been reported that microbiota composition is predominately modified by diet [12, 28, 29]. In mice, changes in diet account for 57% of the variation in gut microbiota and genetic factors for 12% [30]. One cannot exclude that changes in diet composition attenuated the effect of DPP-4 inhibition.

Conclusions

We did not observe major differences in the structure and composition of the colonic bacterial flora after treatment with linagliptin in our preliminary study. However, the study was underpowered, and due to the small study size and short follow-up period, we cannot exclude some effect of linagliptin on the colonic microbiota. Further longitudinal studies based on larger populations should be performed to determine whether incretin-based drugs, including DPP-4 inhibitors, influence the colonic bacterial flora.

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Statement of competing interests

S. Mrozinska received a travel grant form Boehringer Ingelheim and a course grant from EGIS. The remaining authors declared no conflict of interest.

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Role of serum allograft inflammatory factor-1 (AIF-1) in Egyptian type 2 diabetic patients

ABSTRACT

Background. Diabetes mellitus (DM) is a powerful and independent risk factor for cardiovascular disease. The atherosclerosis process in diabetes is indistinguishable from that of the nondiabetic population, but it begins earlier and is often more extensive and more severe. AIF-1 promotes chemotaxis, spreading and migration of macrophages and vascular smooth muscle cells (VSMCs) which suggest a role of AIF-1 in the atherosclerotic plaque formation. Thus, this study determines the role of AIF-1 in the Egyptian type 2 diabetic patients.

Results. The level of AIF-1 was significantly higher in the type 2 diabetic group when compared to the control group ($p = 0.000$). In type 2 diabetic patients group, there was a significant positive correlation between CIMT and AIF-1 ($r = 0.468$, $p = 0.000$). In addition to the positive correlation between CIMT and AIF-1, CIMT in regression model analysis was significantly positive contributing to the outcome variable (AIF-1) ($p < 0.05$), denoting the possible role of elevated serum AIF-1 level in atherosclerotic process with further studies on larger scale needed. (Clin Diabetol 2019; 8, 6: 271–276)

Key words: allograft inflammatory factor-1 (AIF-1), diabetes mellitus (DM), atherosclerosis, inflammation

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Introduction

Diabetes mellitus (DM) is a powerful and independent risk factor for cardiovascular disease which remains to be the major cause of death in type 2 diabetic patients [1, 2]. Low grade inflammation and activation of the immune system play a role in the common pathogenesis of both insulin resistance and endothelial dysfunction and subsequently the development of type 2 diabetes and atherosclerosis [2–4].

Subclinical vascular disease in type 2 diabetic patients was proved to be assessed by carotid intima media thickness (CIMT) [5–7] and the presence of plaques (calcified or not) [8] specially in patients with normal renal function by many researches. The dyslipidemic lipid profile (increased LDL and total cholesterol) usually associated with type 2 diabetes and adds more impact to the process of atherosclerosis process development in these patients [9–11].

AIF-1 (ionized calcium-binding adaptor molecule-1 (Iba1) is a 17 kDa conserved structural cytoplasmic, calcium-binding, inflammation-responsive scaffold protein [12, 13]. It is one of the EF hand proteins' family [14, 15].

AIF-1 was originally identified in rat cardiac allografts with chronic rejection. In humans, AIF-1 is involved in many pathological processes where it has been found to be expressed by activated T cells, vascular endothelial cells and blood vessel smooth muscle cells after a balloon injury ie: vascular trauma inducible cytokine [16, 17]. Thus AIF-1 may play a role in endothelial dysfunction, macrophages and VSMCs activation, migration, reorganization and tissue remodelling as a response to endothelial damage [3, 16, 18].

The in vivo expression of AIF-1 in human VSMCs in atherosclerotic plaques supported the in vitro studies

that have shown the role of AIF-1 in atherosclerotic plaque formation through promotion of chemotaxis, cell attachment, spreading and migration of macrophages and VSMCs [19].

This work aimed was to study serum AIF-1 in type 2 diabetic patients.

Materials and methods

One hundred subjects were included in the study after approval of the ethical committee of Medical Research Institute and informed consents was taken. The included subjects were divided into two groups; Forty apparently healthy volunteers as a control group (group 1), sixty type 2 diabetic patients (group 2). Subjects with obesity, urinary tract infections UTI and renal impairment or increased urinary proteins were excluded. The source of finance was supplied by authors.

To all studied subjects; thorough history taking was done including history of cardiovascular diseases, smoking habits, drinking habits, medications such as anti-diabetic, anti-hypertensive and anti-hyperlipemic drugs. Complete physical examination with special stress on BMI calculation, cardiovascular examination and measuring the carotid intima media thickness (CIMT) and presence of plaques (calcified or not) using a β mode ultrasound.

The following laboratory investigations were done to all participating subjects: quantitation of urinary albumin, urinary proteins, creatinine, calculation of urinary albumin and protein to creatinine ratio, estimation of serum fasting and postprandial glucose, urea, creatinine, calculation of estimated glomerular filtration rate (eGFR) [20], urine cultures and determination of hemoglobin A_{1c} [21]. In addition to estimation of serum lipid profile (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides with calculation of low density lipoprotein cholesterol (LDL-C). Also, serum C-reactive protein (CRP) [22] was estimated and serum AIF-1 by ELISA technique (Biocompare, South San Francisco, USA, catalog no. SEC288Hu) [23].

Data analysis was performed by using SPSS for Windows, version 20 (Statistical Package of social sciences, Chicago, USA) [24]. Normality of the quantitative variables was determined with the use of the Kolmogrov-Smirnov test. Where normally distributed data were presented as mean ± standard deviation and unpaired Student's t-test was used to compare a variable across two subgroups in addition to Pearson's correlation test was used to investigate the relationship between different quantitative variables. The non-normal distributed data were presented as median (minimum–maximum). Non parametric Mann-Whitney test was used to compare the variables across two groups. Stepwise multiple regression analysis was conducted to test the variables contributing in the AIF-1 marker.

Table 1. Statistical significance of clinical data of the studied groups

Item	Group 1 (n = 40)	Group 2 (n = 60)
Age (years)		
Mean	46.15	56.87
SD	± 8.41	± 8.00
p	0.000**	
Sex		
Males	19 (47.50%)	25 (41.79%)
Females	21 (52.50%)	35 (58.30%)
p	0,794	
Weight [kg]		
Mean	68.15	74.62
SD	± 4.38	± 5.45
p	0.000**	
Height [m]		
Mean	1.71	1.75
SD	± 0.05	± 0.04
p	0.001*	
BMI [kg/m^2]		
Mean	23.36	24.38
SD	± 1.39	± 0.75
p	0.000**	
Hypertension		
Yes	0 (0%)	19 (31.7%)
No	20 (100%)	41 (68.3)
p	0.004*	
CIMT [mm]		
Mean	0.285	1.235
SD	± 0.12	± 0.32
p	0.000**	
Plaques and calcification		
Yes	0 (0%)	15 (25%)
No	20 (100%)	45 (75%)
p	0.013*	

χ^2, Chi square test, Mann-Whitney test; p — p value for comparing between the two groups; *statistically significant at $p \leq 0.05$

Results

Blood pressure, CIMT and presence of plaques and calcification in type 2 diabetic patients was significantly increased comparing to controls ($p = 0.004$, $p = 0.000$, $p = 0.013$) respectively (Table 1).

Glycemic control measures showed significant increase in fasting, post prandial blood glucose and HbA_{1c} in type 2 diabetic patients than controls ($p = 0.000$, $p = 0.000$, $p = 0.000$) respectively (Table 2).

Table 2. Statistical significance of laboratory data in the studied groups

Item	Group 1 (n = 40)	Group 2 (n = 60)
Total cholesterol [mg/dL]		
Mean	152.85	189.25
SD	± 11.27	± 51.53
p	0.003*	
HDL-C [mg/dL]		
Mean	51.05	37.55
SD	± 6.70	± 9.71
p	0.000**	
TG [mg/dL]		
Mean	80.05	128.8
SD	± 25.81	± 47.29
p	0.000**	
LDL-C [mg/dL]		
Mean	85.79	125.94
SD	± 10.38	± 43.83
p	0.000**	
CRP [mg/L]		
Median	3.00	10.00
Min–Max	(2.00–4.50)	(2.00–650.00)
p	0.000**	
Fasting serum glucose [mg/dL]		
Mean	87.35	203.53
SD	± 9.4	± 72.13
p	0.000**	
Postprandial serum glucose [mg/dL]		
Mean	108.15	190.025
SD	± 15.39	± 76.33
p	0.000**	
HbA_{1c} (%)		
Mean	4.63	7.93
SD	± 0.37	± 2.49
p	0.000**	
Serum urea [mg/dL]		
Mean	27.6	29.47
SD	± 6.83	± 7.32
p	0.336	
Serum creatinine [mg/dL]		
Mean	0.78	0.78
SD	± 0.14	± 0.11
p	0.957	
eGFR [ml/min]		
Median	103.5	98.6
Min–Max	(91.70–134.7)	(90.10–157.60)
p	0.065	
Urine protein/creatinine ratio [mg/g]		
Mean	100.5	110.93
SD	± 23.74	± 25.17
p	0.108	
Urine albumin/creatinine ratio [mg/g]		
Mean	20.25	22.67
SD	± 4.13	± 4.6
p	0.04*	
Serum AIF1 [pg/mL]		
Median	90.00	1.235
Min–Max	(20.00–500.00)	± 0.32
p	0.000**	

χ^2, Chi square test, Mann-Whitney test; p — p value for comparing between the two groups; *statistically significant at $p \leq 0.05$

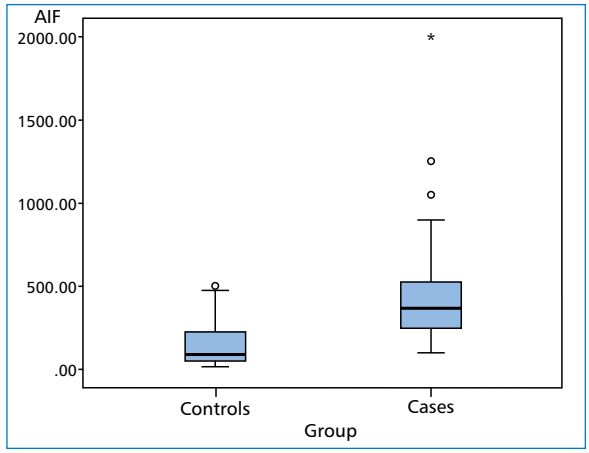


Figure 1. Box plot illustrates the median and range of serum AIF-1 readings [pg/mL] among the studied groups. Box plot represents the interquartile range 25^{th}–75^{th} percentile. The wiskers below and above represents 10–90 percentile. The line across each box represents the median value. Significant increase in AIF-1 in the group of diabetic atherosclerotic patients when compared to the control group (p = 0.000)

Table 3. Statistical significant correlations between the studied parameters in the type2 diabetic group (group 2)

Item	Correlation coefficient (r)	Significance (p-value)
AIF-1 and creatinine	0.338	0.008
AIF-1 and CIMT	0.468	0.000
BMI and HbA_{1c}	0.343	0.007
BMI and fasting serum glucose	0.376	0.003
HbA_{1c} and CIMT	0.257	0.047

Lipid profile and CRP levels showed significant increase in cholesterol, triglycerides, LDL and CRP in type 2 diabetic patients than control (p = 0.003, p = 0.000, p = 0.000, p = 0.000) respectively (Table 2).

Renal functions tests showed only a significant increase in albumin creatinine ratio in the type 2 diabetic patients than controls (p = 0.04) (Table 2).

AIF-1 showed significant increase in type 2 diabetic patients than control group (p = 0.000) (Table 2, Figure 1).

Pearson's correlation test showed positive significant correlations only between AIF-1 and creatinine level in addition to CIMT (r = 0.3380, r = 0.468) (p = 0.008, p = 0.000) respectively in type 2 diabetic patients (Table 3).

Multiple regression analysis showed the overall model was statistically significant (F = 3.309,

p = 0.001). Where, CIMT was significantly positive contributing to the outcome variable (AIF-1) ($p < 0.05$), while other predictors were not significantly contributing to the outcome ($p > 0.05$) (Table 4).

Discussion

Diabetes when accompanied by other major cardiovascular risk factors, such as hypertension, dyslipidemia, and smoking, show marked increase in the incidence of atherosclerosis. The atherosclerosis process begins earlier and more aggressive in diabetes and indistinguishable from nondiabetic population [2]. The atherogenic lipid profile characterized by elevated triglycerides and low levels of high-density lipoprotein (HDL) cholesterol are major modifiable risk factors contributing to progressive cardiovascular risk generally in addition to both type 2 diabetes and metabolic syndrome [25, 26].

Atherosclerosis is known to be an inflammatory disease as an overall process where inflammatory cells (pro-inflammatory cytokines secreting cells) such as neutrophils, lymphocytes and monocytes participate in the overall process of atheroscelerosis [27].

AIF-1 plays a role in endothelial cell, macrophage, T-lymphocyte, and VSMC activation, proliferation and migration which are known to play a role in inflammation [3, 16, 18].

It has been shown that serum AIF-1 was increased in patients with diabetic nephropathy [23], also it is considered as a human adipokine produced mainly by macrophages within the white adipose tissue in obese patients and might participate in the regulation of adipose tissue inflammation and, in turn, insulin resistance [28]. In the present study, all the studied groups were of normal e GFR and BMI.

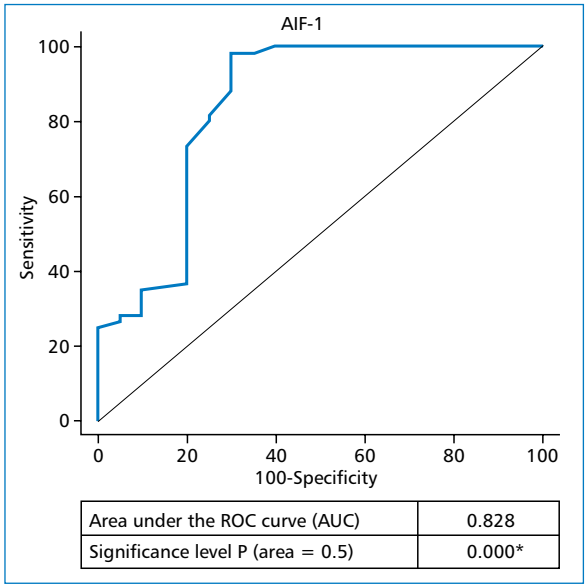
The level of AIF-1 was significantly higher in type 2 diabetic groups when compared to the control group (p = 0.000) (Figure 1). The statistical correlation in our study revealed that there was a significant positive correlation between CIMT and AIF-1 (r = 0.468, p = 0.000) in type 2 diabetic patients group, denoting the possible role of elevated serum AIF-1 level in atherosclerotic process. In addition the significant increase in the presence of plaques and calcification in the patients than controls (p = 0.013) has been seen. Although there is significant increase in dyslipidemic profile of type 2 diabetic patients than controls (cholesterol, triglycerides and LDL (p = 0.003, p = 0.000, p = 0.000) which add to the process of atherosclerosis development in these patients, there were no significant correlations between dyslipidemia and AIF-1 level in these patients thus a study on larger scale of type 2 diabetic patients is needed.

In multiple linear regression analysis, the overall model is statistically significant (F = 3.309, p = 0.001) and it is found that increased CIMT is significantly contributing to elevated serum AIF-1 level in type 2 diabetic patients ($p < 0.05$). Thus, elevated serum AIF-1 level in type 2 diabetic patients in addition to its positive significant correlation with CIMT add to the possible role of AIF1 in contribution to the process of atherosclerotic changes in type 2 diabetic patients. This was consistent with Berglund et al., 2012 [29] who stated that expression of AIF-1 mRNA in human carotid plaques

Table 4. Multiple regression analysis

Model	Regression coefficients		t	Sig.	95% confidence interval for B	
	B	S.E			Lower bound	Upper bound
(Constant)	1235.852	1068.189	1.157	0.251	–895.687	3367.39
Age	–6.52	5.094	–1.191	0.280	–16.687	3.648
BMI	–10.074	40.213	–0.251	0.803	–90.318	70.170
Hypertension	–139.901	94.727	–1.477	0.144	–328.554	49.124
Plaques and calcification	68.936	115.962	0.594	0.554	–162.462	300.334
CIMT	502.386	127.204	3.949	0.001*	248.554	756.217
Fasting blood sugar	–0.449	0.746	–0.602	0.549	–1.939	1.040
Post prandial blood sugar	0.118	0.703	0.168	0.867	–1.284	1.520
Albumin to creatinine ratio	0.467	0.897	0.134	0.890	–2.284	3.652
HbA_{1c}	–20.978	33.729	–0.622	0.536	–88.340	46.383
Cholesterol	0.421	1.264	0.333	0.740	–2.102	2.945
LDL	0.169	1.224	–1.581	0.138	–2.273	2.611
Triglycerides	–0.185	1.286	–0.144	0.886	–2.753	2.383
CRP	–0.353	0.479	–0.078	0.464	–1.640	0.603
e-GFR	–6.404	3.486	–1.837	0.071	–13.361	0.552

*Predictors are significantly contributing to the outcome variable if $p < 0.05$



Area under the ROC curve (AUC)	0.828
Significance level P (area = 0.5)	0.000*

Figure 2. ROC curve for serum AIF-1 in the studied groups at a cutoff value of 120 pg/mL. Receiver operating characteristic curve (ROC) for serum AIF-1 at cutoff value of 120 pg/mL, the area under the ROC curve denotes the diagnostic performance of the test (0.0828), significance p = 0.000*

associates with less extracellular matrix and a more pro-inflammatory plaque and plasma profile, features that may predispose to plaque rupture.

Zhao et al, 2013 [14] reported that AIF-1 is expressed by activated lipid-rich macrophages and VSMCs during the progression of atherosclerosis. In this regard, AIF-1 may be involved in the early stage of atherosclerosis and CAD. Kelemen et al, in 2005 [30] stated that increased AIF-1 expressed in activated T lymphocytes enhance activation of adjacent arterial vascular smooth muscle cells (VSMCs) and development of atherosclerosis. Expression of AIF-1 seems to be involved in vascular smooth muscle cells and macrophages migration, chemotaxis, proliferation and tissue remodeling as a response to endothelial damage which suggest a positive role of AIF-1 in the atherosclerotic plaque formation. These in vitro studies were supported by immunohistochemical analysis which has shown in vivo protein expression of AIF-1 in human smooth muscle cells in atherosclerotic plaques [19].

In the present work, by drawing receiver operating characteristic (ROC) curve of serum AIF-1 at a cutoff value of 120 pg/mL in the studied group (Figure 2), the area under the curve (AUC) for AIF-1 was 0.843, $p \leq 0.0001$. The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of AIF-1 for detection of atherosclerosis were 98.33%, 70%, 91%, 93% and 91% respectively. Thus, the present study supported that AIF-1 can be a helpful marker for early detection of atherosclerosis and prediction of cardiovascular complications in type 2 diabetic patients.

Conclusion

In conclusion, AIF-1 was significantly higher in the type 2 diabetic patient group (group 2) when compared to the control group (group 1). In the group of type 2 diabetic patients AIF-1 showed positive correlation with CIMT denoting the possible relation between increased AIF-1 levels and subsequent vascular damage and the process of atherosclerosis in type 2 diabetes.

Recommendations: further work is needed on a larger number of type 2 diabetic patients with higher focus on the presence of vascular calcification and atherosclerosis.

Compliance with ethical standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

Conflicts of interest

ElDeeb MK, Khalil GI, Zaki MA, Mehana E, Omer S declare that they have no conflict of interest.

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Correlations between biomarkers of oxidative stress, glycemic control and insulin resistance in women with type 2 diabetes

ABSTRACT

Background. The main characteristic of type 2 diabetes mellitus (T2DM) is hyperglycemia due to insulin resistance. Enhanced oxidative stress owing to increased oxygen free radicals and/or reduced antioxidant defense has very important roles in T2DM development and also most of its complications. The aim of the current study was to evaluate correlations between biomarkers of oxidative stress, glycemic control and insulin resistance in women with T2DM.

Materials and methods. Seventy nine women with T2DM were included in the current study and fasting blood samples were collected. Hemoglobin A_{1c} (HbA_{1c}); glucose; oxidative stress biomarkers including malodialdehyde, 8-isoprostane, catalase and total antioxidant capacity (TAC) were measured. The adiponectin/leptin (A/L) ratio and the homeostasis model assessment of beta-cell function (HOMA-B) were calculated. The results were considered significant when the p-value was less than 0.05.

Results. Serum levels of TAC showed a significant positive correlation with the A/L ratio ($r = 0.261$, $p = 0.02$). A significant negative correlation was observed between values of HbA_{1c} and TAC ($r = –0.300$, $p = 0.007$). However, HbA_{1c} correlated positively with 8-isoprostane ($r = 0.236$, $p = 0.036$). Values of HOMA-B correlated negatively with values of HbA_{1c} ($r = –0.327$, $p = 0.003$). Serum levels of 8-isoprostane were significantly higher in obese (BMI > 30 kg/m^2) women than in non-obese (BMI < 30 kg/m^2) women ($p = 0.032$). Values of catalase ($p = 0.022$) and HOMA-B ($p = 0.009$) were significantly lower in women with $HbA_{1c} \geq 7.6\%$ compared with women with $HbA_{1c} < 7.6\%$.

Conclusions. In summary, chronic hyperglycemia results in oxidative stress. This situation might lead to less beta cells function. In addition, low levels of the A/L ratio were associated with increased oxidative stress.
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Key words: type 2 diabetes, oxidative stress, hyperglycemia, insulin resistance, reactive oxygen species

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Introduction

The main characteristic of type 2 diabetes mellitus (T2DM) is hyperglycemia due to insulin resistance. In-

sulin resistance also can lead to metabolic impairment of other biomolecules such as lipids and proteins [1]. Enhanced oxidative stress owing to increased oxygen free radicals and/or reduced antioxidant defense has very important roles in T2DM development and also most of its complications [2]. Studies have shown that oxidative stress has a central role in insulin resistance development [3].

Increased circulating levels of glucose can cause oxidative stress via overproduction of reactive oxygen species [4]. On the other hand, beta cells function is impaired during chronic hyperglycemia because of increased oxidative stress which damages the cells [5].

Since antioxidant enzymes activities are low in beta cells, they are very sensitive to destructive effects of oxidative stress. On the other hand, lipotoxicity induced in beta cells by means of oxidative stress might be a central mechanism of destructive effects of reactive oxygen species in these insulin-secreting cells [6]. Current literature shows that oxidant/antioxidant balance is disrupted in people with obesity that leads to increased oxygen free radicals production which means an oxidative stress condition [4].

The current study was designed to evaluate correlations between biomarkers of oxidative stress, glycemic control and insulin resistance in women with T2DM.

Materials and methods

The current study was performed after approving by the Ethics Committee of the University. Eighty Persian women with T2DM were selected using convenience sampling method according to World Health Organization (WHO) [7] criteria after signing an informed consent form. One of the patients was excluded from the study. Therefore, the final sample size became 79 women with T2DM. Among the patients 54% were in premenopausal status and 46% were in postmenopausal status ($p = 0.574$). Age range of the patients was 40–65 years.

No more than 2 years of T2DM duration and not taking antioxidants supplements during the last three months were inclusion criteria of the current study. Patients under treatment with insulin and other hormone, anticoagulants, diuretics and β-blockers were excluded from the study. Other exclusion criteria were alcoholism, smoking, pregnancy, lactation and any chronic renal, hepatic, thyroid, haematic and gastrointestinal disorders.

Systolic and diastolic blood pressures, waist circumference (WC) and weight were measured and body mass index (BMI) was calculated and reported as kg/m^2.

Subjects were divided into two groups including obese (BMI > 30 kg/m^2) and non-obese (BMI < 30 kg/m^2) [8]. Subjects were also divided into two groups according to hemoglobin A_{1c} (HbA_{1c}) values (HbA_{1c} < 7.6% and $HbA_{1c} \geq 7.6\%$) [9]. As glucose-lowering drugs, all patients were under treatment with metformin or a combination of metformin and glibenclamide.

After 12 hours of overnight fasting, blood samples were collected. Hemoglobin A_{1c} was assessed using column chromatography method (Biosistem, Spain). Serum values of glucose and Gamma-glutamyltransferase enzyme (GGT) were evaluated using commercially available colorimetric methods (Parsazmun, Iran). Other assays were included activity of catalase using spectrophotometric method [10], the ferric reducing ability of plasma (FRAP) assay for evaluating total antioxidant capacity (TAC) [11] and the thiobarbituric acid (TBA) assay for determining malodialdehyde (MDA) [12]. Other assays included insulin (Monobind Inc., USA), leptin and total adiponectin (BioVendor Laboratory Medicine, Inc. Czech Republic) and free 8-isoprostane (Cayman Chemical, Ann Arbor, MI, USA) using enzyme-linked immunosorbent assay (ELISA) on a microplate reader (STAT FAX 4200, USA).

The homeostasis model assessment of insulin resistance (HOMA-IR) [13], the quantitative insulin sensitivity check index (QUICKI) [14], the homeostasis model assessment of β-cell function (HOMA-B) [5], the leptin/adiponectin ratio (L/A) [13] and the adiponectin/leptin ratio (A/L) [15] were calculated.

Statistical analysis were done using Kolmogorov–Smirnov test for exploring normal and skewed distributed variables, independent-samples t-test and Mann-Whitney U-test for analyzing differences in demographic and biochemical data in Tables 1 and 2 and Pearson's and Spearman's correlation analyses for exploring correlations between biochemical variables in SPSS 19 software (SPSS Inc, Chicago, IL). Chi-square test was used for qualitative analysis. The mean ± SEM was used for expressing the variables and were considered statistically significant at a p-value less than 0.05.

Results

Demographic and biochemical characteristics of women with T2DM are presented in Table 1. Correlations were assessed in whole of the subjects. Serum levels of malodialdehyde correlated negatively with levels of adiponectin ($r = –0.30$, $p = 0.007$) (Figure 1). A negative correlation was observed between serum levels of 8-isoprostane and activities of catalase ($r = –0.24$, $p = 0.032$). A significant positive correlation was observed between serum activities of GGT and values of waist circumference ($r = 0.23$, $p = 0.041$). Serum levels of TAC showed a significant positive correlation with the A/L ratio ($r = 0.261$, $p = 0.02$) (Figure 2).

Table 1. Demographic and biochemical characteristics of women with type 2 diabetes mellitus (T2DM)

Variables	T2DM (n = 79)
Age (years)	53.09 ± 0.73
Duration of diabetes (year)	4.94 ± 0.30
Waist [cm]	103.68 ± 0.99
Weight [kg]	72.98 ± 1.05
BMI [kg/m^2]	28.53 ± 0.38
SBP [mm Hg]	12.59 ± 0.17
DBP [mm Hg]	8.03 ± 0.08
FBG [mg/dl]	139.86 ± 5.41
HbA_{1c} (%)	8.68 ± 0.24
Insulin [mIU/l]	13.99 ± 0.50
Adiponectin [μg/ml]	6.71 ± 0.33
Leptin [μg/ml]	21.92 ± 1.35
A/L ratio	0.43 ± 0.04
L/A ratio	3.94 ± 0.38
MDA [nmol/ml]	11.37 ± 0.39
8-isoprostane [pg/ml]	401.19 ± 9.96
Catalase [KU]	2.05 ± 0.11
GGT [U/L]	32.94 ± 2.50
TAC [mmol/L]	0.30 ± 0.01
HOMA-IR	4.82 ± 0.25
HOMA-B	99.84 ± 12.50
QUICKI	0.31 ± 0.002

Results are presented as mean ± SEM. BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure; FBG — fasting blood glucose; HbA_{1c} — hemoglobin A_{1c}; A/L — the adiponectin/leptin ratio; L/A — the leptin/adiponectin ratio; MDA — malodialdehyde; GGT — gamma-glutamyl transferase; TAC — total antioxidant capacity; HOMA-IR — homeostasis model assessment of insulin resistance; HOMA-B — homeostasis model assessment of beta-cell function; QUICKI — quantitative insulin sensitivity check index

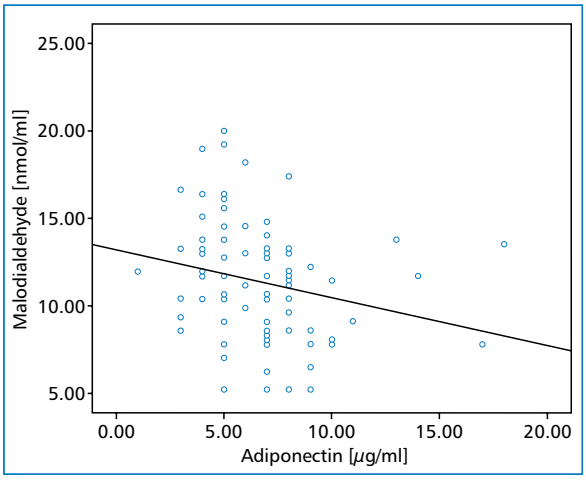

Figure 1. Correlation between serum levels of malodialdehyde and levels of adiponectin in women with type 2 diabetes mellitus (r = –0.30, p = 0.007)

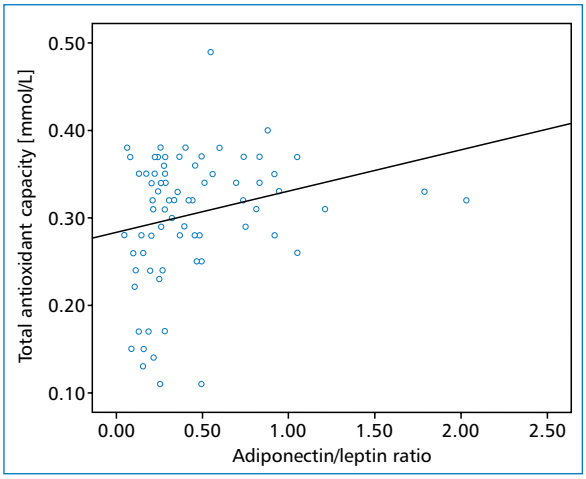

Figure 2. Correlation between serum values of total antioxidant capacity and the adiponectin/leptin ratio in women with type 2 diabetes mellitus (r = 0.261, p = 0.02)

However, TAC correlated negatively with the L/A ratio (r = –0.262, p = 0.02). A significant negative correlation was observed between values of HbA_{1c} and TAC (r = –0.300, p = 0.007). However, HbA_{1c} correlated positively with 8-isoprostane (r = 0.236, p = 0.036) (Figure 3). Values of HOMA-B correlated negatively with values of HbA_{1c} (r = –0.327, p = 0.003) (Figure 4). A significant positive correlation was observed between values of FBS and HbA_{1c} (r = 0.32, p = 0.004). Serum activities of GGT showed a slightly positive correlation with levels of malodialdehyde (r = 0.219, p = 0.051).

Comparison of demographic and biochemical characteristics of obese and non-obese women with T2DM was presented in Table 2. Obese women had higher values of waist circumference (p < 0.001), weight (p < 0.001) and BMI (p < 0.001) than non-obese women. Serum levels of 8-isoprostane were significantly higher in obese women than in non-obese women (p = 0.032). Marginally decreases were observed in values of HOMA-B (p = 0.091) and TAC (p = 0.087) in obese women compared with non-obese women. HOMA-IR was nonsignificantly higher in obese women compared with non-obese women.

Table 3 shows comparison of demographic and biochemical characteristics of women with T2DM according to HbA_{1c} values. Women with $HbA_{1c} \geq 7.6\%$ had higher values of FPG (p = 0.006), HbA_{1c} (p < 0.001), SBP (p = 0.006) and HOMA-IR (p = 0.027) than women with $HbA_{1c} < 7.6\%$. However, values of catalase (p = 0.022)

Table 2. Comparison of demographic and biochemical characteristics of obese (BMI > 30 kg/m^2) and non-obese (BMI < 30 kg/m^2) women with type 2 diabetes mellitus

Variables	Non-obese (n = 50)	Obese (n = 29)	p
Age (years)	52.80 ± 0.93	53.59 ± 1.20	0.609
Duration of diabetes (year)	5.06 ± 0.36	4.72 ± 0.54	0.598
Waist [cm[	100.84 ± 1.09	108.59 ± 1.57	< 0.001
Weight [kg]	68.02 ± 1.06	81.53 ± 0.89	< 0.001
BMI [kg/m^2]	26.31 ± 0.28	32.35 ± 0.25	< 0.001
SBP [mm Hg]	12.54 ± 0.22	12.67 ± 0.27	0.881
DBP [mm Hg]	7.93 ± 0.10	8.21 ± 0.13	0.188
FBG [mg/dl]	134.04 ± 6.43	149.90 ± 9.56	0.154
HbA_{1c} (%)	8.57 ± 0.31	8.86 ± 0.37	0.319
Insulin [mIU/l]	14.16 ± 0.62	13.69 ± 0.87	0.652
Adiponectin [μg/ml]	6.60 ± 0.41	6.90 ± 0.54	0.906
Leptin [μg/ml]	22.16 ± 1.69	21.51 ± 2.29	0.818
A/L ratio	0.44 ± 0.06	0.42 ± 0.05	0.428
L/A ratio	4.27 ± 0.55	3.39 ± 0.41	0.428
MDA [nmol/ml]	11.20 ± 0.51	11.65 ± 0.60	0.585
8-isoprostane [pg/ml]	385.03 ± 13.21	429.05 ± 13.53	0.032
Catalase [KU]	2.02 ± 0.14	2.10 ± 0.18	0.744
GGT [U/L]	32.51 ± 3.30	33.67 ± 3.80	0.473
TAC [mmol/L]	0.31 ± 0.01	0.29 ± 0.01	0.087
HOMA-IR	4.63 ± 0.27	5.16 ± 0.51	0.316
HOMA-B	107.69 ± 15.98	86.31 ± 20.13	0.091
QUICKI	0.31 ± 0.002	0.31 ± 0.003	0.677

Results are presented as mean ± SEM. Abbreviations are given in Table 1

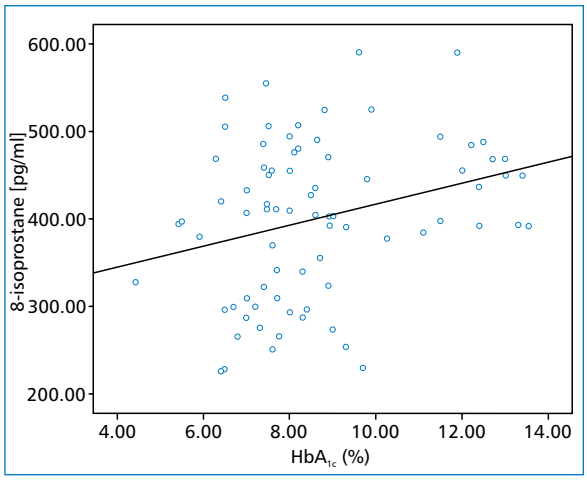

Figure 3. Correlation between values of HbA_{1c} and 8-isoprostane in women with type 2 diabetes mellitus (r = 0.236, p = 0.036)

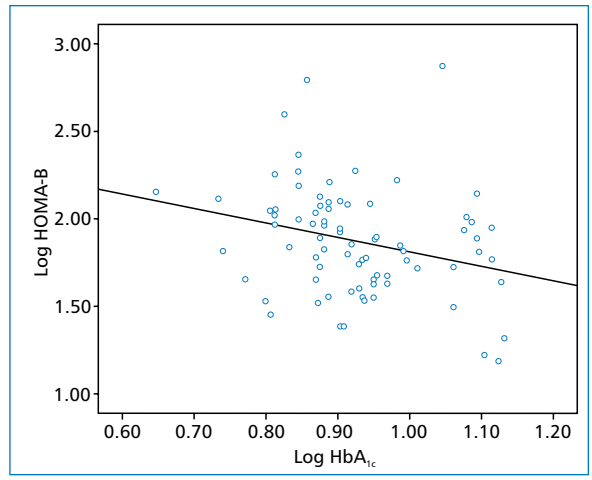

Figure 4. Correlation between values of HbA_{1c} and the homeostasis model assessment of beta-cell function (HOMA-B) in women with type 2 diabetes mellitus (r = –0.327, p = 0.003)

Table 3. Comparison of demographic and biochemical characteristics of women with type 2 diabetes mellitus according to HbA_{1c} values

Variables	HbA_{1c} < 7.6% (n = 27)	HbA_{1c} ≥ 7.6% (n = 52)	p
Age (years)	53.44 ± 1.02	52.90 ± 0.99	0.730
Duration of diabetes (year)	5.15 ± 0.43	4.83 ± 0.41	0.620
Waist [cm]	102.56 ± 1.94	104.27 ± 1.11	0.413
Weight [kg]	71.41 ± 1.76	73.80 ± 1.30	0.282
BMI [kg/m^2]	27.89 ± 0.58	28.86 ± 0.50	0.233
SBP [mm Hg]	11.92 ± 0.24	12.93 ± 0.21	0.006
DBP [mm Hg]	7.91 ± 0.14	8.10 ± 0.10	0.605
FBG [mg/dl]	120.48 ± 6.16	149.92 ± 7.22	0.006
HbA_{1c} (%)	6.74 ± 0.14	9.68 ± 0.26	< 0.001
Insulin [mIU/l]	14.12 ± 0.76	13.92 ± 0.66	0.852
Adiponectin [μg/ml]	6.59 ± 0.59	6.77 ± 0.39	0.619
Leptin [μg/ml]	23.23 ± 2.43	21.24 ± 1.63	0.487
A/L ratio	0.38 ± 0.05	0.46 ± 0.05	0.451
L/A ratio	4.09 ± 0.59	3.87 ± 0.49	0.451
MDA [nmol/ml]	11.94 ± 0.71	11.07 ± 0.46	0.290
8-isoprostane [pg/ml]	383.81 ± 18.31	410.21 ± 11.71	0.211
Catalase [KU]	2.40 ± 0.23	1.86 ± 0.12	0.022
GGT [U/L]	35.28 ± 5.69	31.72 ± 2.42	0.687
TAC [mmol/L]	0.32 ± 0.01	0.30 ± 0.01	0.120
HOMA-IR	4.15 ± 0.29	5.17 ± 0.34	0.027
HOMA-B	130.24 ± 23.86	84.06 ± 14.08	0.009
QUICKI	0.31 ± 0.003	0.31 ± 0.003	0.137

Results are presented as mean ± SEM. Abbreviations are given in Table 1

and HOMA-B (p = 0.009) (Figure 5) were significantly lower in women with HbA_{1c} ≥ 7.6% compared with women with HbA_{1c} < 7.6%.

Discussion

In the current study some important correlations were observed including negative correlation between values of HbA_{1c} and TAC, negative correlation between values of HOMA-B and HbA_{1c}, positive correlation between values of HbA_{1c} and 8-isoprostane and positive correlation between values of the A/L ratio and TAC.

A study conducted by Picu et al. [4] has shown a positive correlation between total oxidant status (TOS) and HbA_{1c} in patients with T2DM. Therefore, it has been hypothesized that prolonged hyperglycemia results in overproduction of reactive oxygen species which leads to oxidative stress. In the current study,

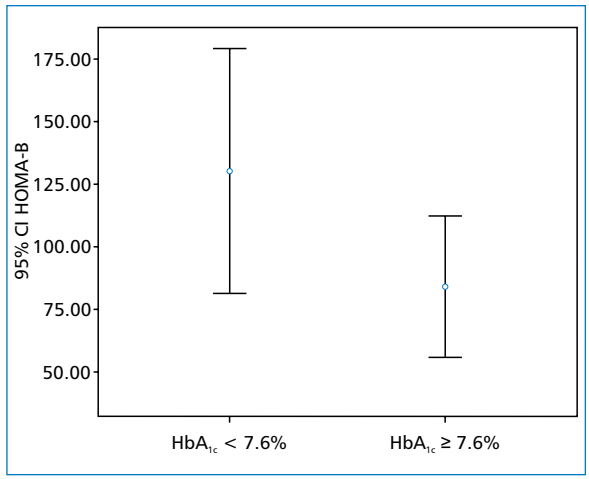

Figure 5. Comparison of the homeostasis model assessment of β-cell function (HOMA-B) of women with type 2 diabetes mellitus according to HbA_{1c} values (p = 0.009)

a significant negative correlation was observed between values of HbA_{1c} and TAC. On the other hand, current study showed a significant positive correlation between values of HbA_{1c} and 8-isoprostane. These results could support the hypothesis.

Hou et al. [5] and Al-Hakeim et al. [9] divided patients with T2DM according to HbA_{1c} values. They observed that patients with higher values of HbA_{1c} have lower values of HOMA-B. Therefore, beta cells function is affected by glycemic control. A negative correlation has shown between values of HOMA-B and HbA_{1c} in males with T2DM [16]. In the current study, Values of HOMA-B correlated negatively with values of HbA_{1c} in the whole patients. In addition, values of catalase and HOMA-B were significantly lower in women with $HbA_{1c} \geq 7.6\%$ compared with women with $HbA_{1c} < 7.6\%$. Increased circulating levels of glucose can cause oxidative stress via overproduction of reactive oxygen species [4]. On the other hand, beta cells function is impaired during chronic hyperglycemia because of increased oxidative stress which damages the cells [5]. Since antioxidant enzymes activities are low in beta cells, they are very sensitive to destructive effects of oxidative stress. On the other hand, lipotoxicity induced in beta cells by means of oxidative stress might be a central mechanism of destructive effects of reactive oxygen species in these insulin-secreting cells [6].

Gamma-glutamyltransferase (GGT) is an enzyme which is well known as a biomarker of fatty liver and alcohol consumption. However, it has been shown that GGT shows a direct relationship with incidence of diabetes independent of popular risk factors such as alcohol consumption. Current literature shows that GGT can also be used as a biomarker of oxidative stress condition in which its activity increases. GGT activity in serum shows a negative correlation with serum levels of antioxidants [17, 18]. In the current study, serum activities of GGT showed a slightly positive correlation with levels of malodialdehyde. On the other hand, plasma GGT activity is also related to obesity with the risk for T2DM [18]. In the current study, a significant positive correlation was observed between serum activities of GGT and values of waist circumference.

Leptin and adiponectin are adipokines that are secreted by white adipose tissue. The principal known role of leptin is energy homeostasis regulation. On the other hand, pro-inflammatory property of leptin has been demonstrated that causes proliferation of monocytes. This property of leptin can cause an increase in activity of enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which leads to an increase in oxygen free radicals production. In other words, leptin can induce oxidative stress [4]. On the other hand, adiponectin has been suggested as the strongest anti-inflammatory cytokine and can promote insulin sensitization effect. Moreover, some antioxidant effects of adiponectin has been reported that can prevent mitochondrial depolarization and dysfunction [19, 20]. It has been reported that in individuals with metabolic syndrome levels of total adiponectin correlates negatively with values of malodialdehyde. Therefore, it has been concluded that lower values of the A/L ratio can cause increased oxygen free radicals which leads to oxidative stress in patients with metabolic syndrome [21]. In the current study, serum levels of adiponectin correlated negatively with levels of malodialdehyde. On the other hand, serum levels of TAC showed a significant positive correlation with the A/L ratio and a significant negative correlation with the L/A ratio. Therefore, low levels of the A/L ratio or high levels of the L/A ratio were associated with increased oxidative stress in subjects of the current study.

Study of Picu et al. [4] has shown a positive correlation between total oxidant status and the percentage of total body fat in patients with T2DM. Therefore, it has been proposed that in obesity oxidant/antioxidant balance is disrupted that leads to increased oxygen free radicals production which means an oxidative stress condition. In the current study, women with BMI > 30 kg/m^2 had higher values of 8-isoprostane and marginally decreased TAC values than women with BMI < 30 kg/m^2 that might indicate an oxidative stress condition.

The current study had some limitations. The most important of them were small sample size and not using a normal group. Other limitation was that the data were not analyzed according to the history of nulli and multiparity pregnancy, gestational diabetes mellitus (GDM), macrosomia and polycystic ovary syndrome (PCOS).

Conclusions

In conclusion, chronic hyperglycemia results in oxidative stress. This situation might lead to less beta cells function. The current study showed that obesity was associated with increased oxidative stress and disrupted oxidant/antioxidant balance. In addition, low levels of the A/L ratio or high levels of the L/A ratio were associated with increased oxidative stress.

Conflict of interest

The authors declared that they have no conflict of interest.

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A case study of eight type 2 diabetic stage 4 chronic kidney disease patients showing lower glycemic variability with faster-acting insulin aspart as compared to insulin aspart

ABSTRACT

Background. Peaks and nadirs of blood glucose level varying daily in a person is referred to as glycemic variability (GV). GV associated with diabetics has been recently linked to cardiovascular disorders (CVD) or even chronic kidney disease (CKD) progression. Faster-acting insulin aspart is the latest ultra-rapid acting bolus insulin which has shown much lesser intra- and inter-patient variability as compared to conventional bolus insulin.

Material and methods. However, inadequate data exist regarding GV in patients with advanced stages of CKD. Hence, with this objective, the present case study was undertaken with eight patients divided into two equal groups, wherein faster-acting insulin aspart and insulin aspart were used as the boluses, respectively. Continuous glucose monitoring data of the patients were taken for the initial four days to calculate mean amplitude of glycemic excursion (MAGE) of the total four days for each individual (mmol/L) to see the difference in GV. A value of > 3.607 mmol/L (65 mg/dL) was considered to be statistically significant.

Results. In this case study of eight stage 4 CKD type 2 diabetic patients, statistically significant lower GV was observed in the faster-acting insulin aspart arm when compared with the insulin aspart arm. The p-value was 0.0004 in unpaired t-test and < 0.05 for U in Mann-Whitney U test after ruling out the baseline confounding factors.

Conclusions. This study confirms the stable pharmacokinetic and dynamic properties of faster-acting insulin aspart and subsequent studies with larger number of patients are required for a conclusive outcome. (Clin Diabetol 2019; 8, 6: 284–291)

Key words: type 2 diabetes mellitus, faster-acting insulin aspart, glycemic variability, mean amplitude of glucose excursion, chronic kidney disease, continuous glucose monitoring system

Introduction

Constant hyperglycemia and bursts of prandial glycemic surges can cause complications in diabetes mellitus (DM) as well as in stress hyperglycemia [1, 2]. Postprandial spikes in blood sugar, along with episodes of hypoglycemia, are responsible for an alarming increase in cardiovascular events in DM [2]. Glycemic variability (GV) comprises these events; thus, minimizing GV will suspend future cardiovascular events. Addressing GV emerges as a target to be pursued in clinical procedures to reduce the mean blood glucose, as GV is now considered to be an independent risk factor in diabetics for cardiovascular disease (CVD) [3]. Contemporary DM treatment modalities with glucagon-like peptide-1-based remedy, newer insulin, newer insulin pumps, bariatric surgery and newer oral anti-diabetic molecules considerably decrease GV [4].

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The mean amplitude of glycemic excursion (MAGE) was intended to encapsulate repast-time related glucose excursions. GV implies to the swings in blood glucose level seen in a person daily. Decreased or missing glycemic auto-modulation or deficits of insulin accessibility are hypothesized to be the etiological causes for these glycemic ridges swings [4]. Intermittent high blood glucose exposure rather than constant high blood glucose exposure has been shown to have deleterious effect in various experimental studies [5].

GV indices derived from continuous glucose monitoring (CGM) are mean ± standard deviation, J index, coefficient of variance, low blood glucose index, high blood glucose index, average daily risk range, MAGE, mean of daily differences and continuous overall net glycemic action [4].

Faster-acting insulin aspart is the latest ultra-rapid acting bolus insulin derived by substituting amino acid proline by aspartic acid in position B28 and adding inactive ingredients L-arginine, niacinamide and others and appearing in circulation after 2.5 minutes of administration [6]. But the data of this insulin in advanced chronic kidney disease (CKD) patients is limited, and therefore, the present case study was undertaken to get newer insights into the use of faster-acting insulin aspart as compared to insulin aspart in relation to the difference in MAGE, as MAGE or rather GV has been described as an independent marker of CVD [3].

Case presentation

We analyzed the clinical records of MAGE of eight type 2 diabetic CKD stage 4 (as calculated by Chronic Kidney Disease Epidemiology Collaboration equation) patients. A total data of thirty seven patients were searched who were meeting the primary criteria of basal-bolus insulin regimen with eGFR (estimated glomerular filtration rate) less than 30 ml/min/1.73 m^2 without any oral anti-diabetic drugs and out of them only eight finally meet the full inclusion criteria. All the patients had to meet the pre-specified inclusion criteria — type 2 diabetics who previously faced either severe or mild to moderate hypoglycemia on regular human insulin treatment, age more than 55 years; giving informed, written consent; MAGE calculated from CGM data; HbA_{1c} of 7.5–9%; estimated glomerular filtration rate (eGFR) 15–30 ml/min/1.73 m^2; duration of diabetes for more than 10 years; all on basal-bolus insulin regimen (basal component being insulin glargine given at a dose to achieve a fasting value of 130 mg/dl); no orally administered agents; and bolus was either insulin aspart or faster-acting insulin aspart given just before meals. The baseline characters of all patients are given in Table 1. Then they were divided into two groups

Table 1. Baseline characters of all patients and insulin doses

Patient serial number	Age (years)/sex	Baseline HbA_{1c} (%)	Baseline eGFR [ml/min/1.73 m^2]	Duration of diabetes (years)	Glargine dose used [units]	Target fasting [mg/dL]	Faster acting aspart dose used as bolus [units] (BBF-BL-BD)	Aspart dose used as bolus [units] (BBF-BL-BD)
1	67/M	7.8	28	11	17	130	8 – 10 – 8	NA
2	66/M	7.7	27	12	19	130	5 – 12 – 8	NA
3	62/F	8.9	28	12	21	130	10 – 12 – 12	NA
4	68/F	8.1	20	11	16	130	9 – 11 – 7	NA
5	66/F	8.8	29	11	20	130	NA	11 – 12 – 9
6	61/M	8.3	28	15	22	130	NA	9 – 12 – 8
7	59/M	8	22	12	15	130	NA	7 – 15 – 12
8	69/M	8.2	19	11	17	130	NA	4 – 7 – 5

BBF — before breakfast; BL — before lunch; BD — before dinner; HbA_{1c} — glycated hemoglobin; eGFR — estimated glomerular filtration rate

Table 2. 95% confidence interval (CI), standard deviation and mean values of baseline characters

	MAGE [mmol/L]	HbA_{1c} (%)	eGFR [ml/min/1.73 m^2]	Duration of diabetes (years)	Age (years)
Standard deviation, δ	1.37	0.40	3.82	1.26	3.38
95% CI	4.87 ± 0.95 (± 19.60%)	8.22 ± 0.28 (± 3.42%)	25.12 ± 2.64 (± 10.54%)	11.87 ± 0.87 (± 7.40%)	64.75 ± 2.34 (± 3.62%)
Average (mean values)	4.87	8.22	25.12	11.87	64.75

MAGE — mean amplitude of glucose excursion; HbA_{1c} — glycated hemoglobin; eGFR — estimated glomerular filtration rate

depending on the types of hypoglycemia faced by each patient namely those with severe hypoglycemia were treated with faster-acting aspart insulin and those who had mild to moderate hypoglycemia were treated with aspart insulin as the bolus insulin. The mean baseline values of the total population were HbA_{1c} 8.22%, age 64.75 years, duration of diabetes 11.87 years and eGFR 25.12 ml/min/1.73 m^2. The 95% confidence interval (CI) and standard deviation (SD) values of the above baseline characters are given in Table 2.

Materials and methods

In the present case study, we analyzed the CGM data (performed with Medtronic iPro2® machine) of the patients for the initial four days to calculate MAGE (mmol/L) for each individual as measured by the software EasyGV Version 9.0.R2 (Nathan R Hill — Copyright University of Oxford 2010–2016) so as to see the differences in GV. A value of more than 3.607 mmol/L (65 mg/dL) was considered to be significantly high, indicating high GV as has been found previously [7]. All the methods were followed as per directions laid down in the declaration of Helsinki.

A CGM sensor was attached to the subcutaneous fat tissue of these patients and adjusted by the standard Medtronic iPro2 working principles. While wearing the CGM, the patients checked their blood glucose levels with a self-monitoring blood glucose device, 4 times a day. The data of the first 4 days from the CGM of each patient was analyzed with the above-mentioned software to calculate MAGE as well as eight other GV indices, namely SD, mean, continuous overall net glycemic action, mean of daily differences, average daily risk range, J index, low blood glucose index and high blood glucose index. After analyzing data, patients were divided into two groups (4 patients each). The first group used only aspart as bolus insulin while the other group used faster-acting aspart as bolus insulin. Unpaired T-test and Mann-Whitney U-test were applied to compare the two groups with regard to each parameter that could have affected the final MAGE outcome, namely baseline HbA_{1c}, age, duration of diabetes, and eGFR. Baseline Pearson correlation and Spearman Rank correlation were also calculated [software: Wessa P. (2017). Pearson Correlation (v1.0.13) in (Free Statistics Software (v1.2.1), Office for Research Development and Education] was used to determine any baseline statistical significance which might affect the MAGE (as MAGE was the dependable variable here).

Results

The baseline correlation equations using both Pearson's formula and Spearman Rank formula (Table 3) confirm no statistical significance with MAGE as dependable variable. Here, the independent variables were HbA_{1c} (p-value 0.347 for Pearson formula), age (p-value 0.344 for Pearson formula), duration of diabetes (p-value 0.188 for Pearson formula) and eGFR (p-value 0.79 for Pearson formula).

After dividing the MAGE calculated and other GV parameters calculated in two groups, we applied Unpaired T-test and Mann-Whitney U test (Table 4) to see the baseline statistical differences between the two groups in terms of HbA_{1c}, age, duration of diabetes and eGFR which might have affected the final MAGE outcome. We found all the parameters to be statistically non-significant in both the tests, reducing the bias at baseline parameters for the outcome.

Further, on applying Unpaired T-test and Mann-Whitney U test in MAGE outcome (Table 5) and Unpaired T-test on other eight GV parameters (Table 6) between the two groups, only MAGE showed statistically significant results in Unpaired T-test (p-value 0.0004) as well as in Mann-Whitney U test (p-value 0.012).

As SD between groups can cause significant changes in the MAGE outcome, we also performed both Unpaired T-test and Mann-Whitney U-test between the two groups taking their SD values (Table 7) and found both to be statistically non-significant, ruling out the probability of SD to be a confounding factor in the final MAGE outcome analysis between the two groups.

The figures of CGM data of one patient from each group are given in Figure 1 and Figure 2 and also the

Table 3. Correlation analysis for MAGE as dependable variable against independent variables duration of diabetes, HbA_{1c}, age and eGFR

Pearson correlation (dependable variable MAGE)	HbA_{1c}	eGFR	Age	Duration of diabetes
T-test	1.01	–0.27	–1.02	1.48
p-value (2 sided)	0.34	0.79	0.34	0.18
95% CI of correlation	[–0.43, 0.85]	[–0.75, 0.64]	[–0.85, 0.43]	[–0.29, 0.89]
Spearman rank correlation (dependable variable MAGE)				
Rho	0.54	0.17	–0.35	0.18
2-sided p-value	0.17	0.68	0.38	0.66

MAGE — mean amplitude of glycemic excursions; HbA_{1c} — glycated hemoglobin; eGFR — estimated glomerular filtration rate; CI — confidence interval

average CGM values of each group is described in Table 8 which also shows no significant difference between the average CGM values between two groups.

Discussion

The postprandial glycemic excursions in glucose level, as well as daily glucose variations, lead to GV [4]. The event of different microvascular and macrovascular complications in diabetes is ascribed to the dysglycemia (peaks and nadirs) seen in a diabetic patient occurring more than the accepted physiological variations for that individual [8]. Two unifying hypotheses have been put forward that accounts for GV, uncontrolled protein glycation termination products and initiation of oxidative stress, resulting in vascular complications [4]. There is a significant relationship between GV and the increased occurrence of hypoglycemia [9]. HbA_{1c} reflects only 8% of severe hypoglycemia; hence, it is a poor marker [10]. But GV can predict around 40 to 50% of future hypoglycemic episodes [4]. Investigations have demonstrated that GV, related to extreme hypoglycemia, could be deleterious to both diabetics as well as non-diabetic patients in intensive care units [11]. Besides CVD, the risk of retinopathy is also increased with GV. The contribution of GV and instability rather than the absolute glucose values have been shown to be responsible for CV mortality as well as for all-cause mortality in elderly type 2 DM patients [12]. Additionally, in 1504 acute ischemic stroke patients with diabetes, it was observed that even after adjusting baseline HbA_{1c}, the functional outcome after 3 months was poorer in patients having increasing glucose level range quartile (used as GV marker). CKD has been shown to be a major contributor to GV [13].

Among the various methods used to determine GV, MAGE is an acceptable tool, but it has some disadvantages, e.g., connection with SD with the presentation of CGM, postprandial excursions can be surveyed using the zone under the curve and the trapezoidal strategy; the determination of MAGE is operator-controlled and not unambiguously characterized [14]. Among non-diabetic patients having coronary artery disease (CAD), MAGE was found to be associated with cardiovascular events [7]. However, in type 2 diabetic patients, MAGE, as one of the GV parameter, was found to be significantly associated with CAD, CKD and stroke(p-value for all three < 0.01). MAGE also showed significant correlation to eGFR and urine albumin:creatinine ratio (p-value for both < 0.03) [15].

Faster-acting insulin aspart is the fastest ultra-rapid acting bolus insulin derived by substituting an amino acid from the regular insulin chain at B28 position by aspartic acid with the addition of few ingredients like L-arginine and niacinamide. It reaches bloodstream by 2.5 minutes with stable pharmacokinetic and pharmacodynamic properties and is suitable for use even post-meals for prandial control of glucose [6]. Niacinamide causes faster absorption of the insulin from subcutaneous tissue [6]. The data of this insulin in stage 4 CKD patients is sparse, and therefore, the present case study has provided some insight into this aspect. However, larger trials are required for further inference. Another switchover study on faster-acting insulin aspart showed significant reduction in nocturnal hypoglycemia risk when compared to human regular insulin by 80% as well as significant reduction in MAGE value over four days [16]. Due to the ultra-rapid onset of action, the risk seems to be reduced for hypoglycemia [17] as well there is documented 74% greater early glucose reductions when compared to aspart insulin [18]. These properties of faster-acting aspart make it an ideal candidate to lower GV with least risk of hypoglycemia when compared to aspart insulin.

Table 4. Unpaired T-test and Mann-Whitney U test between various parameters that might affect the MAGE outcome between two groups (one using faster-acting insulin aspart and the other using insulin aspart)

Unpaired T-test for B/L HbA_{1c}	Mean	SD	95% CI	p-value
Faster-acting-aspart group	8.12	0.54	–0.58 to 0.98	0.55
Aspart group	8.32	0.34		
Unpaired T-test for B/L eGFR				
Faster-acting-aspart group	25.75	3.86	–8.78 to 6.28	0.69
Aspart group	24.5	4.8		
Unpaired T-test for B/L age				
Faster-acting-aspart group	65.75	2.63	–8.45 to 4.45	0.47
Aspart group	63.75	4.57		
Unpaired T-test for B/L duration of diabetes				
Faster-acting-aspart group	11.5	0.58	–1.67 to 3.17	0.47
Aspart group	12.25	1.89		
Mann-Whitney U-test	**For HbA_{1c}**	**For age**	**For EGFR**	**For duration of diabetes**
U value	7	8.5	11	9
Critical value of U at $p < 0.5$	2	2	2	2
Statistical significance of U	Non significant	Non significant	Non significant	Non significant
Z-score	1.04	0.73	–0.20	–0.62
p-value of Z	0.29	0.46	0.83	0.52
Statistical significance of Z-score	Non significant	Non significant	Non significant	Non significant

HbA_{1c} — glycated hemoglobin; eGFR — estimated glomerular filtration rate; SD — standard deviation; CI — confidence interval

Table 5. Unpaired T-test values and Mann-Whitney U test for MAGE values of the two groups

Unpaired T-test	Mean	SD	95% CI	p-value
Faster-acting insulin aspart group	3.66	0.63	1.47 to 3.38	0.0004*
Insulin aspart group	6.08	0.66		
Mann-Whitney U-test				
U value			0	
Critical value of U at $p < 0.5$			2	
Statistical significance of U			Significant	
Z-score			2.50	
p-value of Z			0.01*	
Statistical significance of Z-score			Significant	

*Extremely statistically significant for unpaired T-test and statistically significant for Mann-Whitney U test; CI — confidence interval; SD — standard deviation; MAGE — mean amplitude of glucose excursion

Our case study revealed that faster-acting insulin aspart (as compared to insulin aspart) had a lower GV, indicated by MAGE outcome from CGM in advanced stage 4 CKD diabetic patients, even after excluding confounding factors of baseline HbA_{1c}, age, duration of diabetes, eGFR and SD between the two groups as projected by the p-values of 0.0004 in unpaired t-test and < 0.05 for U in Mann-Whitney U test. Hence, faster-acting insulin aspart can be used therapeutically to achieve acceptable GV in most diabetic patients with CKD, as it showed better results in patients in stage 4 of CKD. Lower GV should clinically produce lower rates of hypoglycemic risk, which should be our target to effectively counter glycemia in advanced CKD patients.

Conclusion

In this case study, we found that faster-acting insulin aspart was associated with statistically significant lower GV, as compared to insulin aspart, in patients

Table 6. Unpaired T-test of glycemic variability parameters other than MAGE in two groups

	Mean	SD	95% CI	p-value
SD				
Faster-acting insulin aspart	2.34	0.74	–0.89 to 0.93	0.96
Insulin aspart	2.36	0.08		
Mean				
Faster-acting-aspart group	8.58	0.64	–0.79 to 1.48	0.48
Aspart group	8.92	0.67		
Continuous overall net glycemic action				
Faster-acting-aspart group	8.10	0.44	–1.37 to 1.02	0.73
Aspart group	7.93	0.87		
High blood glucose index				
Faster-acting-aspart group	6.94	2.28	–2.93 to 3.30	0.88
Aspart group	7.13	1.13		
Average daily risk range				
Faster-acting-aspart group	16.43	4.84	–0.33 to 12.8	0.05
Aspart group	22.66	2.32		
J index				
Faster-acting-aspart group	38.90	6.74	–7.72 to 12.68	0.57
Aspart group	41.38	4.90		
Low blood glucose index				
Faster-acting-aspart group	0.89	0.29	–0.90 to 1.35	0.64
Aspart group	1.11	0.87		
Mean of daily differences				
Faster-acting-aspart group	2.31	0.65	–1.51 to 0.23	0.12
Aspart group	1.67	0.28		

SD — standard deviation; CI — confidence interval

Table 7. Unpaired T-test and Mann-Whitney U-test between the two groups for SD

Unpaired T-test	**Mean**	**SD**	**95% CI**	**p-value**
Faster-acting insulin aspart group	42.18	13.41	16.19 to 16.83	0.96
Insulin aspart group	42.50	1.47		
Mann-Whitney U-test				
U value			7	
Critical value of U at $p < 0.5$			2	
Statistical significance of U			Non significant	
Z-score			1.04	
p-value of Z			0.29	
Statistical significance of Z-score			Non significant	

SD — standard deviation; CI — confidence interval

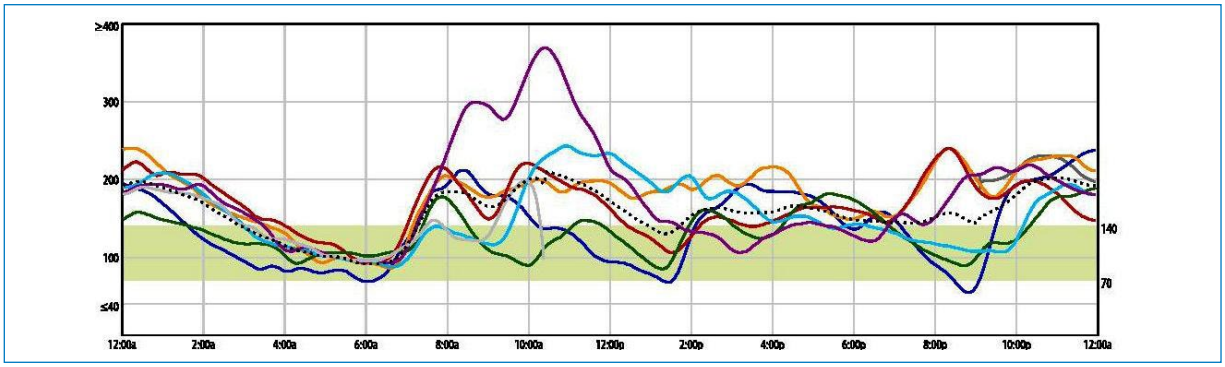

Figure 1. CGM data of one patient on aspart insulin

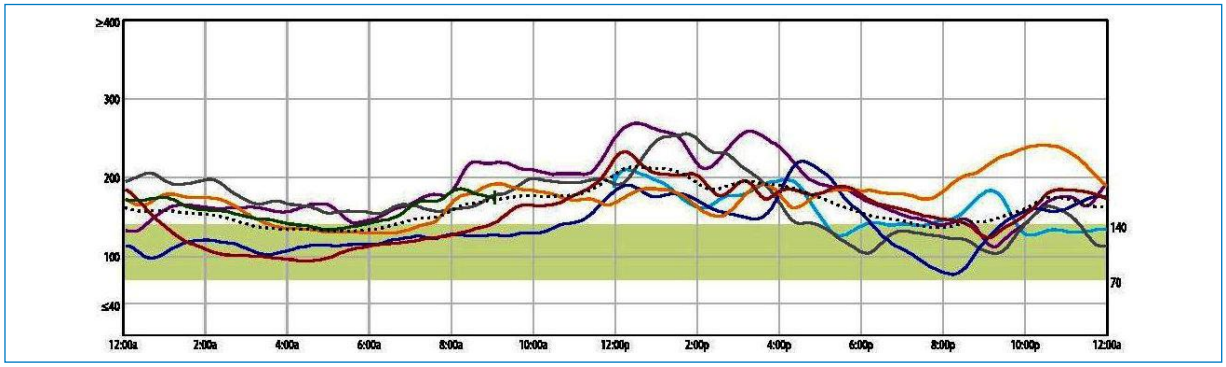

Figure 2. CGM data of one patient on faster-acting aspart insulin

Table 8. CGM values averaged over the four days in two groups

Patient serial number	CGM value averaged for four days	Bolus insulin group	Average value of CGM for each group
1	156.21	Faster-acting aspart group	160.66
2	160.32		
3	177.33		
4	148.79		
5	163.47	Aspart group	154.47
6	138.85		
7	162.91		
8	152.63		

with advanced CKD. Recently, GV has emerged as a target objective in diabetes holistic management due to its association with CVD and CKD progression. Finally, this study confirms the stable pharmacokinetic and dynamic properties of faster-acting insulin aspart, and future studies involving larger number of patients can help draw a conclusion.

Ethics committee approval

Not required, as it is a case study where patients gave proper informed written consent to use their clinical medical records, without exposing their identity, for academic purposes in medical field.

Informed consent

Written informed consent was taken from all the participants.

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Author contributions

All authors certify that they have participated adequately in developing intellectual content and analysis of data. Each author has reviewed the final version of the manuscript and approves it for publication.

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Health care seeking behaviors in type 2 diabetic patients in East Azerbaijan

ABSTRACT

Background. Timely and effective use of health care services is essential to delay or prevent complications and reduce the burden of diabetes. Positive health care seeking behaviors can improve diabetes control and, as a result, reduce the incidence of the complications. So, this study aimed to investigate the status of health care seeking behaviors and affecting factors in type 2 diabetic patients.

Materials and methods. This was a cross-sectional study. There were 1139 patients with type 2 diabetes aged > 18 years selected who referred to educational hospitals, Endocrinologist office, primary health care centers, and Clinics. Data collected using a researcher-made questionnaire and analyzed using SPSS software version 22.

Results. 36.3% of diabetic patients initially referred to a physician in the event of illness symptoms, and 70.7% of patients referred to a physician in case of exacerbating of disease symptoms. 58.4% of patients preferred to consult a specialist directly. 78.85 of patients reported that they had referred to a physician on a regular basis and 59.9% of patients followed up their treatment process on a regular basis. The physician was the main source of information for the majority of patients. Income, education and health insurance status, disease severity, chronicity of DM, the history of hospitalization due to DM were the affecting factors on health care seeking behaviors ($p < 0.05$).

Conclusion. Most of the diabetics often did not follow the official structure of health care providing to manage their illness, and despite the referral system and family practitioner program more than half of the patients went directly to the specialist physician's office. Factors related to the patient (an income and education status), disease characteristics (disease severity, chronicity of DM and the history of hospitalization due to DM) and health care system factors (type of the basic insurance and supplementary insurance status) affect the health care seeking behaviors. (Clin Diabetol 2019; 8, 6: 292–302)

Key words: health-seeking behaviors, healthcare seeking behaviors, help-seeking behavior, information seeking behavior, treatment seeking behavior, type 2 diabetes mellitus

Introduction

In order to delay or prevent complications, diabetic patients have to adopt certain lifestyle changes and comply with drug therapy. These measures need to be preserved for long-term, and so, sustained follow-ups

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are necessary [1]. Also, diabetes management includes multiple health care providers [2, 3].

Timely and effective use of health care services, especially primary health care, is essential to reduce the burden of diabetes [4]. Effective utilization of health care services is affected by health care seeking behaviors. Health seeking behavior (HSB) has been defined as any activity or inaction undertaken by individuals who perceive themselves to have a health problem or to be ill for the purpose of finding an appropriate remedy [5]. It is well established that HSB is influenced by the manifestation of symptoms [1, 6]. Most patients with diabetes are asymptomatic at the time of initial diagnosis; so, no feeling they need to get health care and disease denial is a pivotal barrier in disease management [7]. For diabetes, there is a tendency for patients to delay or ignore health care measures until the complications become evident [8].

Positive health-seeking behavior (i.e., the early recognition of symptoms, presentation to health facilities, and compliance with effective treatment) could improve diabetes control and as a result, reduce the incidence of the complications caused by this devastating disease [9] and ultimately could improve the quality of life of patients with diabetes mellitus [10].

Moreover, understanding community patterns of health care-seeking behaviors, which in the society are the first points of care, would improve public health practice in the society [11] and it is important so as to develop proper health management strategies [12]. The knowledge regarding health-seeking behavior can assist health policymakers in order to identify referral patterns, affecting factors, barriers to access and use of services, and implement appropriate interventions to guide patients in the right way to receive services and remove barriers to the use of services [13, 14]. Since there are few published studies dealing with the health-seeking behaviors in the diabetic patients in Iran, the aim of this study is to describe the health-seeking behavior of patients with type 2 diabetes mellitus and its affecting factors.

Health care system in Iran

The health system structure in Iran consists of two sectors. The public sector structured based on the three-level referral system (primary, secondary, and tertiary health services) and family practitioner. Over the last two decades, the emphasis of the government on primary health care has made the public sector the main provider of primary health care services in the whole country. In addition, the public sector provides a notable part of secondary and tertiary health services in the provinces of Iran. The private sector mainly focuses on secondary and tertiary health care in urban areas and it benefits from a good deal of prestige. Also, there are many nongovernmental organizations (NGOs) active in health issues in Iran, mainly active in special fields like children with cancer, breast cancer, diabetes, thalassemia, and so on [15–17].

Materials and methods

Study design, sample size, and sampling

This was a cross-sectional study. The statistical population included all patients with type 2 diabetes aged > 18 years, without having physical and mental disabilities, who referred to educational hospitals(Imam Reza and Sina's educational and therapeutic centers and Asad Abadi clinic in Tabriz, Iran), Endocrinologist office, health centers and Sheykh Al-Raees Clinic. The sample size includes 1200 diabetic patients. Sampling was performed in Sequential Sampling Method.

Data collection tools

Data was collected using a researcher-made questionnaire. The questionnaire consisted of three sections. The first section contained demographic characteristics and socioeconomic status. The second section contained disease-related variables of diabetic patients. The third section contained the questions pertaining to the justification for health care seeking behaviors in patients with type 2 diabetes. The questionnaire was designed based on literature review and reviewing the models and theories related to the health care seeking behaviors, reviewing existing questionnaires on health-seeking behaviors especially questionnaires related to health care seeking behavior in diabetic patients and also interviews with experts. At the next step, the Content Validity Ratio (CVR) and Content Validity Index (CVI) were used for quantitative assessment of the content validity. The scores for the CVI and CVR were 0.9 and 0.76, respectively, in Table 1. The total number of experts to assess the validity of the questionnaire was 40 people.

Data collection

After approving the study protocol in the Ethics Committee of the University, the questionnaires were given to the participants by referring to the treatment centers. Moreover, the required explanations were given to the patients about the objectives of the study and how to complete the questionnaire. For illiterate people, the questionnaire was read by an interviewer to the participants while they were completing their questionnaire.

Table 1. The scores of Content Validity Ratio (CVR) and Content Validity Index (CVI)

Number	Questions related to health care seeking behaviors	CVI	CVR
1	What is the first action when you have symptoms?	0.89	0.9
2	In the case of the manifestation of illness symptoms, who would you rather consult with first?	0.96	0.7
3	When you get sick, Which health care provider do you refer in order to receive health services? (Public or private)	0.9	0.55
4	When you get sick, which health care provider centers do you refer? (In terms of the referral level of the service providers)	0.8	0.65
5	Under which circumstances do you visit a doctor? (On a regular basis and periodically — in the occurrence of disease symptoms — in case of exacerbate of disease symptoms)	0.87	0.8
6	How often do you visit your doctor?	0.97	0.75
7	From what sources do you get the required information for treatment and how you control diabetes? (Mark the most important resource/just one option)	0.94	1.00
	Total score	0.9	0.76

Data analysis

Data were analyzed using SPSS 22. Frequencies, percent, mean and SD was calculated for the variables. Chi-square test was used to assess the association between the demographic, socioeconomic and disease--related variables and health-seeking behaviors.

Results

Characteristics of the study population are shown in Table 2. 1139 questionnaires out of 1200 distributed questionnaires were included in the final analysis, and 60 questionnaires were excluded due to huge missing data. 66.3% of the patients were women. The mean ± SD of age, disease duration and body mass index (BMI) of patients were 56.93 ± 13.34 years, 9.06 ± 7.12 years and 28.37 ± 5.27, respectively. 41.5% of the patients were illiterate. The type of current treatment of most diabetic patients (54.3%) was oral agents. 30.2% of patients had a history of hospitalization due to complications of diabetes during the past year. 76.1% of patients had at least one comorbidity or complication (Table 2).

In the event of illness symptoms, the majority of patients (36.3%) were referred to a physician. However, a substantial percentage of patients changed their behavior or did self-medication.70.7% of patients reported they referred to a physician in case of exacerbating of disease symptoms and complications. In the case of manifesting or exacerbating the symptoms of the disease, 58.4% of patients reported they preferred to consult a specialist physician. Approximately more than half of the patients (50.9%) referred to public hospitals or university clinics. 78.85 of patients reported they referred to a physician on a regular basis to manage their disease in the event of illness and 59.9% of patients followed up their treatment process on a regular basis. 51.6% of patients visited a general practitioner every three months. The physician was the most frequent source of required information for most patients (65.7%) (Table 3).

As can be seen from Table 4, income status, severity of illness, chronicity of DM and the history of hospitalization due to complications of diabetes during the past year were the affecting factors on the most health-seeking behaviors, and this association was statistically significant ($p < 0.05$). The type of basic insurance and supplementary insurance status were significantly associated with the follow-up pattern and the type of health center receiving the services ($p < 0.05$). Education status was significantly associated with the first care provider for consulting, the type of health center to receive services ($p < 0.05$).

In this study, patients with lower income were more likely to go to a physician in the event of illness symptoms, whilst those with higher income were more likely to do self-medication or change their behavior. In the event of illness symptoms, patients living in urban areas were more likely to refer to a physician and change their behavior, whereas those living in rural areas were more likely to consult family members, friends, relatives, and do self-medication. Patients with no history of hospitalization were more likely to refer to a physician, while those with a history of hospitalization were more likely to do self-medication.

Patients with higher income and education reported that they initially refer to a physician in the event of illness symptoms, whereas those with lower income and education level reported that they initially refer to primary health care providers. Patients with longer disease duration and history of hospitalization,

Table 2. Demographic and clinical profile

Variable	Mood	Frequency	Percent
Gender	Male	384	33.7
	Female	755	66.3
Age	< 40	127	11.2
	40–60	527	46.3
	> 60	485	42.6
Income status	< 10 million Rials	397	52.5
	> 10 million Rials	359	47.5
Education status	Illiterate	473	41.5
	Reading and writing ability	407	35.7
	Diploma	195	17.1
	Academic education	64	5.6
Type of basic health insurance	Social security	707	64.4
	Iranian health insurance	391	35.6
Supplementary health insurance status	Yes	430	39.1
	No	669	60.9
Habitation status	Rural area	135	11.9
	Urban area	1003	88.1
Disease duration	< 5 year	421	37.1
	5 to 10 years	260	22.9
	> 10 years	455	40.1
Type of current treatment	Oral pills	619	54.3
	Insulin	449	39.4
	Change in lifestyle (change in diet and physical activity)	71	6.2
Presence of co-morbidity or complications	Yes	867	76.1
	No	272	23.9
The history of hospitalization due to complications of diabetes during the past year	Yes	344	30.2
	No	795	69.8

those who rated their disease severity as severe, those with diabetes complications and patients whose main treatment strategy was taking insulin were more likely to refer to a physician.

Patients with higher incomes and education, those who are living in urban areas, those with the social security basic health insurance and those who have supplementary insurance, those with longer disease duration and the history of hospitalization due to complications of DM and those who rated their disease severity as low were more likely to refer to tertiary level of heath care providers, such as special hospital, private hospitals and private doctor's office. Patients whose current treatment was based on change in the lifestyle were more likely to refer to primary health care providers.

Patients with higher income and education, those living in urban areas, those with social security insurance and supplementary insurance, those with longer disease duration and those who rated their disease severity as severe were more likely to refer to private centers for receiving services. Also, patients who had no history of hospitalization due to diabetes complications during the past year were more likely to go to private centers; Table 5.

Moreover, older patients, those living in urban areas, those with the social security insurance and supplementary insurance coverage, those with longer disease duration, and patients with no history of hospitalization were more likely to refer to a physician on a regular basis; Table 6.

Discussion

This study examines the health care seeking behaviors and its affecting factors in patients with type 2 diabetes. Despite the implementation of family physician program, just a few percentages of individuals chose to consult a family physician in the event of

Table 3. Health care seeking behaviors profile

Questions	Options	Frequency	%
The first action in the event of illness symptoms	Refer to a doctor	413	36.3
	Behavior change (change diet or/and physical activity level)	327	28.7
	Self-medication	325	28.6
	Consult and get tips from family members, friends and other people around you	73	6.4
The first action in case of exacerbating of symptoms	Refer to a doctor	805	70.7
	Behavior change (diet change and physical activity level)	306	26.9
	Changing physician	28	2.5
The first care provider to consult in case of manifesting or exacerbating the symptoms of the disease	Primary health care providers such as Behvarz and health watch	103	9.1
	Family/general practitioner	371	32.6
	Specialist	665	58.4
The type of health care center to visit and receive the service	Primary health care centers (health house and health center)	211	18.5
	General hospitals	580	50.9
	Special hospital, private hospital or private doctor's office	348	30.6
The type of health care center to visit and receive the service	Public health centers	821	72.1
	Private health centers	318	27.9
Regular referring to the doctor in order to better manage the disease	Yes	896	78.8
	No	241	21.2
Follow-up pattern (How do you see your doctor?)	Asymptomatic/on a regular basis	682	59.9
	In the event of symptoms	276	24.2
	In the case of exacerbation of the symptoms or complications of the disease	181	15.9
Follow-up pattern (How often do you visit your doctor?)	Once a week	49	4.3
	Once every two weeks	22	1.9
	Monthly	136	11.9
	Every three months	588	51.6
	Once every six months	114	10.0
	Once every year	32	2.8
	If needed	198	17.4
The most frequently used information source for disease management	Family members and friends	243	21.5
	Physician	745	65.9
	Radio and TV programs, electronic database, blogs, websites, health portals, mobile applications, social networks	129	11.4
	Join the Diabetes Associations	13	1.2

illness symptoms. By contrast, a greater percentage of patients preferred to go to specialist physicians directly by bypassing the referral system. This issue disrupts the integrity and continuity of care addition to overuse the specialists' services and imposing additional costs on the health care system. This is because basic insurances have not played their role properly, and they reimburse the cost of self-referral to specialist doctors. In other

Table 4. Factors affecting health care seeking behaviors

Variables	The first action in case of the occurrence of disease symptoms		The first care provider to consult in case of incidence or exacerbation of the symptoms of the disease		The type of health care center to receive the services (primary, secondary or tertiary level)		The type of health care center to receive the services (public or private)		Follow-up pattern	
	χ^2	P value	χ^2	P value	χ^2	P value	χ^2	P value	χ^2	P value
Gender	3.95	0.26	3.31	0.19	2.31	0.31	1.18	0.27	2.81	0.24
Age groups	2.96	0.81	5.21	0.26	5.25	0.26	5.20	0.074	13.09	0.01
Income status	23.27	< 0.0001	8.47	0.01	61.09	< 0.0001	27.03	< 0.0001	1.98	0.37
Education status	14.54	0.10	13.61	0.03	55.69	< 0.0001	35.15	< 0.0001	7.49	0.27
Habitant status	18.82	< 0.0001	0.98	0.61	24.88	< 0.0001	13.11	< 0.0001	8.6	0.01
Type of basic health insurance	3.704	0.29	0.41	0.81	12.70	0.002	7.61	0.006	16.44	< 0.0001
Supplementary health insurance status	2.406	0.49	3.17	0.2	40.62	< 0.0001	36.40	< 0.0001	17.18	< 0.0001
Claims to know about the cause of DM	2.72	0.43	17.06	< 0.0001	18.91	< 0.0001	2.75	0.09	2.14	0.34
Chronicity of DM	14.39	0.02	17.78	0.001	27.42	< 0.0001	8.81	0.01	23.58	< 0.0001
Type of current treatment	7.56	0.27	17.82	0.001	30.57	< 0.0001	0.403	0.81	8.73	0.06
Presence of co-morbidity or complications	5.01	0.17	5.76	0.05	5.59	0.06	5.44	0.02	4.85	0.08
History of hospitalization due to complications of diabetes during the past year	10.37	0.01	12.01	0.002	23.44	< 0.0001	4.08	0.04	13.19	0.001
The severity of illness	27.90	< 0.0001	11.03	0.02	16.85	0.002	16.27	< 0.0001	17.43	0.002

words, the appropriate payment mechanisms have not been designed to support the existing structure. In Kavosi et al.'s study (2018), self-referrals and unnecessary referrals are reported as the most important problems of the referral system in Iran [18].

Based on the findings of this study, the majority of diabetics reported that they initially referred to a physician in the event of illness symptoms, and only a few percentages of patients consulted family members, friends, and relatives. This action can be considered as a positive behavior. Although a substantial percentage of patients reported that they did self-medication initially in the event of disease symptoms, it should be noted that self-medication without doctor consultation may result in drug interactions, unwanted consequences and exacerbation of the disease. In a study by Musinguzi et al., self-medication and access to antihypertensive drugs with or without prescription were common as well as the use of herbal remedies [19]. Dawood et al. reported that 66.7% of the participants chose to consult the physician when they experienced any health problems, and only 20.9% do self-medication, and the association between the practice of self-medication and Chinese participants, educated people, people with alone living status and people with more self-care orientation was significant [20]. Dominguez reported that more than half of the diabetic patients sought advice from someone, and the first person they went to was their doctor and 41.2% of the respondents visited their doctor on a monthly basis, and effective factors for seeking consult were the presence of co-morbidities, perceived effectiveness of treatment, observance of proper diet, exercise, and compliance to treatment [8].

In this study, gender solely had no effect on the health seeking behavior variables, and age was significantly associated with the follow-up pattern only. Older patients are more likely follow-up their treatment in a regular basis. Urban patients were more likely to go to a physician in the event of illness, whilst rural patients were more likely to consult family members, friends and relatives, and do self-medication. This issue can

Table 5. The first action in case of the occurrence of disease symptoms

Variables	Categories	Consult and get tips from family members, friends and other people around you	Change Behavior (Change Diet or/and Physical Activity Level)	Self-medication	Refer to a doctor	χ^2	P value
Gender	Male	5.7%	29.2%	31.8%	33.3%	3.95	0.26
	Female	6.8%	28.5%	26.9%	37.8%		
Age	< 40	7.1%	26.8%	25.2%	40.9%	2.96	0.81
	40–60	7.0%	29.3%	29.1%	34.6%		
	> 60	5.6%	28.7%	28.9%	36.9%		
Income status	< 10 million Rials	7.3%	22.7%	22.7%	47.4%	23.27	< 0.0001
	> 10 million Rials	5.8%	32.6%	30.1%	31.5%		
Education status	Illiterate	7.6%	26.0%	26.2%	40.2%	14.54	0.1
	Reading and writing ability	4.4%	30.0%	29.8%	35.7%		
	Diploma	6.2%	31.3%	31.8%	30.8%		
	Academic education	10.9%	32.8%	28.1%	28.1%		
Habitant status	Urban	5.4%	29.8%	28.0%	36.7%	18.82	< 0.0001
	Rural	14.1%	20.7%	32.6%	32.6%		
Type of basic health insurance	Iranian health insurance	6.9%	30.9%	29.4%	32.7%	3.704	0.29
	Social security	5.9%	27.6%	28.0%	38.4%		
Supplementary health insurance status	Yes	5.8%	31.0%	27.0%	36.2%	2.406	0.49
	No	6.8%	27.2%	29.6%	36.4%		
Claims to be knowledgeable about the cause of DM	Yes	6.1%	28.9%	28.0%	37.0%	2.72	0.43
	No	8.3%	28.0%	31.5%	32.1%		
Chronicity (in years) of DM	< 5 years	8.6%	26.6%	27.6%	37.3%	14.39	0.02
	5–10 years	4.6%	25.0%	28.1%	42.3%		
	> 10 years	5.5%	32.6%	29.7%	32.2%		
Type of current treatment	Oral pills	6.0%	28.0%	28.3%	37.7%	7.56	0.27
	Insulin	6.2%	31.0%	29.4%	33.4%		
	Change in lifestyle (change in diet and physical activity)	11.3%	21.1%	25.4%	42.3%		
Presence of co-morbidity or complications	Yes	6.0%	30.3%	28.5%	35.2%	5.01	0.17
	No	7.7%	23.9%	28.7%	39.7%		
The history of hospitalization due to complications of DM during the past year	Yes	7.8%	26.2%	34.0%	32.0%	10.37	0.01
	No	5.8%	29.8%	26.2%	38.2%		
The acuteness of disease	Sever	7.0%	28.8%	30.8%	33.3%	27.90	< 0.0001
	Moderate	8.7%	20.3%	26.0%	45.0%		
	Low	2.5%	36.6%	24.8%	36.1%		

Table 6. The first care provider to consult in case of manifestation or exacerbation of disease symptoms

Variables	Categories	Primary health care providers such as Behvarz	A general practitioner or family doctor	Specialist	χ^2	P value
Gender	Male	11.5%	26.5%	61.9%	3.31	0.19
	Female	7.9%	35.4%	56.7%		
Age	< 40	4.0%	26.0%	70.0%	5.21	0.26
	40–60	9.8%	36.4%	53.8%		
	> 60	10.0%	30.0%	60.0%		
Income status	< 10 million Rials	11.9%	40.3%	47.8%	8.47	0.01
	> 10 million Rials	8.7%	25.2%	66.0%		
Education status	Illiterate	11.9%	37.1%	51.0%	13.61	0.03
	Reading and writing ability	10.2%	31.5%	58.3%		
	Diploma	1.7%	31.7%	66.7%		
	Academic education	4.3%	13.0%	82.6%		
Habitant status	Rural	9.8%	39.0%	51.2%	0.98	0.61
	Urban	9.0%	31.8%	59.2%		
Type of basic health insurance	Iranian health insurance	9.8%	30.9%	59.3%	0.41	0.81
	Social security	8.6%	34.1%	57.3%		
Supplementary health insurance status	Yes	6.1%	30.5%	63.4%	3.17	0.2
	No	10.8%	33.8%	55.4%		
Claims to be knowledgeable about the cause of DM	Yes	8.9%	28.6%	62.5%	17.06	< 0.0001
	No	10.2%	57.1%	32.7%		
Chronicity (in years) of DM	< 5 year	9.9%	42.4%	47.7%	17.78	0.001
	5–10 years	7.2%	33.7%	59.0%		
	> 10 years	9.2%	19.3%	71.4%		
Type of current treatment	Oral agents	11.1%	38.2%	50.7%	17.82	0.001
	Insulin	5.2%	20.7%	74.1%		
	Change in lifestyle (change in diet and physical activity)	10.0%	40.0%	50.0%		
Presence of co-morbidity or complications	Yes	9.8%	28.9%	61.3%	5.76	0.05
	No	7.2%	42.3%	50.5%		
The history of hospitalization due to complications of DM during the past year	Yes	8.4%	18.9%	72.6%	12.01	0.002
	No	9.3%	37.6%	53.1%		
The acuteness of disease	Sever	9.1%	25.3%	65.6%	11.03	0.02
	Moderate	10.6%	37.6%	51.8%		
	Low	7.3%	43.9%	48.8%		

be attributed to easy access to a physician in urban areas. A more percentage of individuals with higher income used behavior change and self-medication in the event of illness symptoms, while those with lower income preferred to go to a physician.

Moreover, more than two-thirds of the patients reported that they referred to their own physician in the event of worsening of symptoms, and only a small percentage changed their own physician. This issue can be regarded as a positive behavior because continuity of care is of paramount importance in diabetes management and this reinforces the patient-physician relationship.

The results showed that the highest percentage of patients referred to the public hospitals and university clinics and approximately one-third of patients reported they referred to the private centers. Patients with higher income and education, patients residing in urban areas and those who were covered by social security and supplementary insurance were more likely to go to private health centers. Private centers provide high-quality hoteling services with a less waiting list but private sector tariffs are high and it is expensive to go to a private health center. So, people who have a better socioeconomic status are more likely to go to the private health centers. Therefore, the cost of private sector services area barrier for poor people to using these services. In Hjelm et al.'s study, men more often turned to private for-profit clinics while females more often used free governmental institutions [1]. In Bhosale and Durgesh's study, only 34.1% of patients used government health facilities [21].

Also, diabetic patients with a longer history of diabetes were more likely to refer to private centers, while those with a shorter history of diabetes were more likely to refer to primary health care centers. Duration of the disease is considered as an effective factor in the selection of the service provider among these patients. This issue may be due to the severity and complexity of disease in patients with a longer history of diabetes. In Iran, private centers provide more advanced secondary and tertiary services.

Patients without comorbidity, those without a history of hospitalization and those who reported their disease severity as being moderate were more likely to go to private centers. Patients whose usual treatment was a lifestyle change (change of diet and physical activity level) were more likely to go to primary health centers, whilst individuals who were under insulin therapy were more likely to refer to private centers. In addition, patients who were under insulin therapy had a more severe and complex condition, and these patients refer more to private and specialized centers. Hence, disease severity is an important factor in the type of individuals' behavior while choosing a service provider. In a study by Inche et al., appropriate health-seeking behavior was significantly associated with age, presence of co-morbidity, family history of diabetes, distance from health facilities, perceived family support, as well as the history of early treatment seeking at diagnosis and duration of disease [22].

Most people visited a physician every three months on a regular basis even if they were asymptomatic. In Iran, after the implementation of the Health System Evolution Plan (HSEP) reform and IraPEN, primary health centers are obliged to monitor and visit diabetic patients every three months. Therefore, policies, regulations and health reforms affect health care seeking behaviors directly and indirectly. In Dominguez's study, 41.2% of the diabetic patients visited their doctor on a monthly basis [8]. In our study, 11.9% and 51.6% of patients visited their doctor monthly and every three months, respectively.

In regard to the follow-up pattern, 78.85 of patients reported they referred to a physician on a regular basis and 59.9% of patients followed up their treatment process on a regular basis. A study in 2017 showed that 82.3% of patients with diabetes were taking treatment regularly [21]. In Makinga and Beke's study, 95% of the patients preferred to consult their physicians on a regular basis [23].

Moreover, older patients, patients with social security coverage, patients with supplementary insurance coverage and patients residing in urban areas were more likely to refer to health care providers on a regular basis. Patients who are covered by social security insurance and supplementary insurance, in addition to having better socioeconomic status, pay a smaller amount of franchise when they refer to the physician. So, these individuals are more likely to go to a physician on a regular basis. In the case of the urban area residents, it can be attributed to better access to the physician. According to Yinzi Jin et al.'s study, the positive influence of increasing the number of physicians available to DM patients in rural and western areas was greater than that for urban and eastern DM patients in China [24]. Musinguzi et al.'s study showed that factors influencing HSB are related to health systems and the patient socioeconomic and structural environment [19].

In this study, the majority of the patients received the required information for the management of their disease from their physician. This can be considered as a positive behavior because they prefer to receive the required information from a well-informed source. Also, a considerable proportion of patients consulted the family members and friends. Furthermore, given that most of the

patients in this study were elderly and had a low education level, just a few proportions of them used the electronic database, blogs, websites, health portals, mobile applications, and social networks. Also, Radio and TV programs and Diabetes Associations failed to play their crucial role in informing diabetic patients. A study by Kuske et al. showed that the Internet and health care professionals were the most frequently reported sources [30].

Conclusion

Most of the diabetics often did not follow the official structure of health care providers to manage their illness, and despite the referral system and family practitioner program, more than half of the patients went directly to the specialist physician's office. Factors related to the patients (income and education status), disease characteristics (disease severity, chronicity of DM and the history of hospitalization due to DM) and health care systems (type of basic insurance and supplementary insurance status) affect the health care seeking behaviors.

Conflict of interest

The authors have no conflicts of interest to declare.

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Review of nuclear medicine methods applied in diabetology

ABSTRACT

The aim of this paper is to present the most relevant clinical applications of positron emission tomography/computed tomography, scintigraphy and single photon emission tomography with different radiotracers allowing to visualize e.g. glucose metabolism, amino acids metabolism, receptor density or inflammation and infections in diabetology. (Clin Diabetol 2019; 8, 6: 303–309)

Key words: diabetology, positron emission tomography, nuclear medicine, inflammation, scintigraphy

Introduction

According to data provided by the Central Statistical Office, it is estimated that in Poland there are more than 2.1 million people with both diabetes types and in 2016, there were 659 new cases and 2,517 407 medical consultations provided in diabetology clinics [1]. Nuclear medicine uses the properties of radioactive isotopes to visualize the various physiological processes taking place in the body. Positron emission tomography/computed tomography (PET/CT) and scintigraphy, the extension of which is single photon emission tomography/computed tomography (SPECT/CT) and three-phase scintigraphy (allowing to visualize the blood flow, tissue and bone phase of the examined area) are now the basic imaging methods used in nuclear medicine. In addition nuclear medicine techniques have been used in oncology (for example to determine the severity of the disease or to assess the response to the applied treatment), cardiology (for example for assessing myocardial perfusion or myocardial viability) and in neurology (i.e. for imaging a brain tumors and in neurodegenerative disease) [2].

The aim of this paper is to present the most important applications of PET/CT, SPECT/CT and three-phase scintigraphy and also to show the future directions which might have implications on management in diabetic patients.

Scintigraphy

Scintigraphy in an imaging technique that uses a gamma camera consisting of one or more detector heads to create a functional 3D distribution of a photon emitter radionuclide from the patient's body. The raw data for the reconstruction of spatial distribution are acquired as a series of discrete planar images at multiple angles over the longitudinal axis of the patient [2].

The main elements of the detector head are a collimator, a scintillation crystal and a photomultiplier (Fig. 1). The collimator creates a radionuclide image of the patient on the scintillation crystal, which converts gamma rays into light. The light is detected by photomultiplier tubes whose digital output is used to calculate the spatial coordinates of each scintillation event. The computer system is used for processing (reconstructing), storing and displaying images [3].

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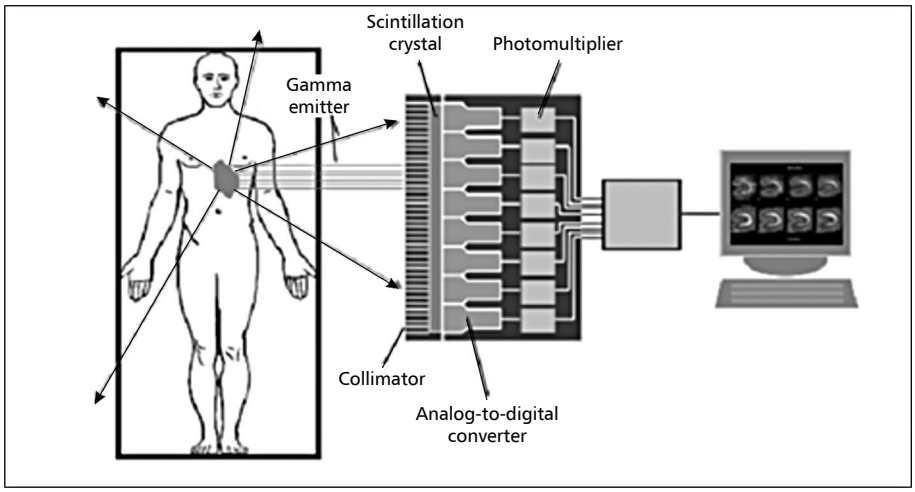

Figure 1. Scheme of a conventional gamma camera used in scintigraphy [3]

Whole body scan (WBS) with 99 metatable technetium (^{99m}Tc) labelled diphosphonates, e.g. methylene diphosphonate (MDP), hydroxyethylidene diphosphonate (HEDP) and hydroxymethylene diphosphonate (HMDP) are a useful tool for imaging malignant otitis externa and scintigraphy with labelled leukocytes, is the most commonly used techniques in inflammation and infection imaging. Infections in diabetology can be divided into frequent and specific ones. Frequent infections include: fungal infections, pulmonary tuberculosis, pneumonia, bacteremia, urinary tract infections, renal replacement infections (hemodialysis or continuous ambulatory peritoneal dialysis), skin and bone infections and diabetic foot infections [4]. Specific infections in diabetic patients include nasocerebral mucormycosis, malignant otitis external, emphysema, and emphysema cholecystitis [5–7].

Diagnosis of diabetic foot

Magnetic resonance imaging (MRI) is the method of choice in imaging diabetic foot infection and osteomyelitis [8], however, also radioisotope methods, including scintigraphy, are beginning to play a significant role in the diagnosis of this disease. Three-phase scintigraphy allows to obtain additional information on the vascularization of the examined area. The first phase of the study (blood flow) exposes increased blood circulation around the ongoing inflammatory process, while the second phase of the study (tissue phase) evaluates the volume of blood in the area. The last part of the study is the metabolic phase (delayed phase), which illustrates the increased accumulation of the radiotracer in the area in which increased osteoblastic activity is noted [4]. The image obtained with this technique does not allow differential diagnosis of fracture with Charcot neuro-osteoartropathy [9] due to low specificity (from 10–67%, mean 40%) [10], therefore, a modification of the study was introduced by adding 4th phase after 24 hours from radiopharmaceutical administration. This is due to the fact that in the osteomyelitis, the accumulation of the radiotracer lasts for several hours, while in uninfected bones up to 4 hours. If in phase 4 the target-to-background ratio increases, this is the sufficient basis for confirmation of osteomyelitis [11], while the ratio of target-to-background with a point of 1.06 with 82% sensitivity and 92% specificity allows to diagnose osteoarthritis. Compared to standard three-phase bone scintigraphy, adding 4th phase increases the specificity of the method to 87% [12].

A significant improvement in the diagnosis of osteoarthritis has been observed with the use of labeled leukocytes (*in vitro* or *in vivo*), which show increased accumulation in inflammation. One of the most commonly used ligands labeled with ^{99m}Tc is hexamethylpropylene amine oxime (HMPAO). The sensitivity and specificity of a static scintigraphic study using ^{99m}Tc-HMPAO is 90–93% and 86–100% respectively [13, 14], while the sensitivity and specificity for three-phase scintigraphy with ^{99m}Tc-HMPAO is 92.6% and 97.6% respectively in bone inflammation diagnosis [15].

Myocardial ischemia and myocardial infarction are much more common in patients with diabetes due to the asymptomatic course of cardiac complications in these patients, therefore, an appropriate strategy can have a key impact in the diagnosis of silent ischemia. Methoxyisobutylisonitrile (MIBI) labeled with ^{99m}Tc is the most widely used radiotracer for assessing myocardial perfusion. The ^{99m}Tc-MIBI-SPECT/CT examination performed after exercise is characterized by high sensitivity and accuracy in the diagnosis of coronary

heart disease. Authors from the International Atomic Energy Agency found that patients with diabetes more often suffer from myocardial ischemia and that diabetes was an independent factor associated with the occurrence of myocardial ischemia in relation to the control group. In addition, in people suffering from diabetes, myocardial ischemia was more frequent, visible in the electrocardiogram (ECG) picture during exercise, while no significant differences in detecting myocardial ischemia between the group of patients and the control group in ECG and ^{99m}Tc-MIBI-SPECT/CT were found [16].

Diabetic gastroparesis

Gastroparesis is defined as a delay in gastric emptying and one of causes is a complication of diabetes mellitus [17]. According to various sources diabetic gastroparesis is diagnosed in 30–50% of patients in type 1 and 2 diabetes and is more common in female than in men [18]. Diabetic gastroparesis might be divided into two types: reversible (when normal gastric motility returns after glycemic normalization) and irreversible (when after normalization of glycemia there is still incorrect gastric motility function). There are several methods to measure gastric emptying, however scintigraphy with ^{99m}Tc is the gold standard. This is a noninvasive imaging techniques which involves measuring the rate of gastric emptying with the meal (usually 2 slices of bread with jam and 2 large eggs with ^{99m}Tc). Imaging is performed at baseline, 30 minutes, 1 hour, 2 hours and 4 hours after the meal. The 1 hour postprandial scan is use to visualize the rapid gastric emptying while the 2- and 4-hour scans are used in delayed visualization of gastric emptying. Extension of gastric emptying by 10% over a 4-hour norm in the case of men allows to diagnose a diabetic gastroparesis, while in women the prolonged time must exceed 25%. Other radionuclide methods which allow to measure gastric emptying include breath testing utilizes a nonradioactive carbon isotope (^{13}C) bound with spirulina or octanoic acid which is mixed with eggs, absorbed from small bowel and metabolized by the liver. Then it is expelled from lungs and measured in exhale breath. Specificity and sensitivity of this method is similar to the scintigraphy with ^{99m}Tc (80% and 86%, respectively), however in a patients with concurrent lungs, pancreatic or liver disease, this examination might give a false positive results. That's why scintigraphy with ^{99m}Tc is the gold standard in diagnosis of diabetic gastroparesis [17, 18].

Other infections

In case of malignant otitis external (MOE) ^{99m}Tc-MDP study is helpful in differential diagnosis from simple external otitis by the increased accumulation of the radiotracer in the temporal bone and the base of the skull. ^{99m}Tc bone scan is more sensitive than other imaging techniques like CT scans or radiography and allows for earlier diagnosis of MOE, because it shows functional changes which occur earlier than anatomic ones. Additionally, a scintigraphy with gallium 67 has been proposed to assess the response to treatment in patients with MOE [19].

Diagnosis of kidney diseases with labeled dimercaptosuccinic acid ^{99m}Tc (^{99m}Tc-DMSA) allows to determine the distribution of kidney function, the focal changes in the parenchyma, to determine the shape and position of the kidneys or to assess the transplanted kidney. The degree of radiopharmaceutical accumulation is dependent on the functional state of the proximal tubules and blood flow [20]. In case of acute pyelonephritis, there are three types of abnormal results: regional, multifocal and the diffused one. As a result of scar formation in the course of chronic pyelonephritis, a reduced uptake of the radiopharmaceutical (usually in a triangular shape, the base directed towards the kidney surface) is observed in the damaged part of the kidney parenchyma [21, 22].

Scintigraphy with ^{99m}Tc labeled iminodiacetic acid (IDA) allows to assess the function of the liver and bile ducts and imaging of a gallbladder. Presence of gallbladder in ^{99m}Tc-IDA scintigraphy suggest cystic duct patency and absence — obstruction. This is very important imaging method, especially in type 2 diabetes, because allows to differentiate non-alcoholic fatty liver disease (NAFDL) with non-alcoholic steatohepatitis (NASH), hepatitis and liver cirrhosis. NAFDL is associated with insulin resistance and obesity which are one of the most common causes of type 2 diabetes. The most commonly used are 3 ligands: trimetyloacetanilido acid (MBrIDA), hepatoiminodiacetic acid (HIDA) and diisopropyliminodiacetic acid (DISIDA) [23]. This imaging shows a higher sensitivity compared to ultrasound (86% to 48% respectively), while the combination of both techniques increases the sensitivity to 90% [24]. The secondary function of the ^{99m}Tc-IDA examination is the liver uptake rate of the radiopharmaceutical (in a well-functioning liver within a few minutes a marked decrease in radioactivity in the bloodstream and visibility of the liver parenchyma is noticed) and the rate of passage of the radiopharmaceutical from the bile ducts to the small intestine (properly functioning gallbladder becomes visible between 15 and 30 minutes of the study, while at the end of the acquisition the radiopharmaceutical should be visible in the bowel projection). Decreased radiotracer uptake from the bloodstream suggests damage to hepatocyte function, delaying bile

excretion to the intestines is an evidence of impaired bile duct patency, and total intestinal uptake after 24 hours from radiopharmaceutical administration — on arthritis or total obstruction of the bile ducts [22, 23].

Positron emission tomography (PET)

The physics of imaging in positron emission tomography (PET) is based on the emission of positrons from nuclei with excessive amounts of protons. Positron moves a short distance in the patient's tissue and annihilates on contact with electron. The effect of the annihilation of the electron-positron pair is the generation of two photons with 511 keV that are emitted in opposite directions. Due to the nature of the photon pair emission process, a single detector system used in SPECT imaging does not provide the appropriate geometry for the simultaneous detection of two photons [24], thus a typical PET scanner consists of many rings of scintillation detectors. Due to the registration of photon coincidences, a collimator is not required, which significantly increases the sensitivity of PET in comparison with the gamma camera [2]. When both photons from an annihilation are absorbed simultaneously (in coincidence) in two opposing detectors, a count will be made (Fig. 2). The photon registration process looks the same as in the gamma camera. As a result of annihilation of positrons of the radionuclide with electrons in tissue, the emission of photon pair of 511 keV in opposite directions takes place. Registration of photons on a scintillation crystal within a given time interval means coincidence detection. Further processing of the recorded signal using a photomultiplier allows to determine the spatial coordinates of each count [25]. Using an electronic system consisting of a photomultiplier tube and an analog-digital converter, an electrical signal from a scintillation in the detector's crystal after interaction with the photon is recorded. After the acquisition, first the acquired images are corrected for damping and scattering, etc., and then processed mathematically to obtain transverse images of the layers of the studied area [26].

One of the most commonly used radiopharmaceuticals in the PET technique is the fluorine-labeled glucose analogue (^{18}F-FDG). Due to the metabolic vector used, this radiopharmaceutical, in addition to uptake in cells showing increased request/needs for glucose (including cancer cells), also accumulates in inflammatory lesions. The sensitivity of the ^{18}F-FDG--PET/CT study decreases with the increase glucose level (should not exceed 200 mg/dL; 11.1 mmol/l), hence new, alternative markers for ^{18}F-FDG that can be used in diabetic patients, regardless of the level of glucose in the blood are needed.

For neuroendocrine tumors imaging (including insulinoma and glucagonoma), which has the presence of somatostatin receptors, the PET/CT study with ^{68}Ga-DOTA-peptides is used. Several derivatives of ^{68}Ga-labeled somatostatin are available, which differ in their affinity for various somatostatin receptor subtypes. ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC, ^{68}Ga-DOTA-TATE and ^{68}Ga-DOTA-LAN they bind to the somatostatin receptor subtype 2 (SSTR2), showing different affinity for other receptor subtypes SSTR: ^{68}Ga-DOTA-NOC and ^{68}Ga-DOTA-LAN to SSTR 3 and 5, ^{68}Ga-DOTA-TOC connects also with SSTR5 (however with less affinity than DOTA-NOC), whereas ^{68}Ga-DOTA-TATE shows the highest affinity for the SSTR2 receptor among all peptides [27, 28].

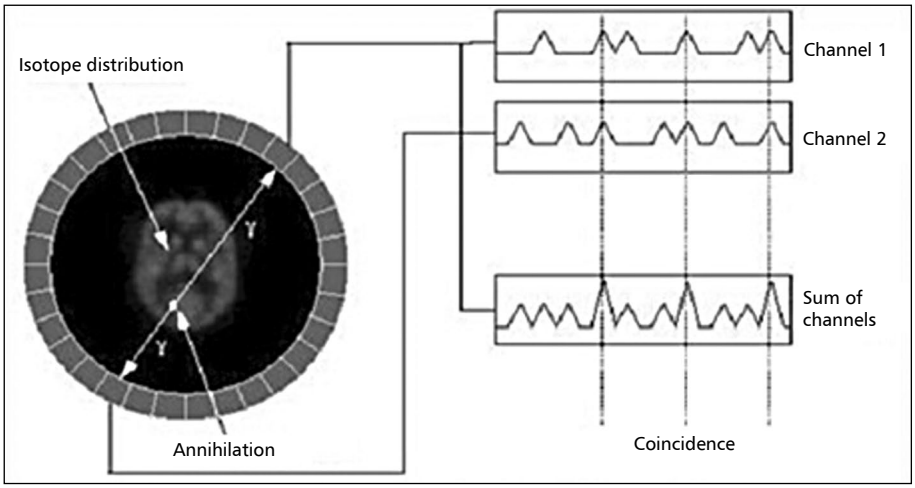

Figure 2. Schematic diagram of a coincidence [25]

Another radioisotope used in the diagnosis of insulinomas in children and infants is Fluorine-labeled dihydroxyphenylalanine (^{18}F-DOPA) [28]. Congenital hyperinsulinism is one of the most common causes of hypoglycaemia in newborns, among which two histological subtypes are distinguished: diffused (about 95–98% of cases) and focal one. In half of infants requiring pancreatic resection, a curable focal form is found, and the performance of the ^{18}F-DOPA-PET/CT study allows for differential diagnosis of diffused and focal forms and the selection of an appropriate treatment method. Identification and exact location of the lesion in the case of focal hyperinsulinism allow resection limited to the focal point leading to a reduction of postoperative complications in treated children including diabetes and pancreatic enzyme substitution [29, 30]. ^{18}F-DOPA-PET/CT study diagnose 75–100% focal changes [31].

One of the latest developments in nuclear medicine is the ability to label glucagon-like peptide-1 receptor (GLP-1R) present in insulinoma. It was found that no other peptide receptor showed such high levels of expression in this type of tumors (frequency > 90% and density 8.133 dpm/mg tissue) [32]. PET/CT study with labeled gal 68 exendin-4 (^{68}Ga-DOTA-exendin-4 PET/CT) showed that it is a sensitive diagnostic tool for detecting insulinoma tumors and locating latent tumors [33, 34]. Sensitivity of ^{68}Ga-DOTA-exendin-4 PET/CT exceeds conventional imaging methods (including computed tomography, MRI and transesophageal ultrasound) in detecting this types of tumors [35].

Future directions

A huge development took place in the production of radiopharmaceuticals for imaging bacterial infections. ^{64}Cu-MAb 1D9, ^{89}Zr-MAb 1D9, ^{68}Ga-UBI29–41, m-[18F]-fluoro-PABA, [methyl-11C]-D-methionie, ^{18}F-FDS, ^{18}F-fluoromaltose and ^{18}F-fluoromaltohexaose are one of the most recent radiopharmaceuticals used in PET/CT studies for imaging bacterial infections.

MAb (1D9) against *Staphylococcus* antigen A or a gram-positive bacterium has been recently labeled with a copper 64 isotope (^{64}Cu, $T_{1/2}$ = 12.7 h) and zirconium 89 (^{89}Zr, $T_{1/2}$ = 78.4 h) and evaluated in PET on mice. MAb 1D9 clearly targeted *S. aureus* on the 3rd day after the radiotracer injection, the ratio of abscess to background was 2–3 times higher than in control groups and comparable to the results obtained from the ^{18}F-FDG-PET study. MAb 1D9 also showed nonspecific uptake in *E. coli* infections and lipopolysaccharide-induced sterile inflammations, attributed to the binding of MAb to Fc receptors present in the membrane of the cell-infiltrating macrophages [36–38].

Trimethoprim (TMP) is an organic chemical compound, a chemotherapeutic agent that inhibits bacterial dihydrofolic acid reductase, an enzyme in the synthesis of DNA and the folate pathway of most bacterial species (including Gram-positive and Gram-negative), mycobacteria and some parasites. As a PET radiotracer, ^{18}F-fluoropropylotrimethoprim (^{18}F-FPTMP) showed a high uptake in bacteria, in *in vitro* studies performed in mice and a high ratio of abscesses to muscles, which ranged between 2 and 3 in mice. However, due to the high activity in the liver, gallbladder and intestines, imaging of infections in the abdomen appears to be limited [39].

Antimicrobial peptides (AMP) designed to quickly kill a wide spectrum of pathogens, including Gram-positive and Gram-negative bacteria, fungi, parasites and even capsular viruses. $UBI_{29–41}$ most commonly labelled with ^{99m}Tc, is a cationic synthetic particle derived from natural cationic vicbuicidin AMP ($UBI_{1–59}$) however, recently this ligand has been used for PET/CT studies in combination with ^{68}Ga. Both PET analogues ^{68}Ga-NOTA-$UBI_{29–41}$ and ^{68}Ga-NOTA-$UBI_{31–38}$ showed comparable characteristics of uptake in infected femoral muscles in mice and rabbits. However $UBI_{29–41}$ was also accumulated in yeast-induced infections, which reduces the specificity of the use of this marker to visualize only the bacterial infections [40, 41].

PET with a radiofluorinated analogue of p-aminobenzoic acid *m*-[18F]-fluoro-PABA, which is a substrate for the synthesis of folic acid in prokaryotic organisms, was performed on rats infected with methicillin or staphylococcus aureus. PET study with *m*-[18F]-fluoro-PABA showed rapid uptake in bacterial infections (core to muscle ratio ≈ 8) and low uptake in sterile inflammation (about nine times lower), which meant that this radiopharmaceutical can be considered as specific for imaging bacterial infections. *m*-[18F]-fluoro-PABA showed reduced uptake in tissues infected with *Staphylococcus* aureus treated with oxacillin, indicating the possibility of using this radiopharmaceutical also to monitor response to treatment [42].

Another substrate associated with the folic acid synthesis pathway is methionine, which has been studied in the context of Staphylococcus aureus and *E. coli* infection imaging. Based on the uptake in infected muscles in mice, a PET study with carbon-labeled (^{11}C) methyl-D-methionine was helpful in differentiating (at 6–9 times higher abscess to inflammation ratios) between active non-active *E. coli* and staphylococcus infection [43].

Siderophores, low molecular weight iron transporters, are used by most bacteria, fungi and some plants to remove iron from the environment, which is key

because of many different metabolic processes. In PET studies siderophores are usually labelled with ^{68}Ga. An *in vivo* study with ^{68}Ga-labeled triacetylfusarinine (^{68}Ga-TAFC) performed on rats infected with *A. fumigatus* (Aspiroplasma), *S. aureus* and sterile inflammation in the thigh and lung muscles infection, showed increased radiotracer accumulation in *A. fumigatus* infected sites (ratio abscess to the background between 5.8 and 6.6). At the same time, there was also an increased uptake of the radiotracer in sterile inflammatory sites (although lower than in the case of *A. fumigatus*), while in tissues infected with *S. aureus*, no ^{68}Ga-TAFC uptake was observed [44].

In the imaging of bacterial infections, [18F]-fluorosorbitol is also used (^{18}F-FDS), sorbitol analogue, which is a substrate metabolized only by enterobacteria. It has been proven that ^{18}F-FDS-PET imaging is a promising diagnostic tool which helps to differentiate *E. coli* infections or a pneumonitis bacillus (*K. pneumoniae*) with Gram-positive infection. *E. coli* infections can be adequately visualized with ^{18}F-FDS in mice, with uptake in infected tissues is about eight times higher than sterile inflammation. Additionally, ^{18}F-FDS-PET after ceftriaxone antimicrobial therapy in *E. coli* infection showed that in infected tissues, radiotracer uptake is eight-fold lower for those treated with ceftriaxone. This showed that ^{18}F-FDS can be used in antimicrobial therapy monitoring [45].

Type 1 diabetes is characterized by the loss of β-cells in the islets of the pancreatic Langerhans, followed by a deficiency of insulin secretion in response to hyperglycaemia. The development of an *in vivo* test that would allow the assessment of β-cell mass (BCM) mass measurement would significantly increase the ability to track diabetes therapy. The β-cells and neurological tissues share common cellular receptors and transporters, therefore a study using brain radioligands for their ability to identify β-cells has been performed. The D2/D3 receptor agonist, radioligand ^{11}C-(+)-propyl-hexahydro-naphthooxazin (^{11}C-PHNO) was the only one showing high uptake in the pancreas with respect to the abdominal organs, such as the kidneys, liver and spleen. However, further *in vitro* and *in vivo* studies to determine the specificity of the D3 receptor for β-cells are indicated to introduce ^{11}C-PHNO as a specific radiotracer used to measure BCM [46].

One of the most advantages of imaging in nuclear medicine is that it allows imaging of functional changes which usually occur much earlier than anatomical changes that can be visualized by standard imaging methods. All the new methods which are in the clinical trial phase, if will be approved for clinical use, might allow in earlier detection of changes, especially in the diagnosis of infection in diabetics, and thus less diabetic complications and better care for patients. Also early detection of changes which may suggest future diabetes development by using nuclear imaging modalities, will allow close control and better care of this group of patients.

Conclusion

Nuclear medicine plays a significant role in the diagnosis of infection and other diseases occurring in diabetic patients. The use of proper radiopharmaceuticals obtain accurate location and diagnosis of diseases, and the combination of nuclear medicine techniques with commonly used imaging methods in diabetic patients permits the implementation of appropriate treatment and its control.

Conflict of interest

All the authors declare no conflict of interest in the field covered by this paper.

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REVIEW ARTICLE
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Muscle cramps — a mini review of possible causes and treatment options available with a special emphasis on diabetics — a narrative review

ABSTRACT

Muscle cramps are characterized by sudden, painful involuntary contraction of the muscles. The cramps sometimes become disabling and the prevalence is more in the elderly. The etiology of the cramps are diverse and some time the cramps are idiopathic. There are many underlying pathophysiological disorders like hypocalcemia, hypomagnesemia, hypothyroidism, and hepatorenal dysfunction which causes muscle cramps. Similarly, diabetes mellitus results in muscle cramps due to electrolytic imbalance, hypoglycemia, peripheral arterial insufficiency, and neuropathies. Persistent muscle pain in diabetic patients degrades the quality of life of those patients. Although the pathophysiology and etiology of the muscle cramps are understood to some extent, the same is less explored from diabetes mellitus perspective. Hence the objective of this review is to explore the underlying factors responsible for muscle cramps in diabetes so that proper strategy for pharmacotherapy can be made to manage this condition. (Clin Diabetol 2019; 8, 6: 310–317)

Key words: muscle cramps, diabetes mellitus, hypocalcemia, hypomagnesemia

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Introduction

Muscle cramps are generally painful involuntary contraction of muscles caused due to ectopic discharge from nerve terminals or nerves [1]. These muscle cramps differ from benign to sometime disabling. Hence, a detailed history and neurological examination is indispensable to identify diverse etiology of the muscle cramps [2]. According to a cross sectional study on 365 older outpatients in UK, 50% of them reported frequent muscle cramps [3]. In another review 56% out of 515 old patients reported to have muscle cramps at least once in a week [4]. However, a very limited epidemiological data is available on the prevalence of muscle cramps in patients with diabetes and in general population [5]. A demographic study conducted on diabetic patient in Toronto revealed, a 75.5% of type 2 diabetic and 57.5% of type 1 diabetic patients encountered muscle cramps [6]. In the above study, diabetic neuropathy was found to be the most important independent risk factor for muscle cramps. Bharucha et al. in their door-to-door survey analysis in a parsi community found diabetes to be the most common cause for non-compressive neuropathy [7]. The high rate in Parsis is probably related to aging population, and in urban slum, it may be due to nutritional and adverse environmental factors and needs further study. Saha et al. in his study showed an increase in age and sex specific prevalence of neurological disorders in both sexes although there was a minor dip in female population in their fourth and fifth decades [8]. A population-based survey by Gouri-Devi et al. showed a two times high rate of neurological disorders as compared to urban parts but the reasons could not be determined [9].

In diabetes mellitus, muscle cramps are a common symptom which may occur due to electrolytic imbalance, hypoglycemia, peripheral arterial insufficiency and neuropathies. These cramps generally occur in the lower extremities and the patients mostly experience it during night. The symptoms range from cramping muscle pain to burning sensation. Muscle infarction is a rare cause of acute muscle pain in diabetic patients [10]. The cramp-fasciculation and peripheral neuropathies are closely associated with muscle cramps [2]. Besides, hypocalcemia, hypomagnesemia, hypothyroidism, and hepatorenal dysfunction may also contribute to muscle cramps. Sometime these muscle cramps are idiopathic which vary in presentation from subject to subject. There are only few studies available which completely address the issue of muscle cramps in relation with diabetes. Hence, the objective of this review is to discuss completely on the pathology, cause and possible treatment modalities of muscle cramps with special attention on diabetes.

Pathophysiology and etiology of muscle cramps

Various theories have been postulated to explain the pathophysiology of muscle cramps [11].

General pathophysiology of cramps

1. Old theories

Psychosomatic theory

The psychosomatic disorder causing muscle cramps is one among the major theories postulated by olden French and German neurologist. As per Féré, muscle cramps result due to heavy exercise, nervousness, neurasthenia, hysteria or epilepsy [12].

Vascular theory

In the 1920s, vascular insufficiency during muscle contraction was believed to be the major cause of muscle cramps. Accumulation of lactic acid was believed to cause persistent involuntary contraction and muscle cramps [13]. Vascular theories were believed until the mid-1980s in central Europe. However, these theories are rejected by Santler, based on clinical common sense [14, 15].

Deformity theory

Static origin of muscle cramps is one among the theories postulated in the past responsible for muscle cramps. Muscle cramps in feet and calves' muscle are believed to be due to static deformities of the back, the pelvis, legs and feet [16, 17]. As rest removes the sources of irritation, this theory supports the argument of healing effect of the rest on exercise induced muscle cramps [18].

Myogenic theory

Another theory is postulated by Strümpell [19] who said that muscle cramps have a myogenic origin, like myotonia. He postulated that the contraction of the sarcoplasma in the muscle fibrils cause muscle cramps. Hence this pain resembles violent colics. This view was opposed by Grund, 1971 [20].

ATP deficiency theory

It is a well-known fact that muscle cramps occur due to lack of relaxation of skeletal muscles. Upon relaxation of skeletal muscles, myosin fibers get dissociated from actin. For this process to take place ATP must get attached to myosin. A paucity of ATP produces insufficient dissociation of myosin from actin [21]. In one recent study, L-carnitine proved to improve the prognosis cirrhotic cramps [22].

2. New theory

Neural origin theory

Very recent theory on muscle cramps was neural origin of cramps. A strong clinical association exists between muscle cramps and lower motor neuron diseases like amyotrophic lateral sclerosis, neuropathy and radiculopathy. These are not associated with upper motor neuron or muscular disease. Spinal reflex produced due irritation of intramuscular sensory nerve endings by toxins like arsenic, alcohol, diabetes mellitus and cholera. This leads to prolonged irritation of anterior horn nerve cells there by result in muscle cramps [23]. In another study, Klimke reported sympathetic stimulation of skeletal muscle by creatine or neurovegetative irritation is a major factor of muscle cramps [24]. The loss of motor neurons with increased age leads to muscle cramps in older people [2]. This result is espoused by a case-control study where older subjects with nocturnal cramps found to have lesser lower limb muscle-strength compared to their counter parts without nocturnal cramps [25]. Tendon shortening in advanced aged individuals due to long immobility leads to excitation of nerve terminals which in turn leads to the development of cramps.

Site of origin

The site of origin of the muscle cramps plays pivotal role in pathophysiology of muscle cramps. Many studies have suggested that muscle cramps result from a rapid penetrative firing of motor unit action potential which are set at a rate much higher than those needed for involuntary contractions. This is due to spontaneous discharges of motor nerve ending rather than a central or muscular origin [2]. The factors which contribute to muscle cramps include excitability of anterior horn cells or the intramuscular motor nerve endings.

Etiology of muscle cramps

Table 1 is a crisp presentation of etiology of muscle cramps. Table 1 summarises few of the huge number of etiologies of muscle cramps in a diabetic population and it has been found that most of them are either due to idiopathic reason or due to underlying peripheral neuropathy [6, 31]. A study of outpatient veterans reported leg cramps in 75% of those with peripheral vascular disease, 63% of those with hypokalemia, and 62% of those with coronary artery disease [5].

There is a famous case report of a 56 year old poorly controlled type 2 diabetes who experienced severely painful muscle cramps of bilateral upper and lower extremity shortly after analogue insulin injection the cause of which had been attributed to a 16% drop in potassium due to insulin injection from baseline on top of an already existing neuropathy in that patient [26]. A similar finding was observed long back in Duke University Medical Centre way back in 1992 [32].

Diabetic neuropathy and nephropathy were reported to have been associated with high incidence of muscle cramps but neuropathy seems to be an independent risk factor as well as the type of diabetes like type 2 > type 1 [6]. Towards the end of hemodialysis one third of the patients experience muscle cramps [33] which gets subsided by volume expansion with hypertonic dextrose or saline solution. Intentionally changing the sodium concentration of the dialysis fluid during the dialysis is sometimes used to preserve the plasma volume towards the end of the dialysis. This process reduces the incidence of muscle cramps in some cases [34, 35]. It is also known that any acute extra cellular fluid volume contraction cause muscle cramps. This occurs during excessive vomiting, diarrhoea or excessive sweating or diuretic therapy. About 60 percent of patients with cirrhosis reportedly have leg cramps, most of whom are older patients with advanced disease [29]. Chronic venous insufficiency also results in cramps but strangely enough the treatment has not lead to the slowing down of the course of muscle cramps [11]. Nerve damage from cancer treatment may be a cause of legs cramps, with a small study demonstrating that leg cramps were present in 82 percent of patients with cancer [30].

Muscle cramps caused due to adverse event of number of drugs, however, very few drugs reported to have caused leg cramps. The medication with intravenous iron source, raloxifene (Evista), naproxen (Naprosyn), conjugated estrogens, and teriparatide (Forteo) produced very low incidence of leg cramps [31]. Besides, clonazepam (klonopin), celecoxib (Celebrex), and gabapentin (Neurontin) also produce leg cramps, although they are prescribed for treatment of muscle cramps. It is also believed that diuretics like hydrochlorothiazide cause leg cramps [36].

Table 1. Etiology of muscle cramps (decreasing frequency in terms of association to diabetes) [11, 26–30]

Sl. No	Cause
1	Idiopathic
2	Peripheral neuropathy
3	Peripheral vascular diseases
4	Cardiovascular disorders
5	End stage renal disease on maintenance hemodialysis and acute electrolyte changes
6	Insulin induced acute drop in serum potassium levels
7	Cirrhosis of liver
8	Venous insufficiency
9	Cancer chemotherapy
10	Drug induced — intravenous iron sucrose, raloxifene, conjugated estrogens, naproxen, teriparatide, daclizumab, levosalbutamol, etc.
11	Neurological disorders like amyotropic lateral sclerosis, parkinsonism, etc.
12	Lumbar canal stenosis

Muscle cramps are associated with various diseases which cause damage to lower motor neurons including amyotrophic lateral sclerosis (ALS) [37], peripheral nerve injury [38], and polyneuropathies [39]. Cramps are more common in the above diseases compared to other lower motor neuron ailments although the cause is not very clear till date.

Due to metabolic changes during pregnancy 30% of women in their third trimester of pregnancy experience muscle cramps [40].

Pathophysiology of muscle cramps in diabetes

Data is sparse on explaining the pathophysiology of muscle cramps in diabetics and more specifically why it actually happens mostly in early morning hours. In a rodent model study on C57BL/6 male mice, it was seen that mice quadriceps centralized nuclei and caspase 3 protein increased significantly reflected by a p value of < 0.05 in both cases [41]. The data suggested that diabetes induced muscle damage by promoting a profibrotic profile. In another human study on muscle biopsy it was found that rate of ADP depletion with rest ($p = 0.008$) and oxidative phosphorylation ($p = 0.046$) in type 2 diabetic gastrocnemius muscle was impaired as evidenced by ^{31}phosphorus magnetic reso-

nance spectroscopy [42]. L-carnitine deficiency might also play an important role in diabetic mitochondrial dysfunction which has been frequently associated with muscle cramps as the deficiency has been linked to insulin resistance [43].

Mitochondrial dysfunction is another key player in causing muscle cramps in diabetics [44]. One study revealed that almost two thirds of diabetic patients suffer from muscle cramps and often they seem to harbour diabetic neuropathy [6]. Another important aspect is a high prevalence of dyslipidemia in diabetic patients for which statins have to be used and this statin cause a reduction of coenzyme Q10 which is often responsible for the muscle cramps [45, 46]. The other suggested etiologies for muscle cramps in diabetics are hypoglycemia, peripheral arterial disease, neuropathy or electrolyte imbalances [47]. In an epidemiological study it was seen that neuropathy and not nephropathy as well as type of diabetes (type 2 > type 1) were important independent predictors of muscle cramps [6].

Characteristic changes of muscles at molecular level in diabetes

Muscle related pathologies in diabetics have multiple reasons and these are summarized below.

Genetics

Gene expression alterations have been reported with type 2 diabetes. In a study, skeletal muscle biopsies taken from male subjects with type 2 diabetes, their first-degree relatives, and healthy controls were investigated at the gene expression level using the microarray technology [48]. An important finding in another study was the substantial increase in expression of genes that are involved in insulin signaling in skeletal muscle from first degree relatives of type 2 diabetics, and the significant downregulation of the same pathway in samples of type 2 diabetic skeletal muscles (Table 2) [49].

Microangiopathy

Small vessels are often abnormal in the tissues of diabetic patients. In recent years, the center of attention has been focused on the capillary basement lamina, which is a layer of amorphous material chemically resembling collagen coating the exterior of the endothelial cells. There has been widening of the capillary basement lamina in the skeletal muscle biopsies from diabetic patients. The endothelial cells appear unremarkable, but the basement lamina seems greatly widened and is often redundant and laminated with various materials in-between the lamina. In more recent years, attention has been directed to the capillary basement lamina, a layer of amorphous material chemically resembling collagen that coats the exterior of the endothelial cells. This layer is located between the blood carrying oxygen and nutrients and the tissues. Widening of the capillary basement lamina in skeletal muscle biopsies from diabetic patients. In any event, the abnormal production of basement lamina appears to be widespread in the capillary bed of patients suffering from diabetes mellitus and these capillary abnormalities have important consequences with respect to many of the lesions that occur in this disease [50].

Table 2. Significant genetic changes in pathways/functions

Gene pathway/function	p-value	Remarks
First degree relatives		
Insulin signalling	0.005	Up regulated
TGF-beta signalling	0.068	Up regulated
RNA splicing	0.089	Up regulated
Inorganic anion transport	0.74	Down regulated
Focal adhesion	0.14	Down regulated
Inflammatory response pathway	0.326	Down regulated
Type 2 diabetic patients		
Protein modification	0.979	Up regulated
Cell cycle G1 to S control reactome	0.676	Down regulated
MAPK signalling	0.002	Down regulated
Insulin signalling	0.002	Down regulated
G-protein signalling	0.078	Down regulated
Apoptosis	0.388	Up regulated

Mitochondrial dysfunction

In one recent study, it has been observed that in type 2 diabetic patients the muscle cramps are produced due to impaired bioenergetic capacity of skeletal muscle mitochondria. The study was done by taking the biopsy of vastus lateralis muscle from lean and obese nondiabetic subjects and type 2 diabetic volunteers. The electron microscopy view of the skeletal muscle revealed the presence of smaller mitochondria in type 2 diabetic and obese volunteers compared to their lean counterparts ($p < 0.01$). Similarly, the activity of rotenone-sensitive NADH:O_2 oxidoreductase enzyme was reduced in type 2 diabetic skeletal muscle compared to the healthy subjects. Hence, it was concluded that in the skeletal muscle of diabetic patient the mitochondria lose its bioenergetic capacity.

Table 3. Differential diagnosis of leg cramps

Condition	Clinical features	Diagnosis	Treatment
Claudication	Aching, sometimes cramping, deep pain brought on by exercise; relieved with rest	History Atherosclerotic risk factors Ankle-brachial index Radiographic studies	Risk factor modification Graded exercise Invasive interventions
Exercise-associated muscle cramping	Painful cramps during or immediately after exercise Palpable muscle tightening	History	Graded exercise and stretching
Hypnic myoclonus	Sudden involuntary jerking at the onset of sleep May awaken the patient	History (from bed partner)	Reassurance
Myositis, myalgias	Deep, aching pain unrelated to exertion Weakness and poor exercise tolerance Often occurs in legs, but can affect any muscle	History Elevated creatinine kinase levels (myositis) Statin use Evaluation for polymyositis and dermatomyositis	Treat underlying cause Discontinue statin
Periodic limb movement disorder	Nonpainful, repetitive, rhythmic, slow dorsiflexion of toes, knees, and hips during sleep Daytime fatigue Patient is unaware	History (from bed partner) Polysomnography	Sleep modification, including medications

Skeletal muscle lipid content and oxidative enzyme activity

In type 2 diabetes and obese patients, a reduced oxidative enzyme activity, increased lipid content and increased glycolytic activity was observed in the skeletal muscle. Insulin resistant glucose metabolism is associated with this metabolic characteristic of the muscle [38].

Differential diagnosis and evaluation of muscle cramps

Cramps related to diabetes need to be identified carefully, condition which might mimic the diabetic cramps needs to be excluded by performing relevant test or checking the medical history of the patient. Upon exclusion of the etiologies and symptoms mentioned in Table 3, the muscle cramps related to diabetes are confirmed [51].

Treatment of muscle cramps

It is indispensable to detect underlying cause of structural and metabolic disorder leading to muscle cramps in diabetic patients. Different treatment strategies can be followed to treat acute and chronic pain in diabetes. In case of acute pain non-pharmacological strategies are adapted. Stretching or lengthening the cramps muscle stops most of the acute cramps [52, 53].

In the recent past, various clinical trials were conducted to check the safety and efficacy of various drugs and nutraceuticals in diabetes induced muscle cramps. Miller et al., in the year 2001, conducted a phase III clinical trial to study the effect of gabapentin (a glutamate blocking drug) on patients with ALS. It was a double-blind randomized controlled trail on 204 patients with ALS. The result of this trial was compared with the phase II trial of the same drug conducted in smaller population and for a shorter period of time [54, 55]. Despite the positive phase II trial report, the phase III trial failed to prove the therapeutic efficacy of gabapentin in patients with ALS. The encouraging phase II data was believed to be due to by chance. Finally, it was concluded that gabapentin had no therapeutic effect on patients with ALS. In another study, 28 elderly patients were enrolled to check the efficacy of vitamin B complex (including vitamin B_6 30 mg/day) in reducing the nocturnal leg cramps. After three months of treatment (vitamin B complex capsule TID) 86% of patients experienced remission from muscle cramps [56]. The patients were not known to be suffering from any vitamin deficiency compared to placebo group.

Naftidrofuryl, a vasodilator was studies in a cohort of 14 subjects suffering from rest cramps (night cramps). The effect of this drug on cerebrovascular and peripheral vascular disease was well established before. However, Young and Connolly in the year 1993, could establish its significant remedial effect on the rest cramps [57]. Naftidrofuryl could significantly reduce cramps frequency in the patients ($p < 0.004$) in this double-blind placebo-controlled trial. This drug enhances the utilization of glucose and oxygen in peripheral vascular disease and protect brain parenchyma during anoxia.

Diltiazem hydrochloride, a calcium channel blocker, used in hypertension was studied for the management of muscle cramps. In this cross-over double-blind study, 13 patients who experience two or more cramps per week were treated with 30 mg of diltiazem hydrochloride. There was a significant ($p < 0.04$) reduction in the number of cramps in drug treated group compared to placebo [58]. This study proved the therapeutic potential of diltiazem in the management of muscle cramps.

As per recommendation of American Academy of Neurology, vitamin B complex, calcium channel blockers such as diltiazem and naftidrofuryl are effective and can be considered for use in the management of muscle cramps (level C). It has also been recommended to avoid the use of quinine derivatives (level A) owing to their potential toxicity [59]. However, quinines can be used for an individual therapeutic trial upon confirmation on the management of potential adverse effect if any.

Oxidative stress was believed to have an effect on the development of insulin resistance [60] and in some studies treatment with antioxidants seemed to improve glycine control in type 2 diabetic patients by scavenging the reactive oxygen species [61, 62]. In one recent study, high dose of vitamin E supplementation found to improve insulin action by reducing the plasma fasting insulin and glucose levels. There was also substantial decline in cellular oxidant stress and inflammatory activity [63]. Hence, in case of diabetic muscle cramps vitamin E can be used as supplement with the first-line treatment.

The effect of L-carnitine supplementation in the management of cirrhotic muscle cramps is discussed in the previous section. However, in a recent study [64], L-carnitine supplementation (600 mg/day PO for 4 months) was found to improve the quality of life of diabetic patients suffering with muscle cramps. Hence, L-carnitine supplementation can be considered as an ideal strategy to manage muscle cramps in diabetic patients.

Role of ubiquinone

Two forms of Co-enzyme Q10 (CoQ10) namely ubiquinone (oxidized form) and ubiquinol (reduced form occurs naturally in human body [65] and other anaerobic organisms. The production of this enzyme reaches at its highest during mid-twenties and gradually declines with age. The concentration of this enzyme gets reduced to 50% by the time people reach 60 years of age [66]. In the recent past, it was reported that a significant decline in the level of CoQ10 in patients with type 2 diabetes is correlated with an increased plasma glucose level, HbA_{1c} and other oxidative stress markers [67].

The rate limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is instrumental in the mevalonic acid path way, cholesterol biosynthesis and synthesis of CoQ10.The inhibition of HMGCR, serious muscle injuries (e.g. myopathy, myositis and rhabdomyolysis) are the adverse events of statins (e.g. atorvastatin) used in the treatment of hypercholesterolemia in type 2 diabetes [68, 69]. Hence, CoQ10 supplementation for statin induced muscle symptoms have opened a new avenue in the complementary management of statin-induced myopathy in type 2 diabetic patients [70].

Special focus of muscle cramps in diabetics

The complication of muscle cramps is a very common one in our type 2 diabetic patients and the treatment should be addressed to the underlying cause if possible or else it should be treated as per the above regimen of use of co-enzyme Q10 as part of idiopathic etiology. A summary of treatment in diabetics is shown below in Table 4 as per the major contributing factors towards cramps.

Table 4. Causes and treatments of muscle cramps in diabetics

Cause	Treatment
Peripheral neuropathy	Standard treatment for neuropathy with tricyclic antidepressants, pregabalin, gabapentin
Peripheral vascular diseases and cardiovascular diseases	Stenting if needed, smoking cessation, cilostazol, standard treatment with statins and aspirin and clopidogrel
ESRD on maintenance hemodialysis	Changing the sodium concentration of the dialysis fluid during the dialysis; treating underlying co-existent neuropathy

Conclusion

Muscle cramps in diabetes reduce the quality of life of patients. Diagnosis of muscle cramps in diabetic patients is a common problem in clinical practice. However, the mechanism and underlying cause is less well understood till date. Hence, the diagnosis of muscle cramps should prompt the physician to look for any associated comorbidities like peripheral neuropathies, metabolic disorders, cirrhosis, immune mediated myositis, and ALS etc. Although the availability of treatment is less, the physician should carefully plan the treatment protocol to manage the cramps thereby improve the quality of life of patients living with diabetes.

Ethics policy

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest

All the authors have declared to have no conflict of interest.

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Experts opinion: implantable continuous glucose monitoring system — innovation in the management of diabetes

ABSTRACT

Continuous glucose monitoring (CGM) systems have revolutionized the treatment and monitoring of diabetes. These devices are recommended for diabetic patients treated with insulin, especially with recurring episodes of hypoglycemia or large circadian variation of glucose levels. CGM allows more effective adjustment of insulin doses to blood glucose trends, resulting in better metabolic control: more time spend in glucose target range, lower time spend in hypoglycemic range, lower glucose variability and improved quality of life of patients and their family members. Real time CGM provides patients not only with continuous information on glucose levels but also alerts for hypo- or hyperglycemic events.

Traditional transcutaneous CGM have some limitations, which can be resolved by the system with an implantable sensor. The Eversense CGM is the only long-term implantable rtCGM. The subcutaneous implantation procedure has proved to be simple and uncomplicated. This CGM system can be recommended in particular for patients who, due to their profession and sports discipline, cannot or do not want to use traditional transcutaneous sensors. Further groups are patients with skin complications associated with the use of traditional sensors, people who perceive frequent sensor replacement as a burden or would benefit from on-body vibration alerts. The studies showed that the patients were satisfied with Eversense, and the majority used it several times after the study ended. The main reason for resigning from the next implantation was cost of the device. (Clin Diabetol 2019; 8, 6: 318–328)

Key words: long-term implantable glucose monitoring, real-time continuous glucose monitoring system, clinical practice opinion

Introduction

The burden of diabetes is steadily increasing. Current estimations implicate that there are more than 450 million people with diabetes worldwide and almost half of them are undiagnosed. According to the International Diabetes Federation, it is expected that the prevalence of diabetes will rise to 693 million people by 2045 [1].

It is commonly known that chronic hyperglycemia can affect the structure and impair the function of many tissues in the body, especially the vascular system. Diabetic complications and comorbid conditions primarily determine the quality of life, and they are mainly responsible for the increased mortality of patients [2].

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So far, the main marker which is used to evaluate the risk of long-term diabetes complications is glycated hemoglobin (HbA_{1c}). HbA_{1c} level is an important indicator of glycemic control. Regular HbA_{1c} level measurements are also helpful in the evaluation of diabetes treatment efficiency [3].

Nowadays, the overall goal of diabetes control in diabetic patients is HbA_{1c} level $\leq$ 7.0% [4]. Better long-term diabetes control resulting in a reduction in HbA_{1c} is associated with decreased risk of chronic complications and mortality [5]. However, it should be emphasized that HbA_{1c} level does not reflect glycemic variability, which emerged recently as another possible risk factor for vascular dysfunction in diabetes [6]. The limited control of glycemia drives also the higher risk of hypoglycemia. Fear of hypoglycemia results in maintaining elevated blood glucose levels and it is the important cause of insufficient metabolic control of the disease and reduces the possibility of treatment intensification [7]. Currently, hypoglycemia is considered to be the greatest obstacle to achieve metabolic control of diabetes. Moreover, severe hypoglycemia has been also considered to be one of the predictors of macrovascular events and also increased mortality in patients with diabetes.

The evaluation of glucose variability and the risk of hypoglycemia has a great impact on the management of diabetes. It allows assessment of the effectiveness of therapy and provides guidance in selecting the appropriate insulin dosage schedule.

Therefore, blood glucose monitoring and appropriate management of glycemia is important not only to prevent chronic complications by reducing hyperglycemia but also to avoid hypoglycemic episodes and to decrease glycemic variability [8, 9].

Blood glucose monitoring as a part of the integral care of diabetic patients

Current monitoring and retrospective evaluation of blood glucose levels are essential parts of adequate diabetes treatment [4]. BG monitoring allows patients to assess their response to the treatment, decrease the risk of hypoglycemia, and to determine whether they are achieving glycemic control. Detailed information about blood glucose levels can be helpful in the adjustments in therapy and lifestyle activities and simultaneously to prevent diabetes-related complications. This is typically achieved using conventional personal blood glucose meters to measure finger-prick capillary blood samples [10]. The recommended frequency of self-monitoring of blood glucose (SMBG) is mainly dependent on the type of diabetes, treatment regimen and susceptibility to hypoglycemia. Intensification of the treatment is associated with the need for more frequent blood glucose monitoring [4].

Regardless of the treatment used, all patients should check blood glucose levels in case of feeling unwell, a sudden illness or suspected hypoglycemia. They should monitor blood glucose before planned physical activity and before activities associated with particular dangers of hypoglycemia (e.g. driving).

However, testing six to eight or more times daily SMBG may burden patients and may result in noncompliance. Therefore, it is also recommended to ensure that patients are properly instructed and are given regular evaluation and follow-up. Proper SMBG requires patient education regarding glucose meter use, interpretation of readings, and further management steps [4].

Self-monitoring of blood glucose was the standard of care for patients with diabetes for a few decades. So far, it is a widely used method of current glucose monitoring in Poland. However, SMBG has notable limitations. It is insufficient to diagnose all acute episodes of hyper- and hypoglycemia and thereby to get a full, daily profile of glycemia, which can allow for rapid patient reaction or adjustment of diabetes treatment. A glucose meter measures glucose levels at a single moment in time, and therefore, it does not indicate the direction or rate of change of glucose levels. Using glucose meter data alone may result in inappropriate therapy decisions (such as administering correction insulin when blood glucose levels are falling). Accordingly, SMBG often fails to detect nocturnal and asymptomatic hypoglycemia [10, 11]. Moreover, obtaining glucose data via glucose meter is dependent upon the patient's decision to self-monitor. It requires a finger prick to obtain a blood sample, and it results in pain for the patient, which also affects patient compliance with glucose measurements.

Continuous glucose monitoring

The introduction of continuous glucose monitoring (CGM) systems in 1999 have slowly changed standards of medical care in diabetes. In recent years, blood glucose monitoring has been revolutionized by the development of different systems for continuous glucose monitoring (CGM).

Currently, there are two types of new technological devices available to measure glucose levels in the interstitial fluid through sensors inserted subcutaneously: real-time CGM and Flash Glucose Monitoring (FGM, intermittent scanning continuous glucose monitoring — is-CGM). Both systems provide information about current and previous glucose levels, as well as glucose trends and anticipated future glycemic status, but each

technology has its unique features. FGM provides glucose information on demand. CGM measures glucose automatically and, as often as every five minutes, which generates 288 measurements per day. RtCGM provides for patients not only continuous current information on glucose levels in interstitial fluid over the whole day, but also alerts for hypo- or hyperglycemic events and rapid glucose trends [11].

Comparison of CGM systems

In Poland, there are a few real-time CGM systems available, e.g., Guardian™ Connect (Medtronic), Dexcom G4, Dexcom G5 and the Eversense CGM. Patients who choose to use CGM have several options available to them, standalone devices or insulin pumps integrated with CGM (MiniMed® Real Time™ 722, MiniMed® Veo™, MiniMed® 640G™ Medtronic) [12].

The Medtronic and Dexcom systems utilize transcutaneous sensors (transcutaneous real-time CGM — TC rtCGM). They consist of three components: a disposable sensor that is inserted into the subcutaneous tissue to measure glucose levels, a transmitter that attaches to the sensor, and a receiver (stand-alone device, insulin pump, smartphone, smartwatch) that displays and stores glucose information [12].

The Eversense CGM is the only, long-term implantable real-time CGM (LTI rtCGM). The system consists of an implantable, fluorescence-based, cylindrical glucose sensor, a removable external smart transmitter and a mobile medical application that displays glucose information and operates on a mobile device that allows users to review current and historical glucose data in real-time. The Eversense system is indicated for up to 180-days wear time in adults only [12].

FreeStyle Libre, the first flash glucose monitoring (FGM) system, was approved in Autumn 2014. FGM also measures glucose concentration in the interstitial fluid. However, it differs from other CGM technology in several ways. FGM is factory calibrated and does not require capillary blood glucose calibration. Sensor glucose levels are not continually displayed on a monitoring device but instead are displayed when the sensor is "flashed" with a reader device on demand. The FGM reader also displays a plot profile of the last 8 hours, derived from interpolating glucose concentrations recorded every 15 minutes. Therefore, when the patient with diabetes performs ≥ 3 sensor scans per day at ≤ 8-hour intervals, the FGM records 24-hour glucose profiles. The sensor can be worn continuously for up to 14 days, but it does not provide low or high glucose alarms in first-generation system. FGM system provides protection against hypoglycemia during the day, but it cannot detect nocturnal hypoglycemia when the user is sleeping or warn the physically active individual about pending hypoglycemia [13–15].

Also, all CGM systems can simultaneously transmit data to the cloud to share information in real-time. To date, insulin pumps integrated with CGM do not have such a function.

Jafri et al. compared available CGM by testing the performance of the Dexcom G5, Abbot Freestyle Libre Pro, and Senseonics Eversense during a 6-week, free life, outpatient bionic pancreas study involving 23 subjects with type 1 diabetes who wore all 3 devices concomitantly. The primary outcome was the mean absolute relative difference (MARD) vs. plasma glucose level measured with a glucometer. All 3 CGM systems produced higher average MARDs than during in-clinic studies. In the 3-way comparison Eversense achieved the lowest nominal MARD (14.8%) followed by Dexcom G5 (16.3%) and Libre Pro (18.0%). The pointing accuracy of the Eversense was significantly better than two other CGM systems [16].

Clinical use of continuous glucose monitoring

CGM and FGM systems are becoming increasingly prevalent in clinical practice because using it can reduce patients' discomfort and provide vastly more detailed glucose variability data. These systems can supply insight into the duration, frequency of hypo- and hyperglycemia and fluctuations in blood glucose levels. Therefore, they can be helpful to improve overall glycemic control by identifying episodes and preventing periods of hypoglycemia and hyperglycemia [15, 17].

Continuous glucose monitoring allows more effective adjustment of insulin doses to blood glucose trends, resulting in better metabolic control: more time spent in the glucose target range, a reduced number of hypoglycemia episodes (or lower time spend in hypoglycemic range), lower glucose variability and improved quality of life of patients and their caregivers [16].

According to the Diabetes Poland guidelines, the use of continuous glucose monitoring systems, including rtCGM and FGM is particularly indicated in patients with labile diabetes type 1, in patients with frequent hypoglycemic episodes. RtCGM is particularly indicated in patients with hypoglycemia unawareness, frequent nocturnal hypoglycemia and children < 10 years of age. In these patient groups, it is also recommended to use insulin pumps integrated with CGM, with a function of automatic temporary insulin suspension of the insulin infusion at low blood glucose values or at risk of hypoglycemia [4].

Initially, as recommended, patients using CGM prior to making therapeutic decisions should confirm the reading with a conventional meter. Currently, pa-

Table 1. Decision algorithm for glucose monitoring according to Clinical Practice Recommendations on the Routine Use of Eversense [15]

	SMBG	isCGM/FGM	TC rtCGM	LTI rtCGM
Intensified insulin therapy	+	+	+	+
High glycemic variability				
Committed to improved self-management		+	+	+
Treatment goals not achieved despite intensive treatment and training				
Frequent hypoglycemia			+	+
Unawareness of hypoglycemia				
Fear of pain or needle phobia				
Physically handicapped (visual, hearing, dexterity impairment)				
History of skin problems				+
Need for vibration alerts				
Need for transient removal of external devices				

isCGM — intermittent scanning continuous glucose monitoring; LTI rtCGM — long-term implantable real-time CGM; SMBG — self-monitoring of blood glucose; TC rtCGM — transcutaneous real-time CGM

tients can make therapeutic decisions based on the Dexcom G5, the Dexcom G6, FGM. In June 2019, the FDA approved a nonadjunctive indication for the 90 days system Eversense CGM. The FDA reviewed device performance data and proposed changes to the Eversense App (that support non-adjunctive use) and determined that Eversense is safe for making treatment decisions (such as dosing insulin and consuming carbohydrates) based on CGM glucose readings and trend arrows.

Szypowska et al. conducted a meta-analysis of seven RCTs to explore the potential beneficial effects of the use of real-time CGM in patients with type 1 diabetes mellitus. This analysis showed that the use of real-time CGM for over 60–70% of time provides a superior benefit over self-monitoring of blood glucose concerning HbA_{1c} reduction. The improvement in HbA_{1c} in patients using real-time CGM was achieved without an increase in severe hypoglycemia. The reduction in HbA_{1c} was noted not only in patients with poorly controlled type 1 diabetes but also in well-controlled subjects. The superiority of real-time CGM over SMBG in lowering HbA_{1c} was also confirmed in pump users [18].

In general, the choice of an optimal glucose monitoring device should be dependent on the patient's clinical indications or lifestyle restrictions [13, 19]. Patients treated with intensive insulin therapy have a wide choice of glucose monitoring devices according to their personal preferences. The proposition of the decision algorithm for the use of specific devices for glucose monitoring was presented in Table 1 [20].

Education process of patients and physicians about optimal using of CGM

CGM systems provide real-time data on interstitial glucose level, direction and rate of change in blood glucose levels. However, users of CGM may not be able to make optimal usage of this additional information without proper education. The appropriate interpretation of measured parameters to make correct therapeutic decisions is very important. Therefore, using CGM has to be supported with education of patients. Use of CGM/FGM requires structured education regarding appropriate expectations towards this system and proper interpretation of current readings including glucose trends [4].

The training for rtCGM should also include the principles of system functioning, its calibration and placing a sensor. Multiple alarms can be set to alert users if blood glucose increases or drops beyond defined target ranges. The most successful education programs emphasize training on self-management, including specific rules to adjust the insulin dose based on glucose data. Patients who understand how to interpret trend arrows have the best outcomes. At the time, when the trend arrow indicates a falling glucose level, then carbohydrates should be consumed, or dose of the prandial insulin should be decreased, and when the trend arrow indicate a rising glucose level, then the dose of insulin should be increased or physical activity should be started. Optimal using of CGM allows the patients to make lifestyle adjustments and therapeutic changes to improve their glycemic control [11].

The optimal training includes three parts: the principles of sensor technology, the operational aspects of the device, and the interpretation of the provided data. This training should commence 1 week before starting using the system.

Also, some physicians lack the appropriate level of knowledge to use CGM as part of their practices. There is need for considerable education regarding inter-

pretation of CGM results for the physicians and other healthcare workers. Educational programs focused on these knowledge and coverage gaps will enable professionals to provide improved care of diabetic patients [11]. According to the Recommendations from the International Consensus on Time in Range, both medical staff and patients should know clinical targets for CGM data interpretation [21].

Senseonics Eversense CGM System

Description of the system and insertion procedure

Eversense is a novel implantable subcutaneous CGM system produced by Senseonics Inc. It was designed to address several of the currently available CGM systems limitations. Eversense CGM sensor is the first one approved for long-term use and it can monitor blood glucose levels every five minutes for up to 90–180 days, and thus it allows to reduce the inconvenience and discomfort of frequent sensor insertion procedures. Eversense CGM system differs from other systems currently on the market in that the sensor is implanted subcutaneously by a doctor; the removable smart transmitter is placed on the skin over the sensor and can be taken on and off as desired by the patient [22].

CGM systems based on electrochemical- and enzymatic- methods are often a subject of interference with substances such as ascorbic acid, paracetamol, dopamine or maltose. Eversense is a fluorescence-based, therefore, these substances do not affect the sensor readings. It has also a silicone ring that contains an anti-inflammatory steroid drug (dexamethasone acetate). The system provides continuous glucose measurements over a 40–400 mg/dL range. The sensor requires twice-daily calibration. The Eversense smart transmitter is worn over the sensor and wirelessly powers it to initiate the glucose measurement and the transfer of data to the Mobile Medical Application (MMA). The transmitter can be removed at any time without the need for sensor replacement, allowing greater convenience and lifestyle flexibility. In addition, hypoglycemic and hyperglycemic alerts and notifications are provided on a mobile device. Additionally, the patient experiences on-body vibratory alerts from the transmitter even when the mobile device is not nearby. Thus, Eversense CGM system gives the desired flexibility and freedom to test blood glucose anywhere at any time and share and analyze their personalized data securely via a smart app. It is also water-resistant submerged in 1 meter for up to 30 minutes and it functions perfectly while the users take a shower or swim. The Eversense Mobile App runs on a compatible mobile device to receive and display the sensor glucose data from the Eversense Smart Transmitter. It eliminates the need to carry a separate receiver device. However, it is also important to check phone compatibility for CGM mobile applications before insertion. It is also able to set up a temporary glucose profile with custom high and low target and alert levels [22].

The Eversense CGM System Kit comes in three packages: the sensor pack, the insertion tools pack, and the smart transmitter pack. The sensor is shipped sterile inside a protective holder for safe handling purposes. The Eversense insertion tools pack contains the incision template, blunt dissector, insertion tool and adhesive patches. The incision template is used to guide and mark the incision area on the skin surface by aligning the marking template to the marked outer edges of the smart transmitter when placed in a comfortable position. The suggested insertion location is approximately at the midway point between the acromion process and the lateral epicondyle. The insertion should avoid areas with loose skin (such as back of arm), scar tissue, tattoo or nevus. During the procedure, the patient should be positioned in a reclined position, preferably on their side, with their elbow flexed up to 90 degrees and their palm resting on their chest or abdomen. The insertion area should be cleaned and disinfected before the application. Local anesthetic should be injected approximately 5 mm along the planned incision and approximately 30 mm perpendicular to the planned incision which is the planned canal of the blunt dissector tool [22].

The attached blunt dissector (Fig. 1A) is used to create the subcutaneous pocket for insertion of the sensor. This tool has two depth guards to help prevent the pocket from being made too deep in the skin. The depth guards have guide marks to assist in determining the length of the subcutaneous pocket for placing the sensor. The insertion should be made approximately 5 mm at the insertion location in such way that it will be able to create an appropriately sized subcutaneous pocket approximately 3–5 mm below the skin surface. The blunt dissector should be moved toward the shoulder while maintaining the metallic and plastic parts of the tool in close contact with the skin to ensure the smallest possible angle of the pocket with respect to the skin. The pocket should not be created more than 3–5 mm below the skin. If the sensor is placed too deep, it may be difficult to communicate with the smart transmitter or to be removed, therefore recent changes made to the blunt dissector should prevent placement mistakes [22].

To insert the sensor inside the subcutaneous pocket, the insertion tool has to be used. It has two guide marks on the cannula to assist in proper placement.

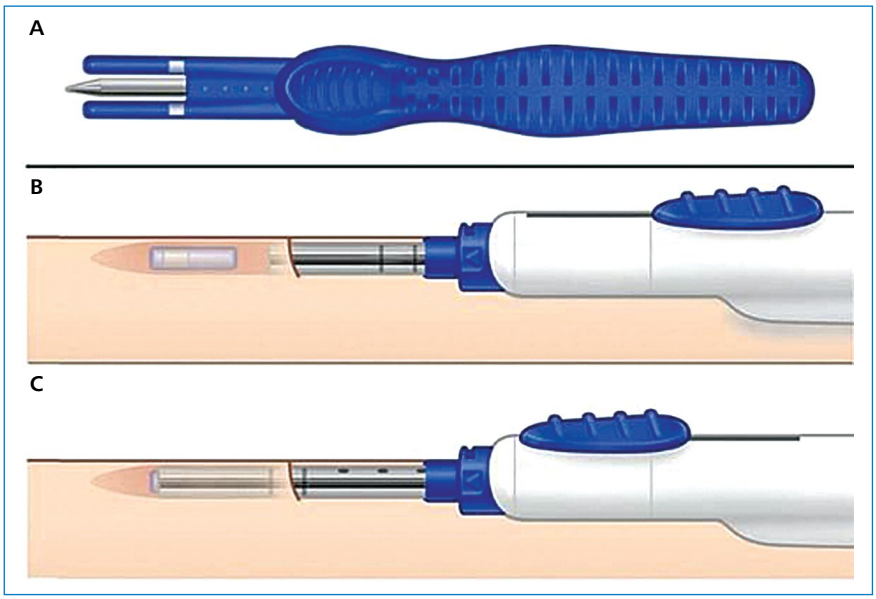

Figure 1. Insertion tools provided with the Eversense system

The tip of the insertion tool should be placed into the incision opening. Firstly, the insertion tool has to be unlocked by a pushing down on the back of the thumb slide (Fig. 1B). The sensor is deployed into the pocket by a retracting of the thumb slide (Fig. 1C). Before removing the insertion tool from the incision, the insertion area should be lightly palpated to confirm that the sensor is in place. The incision should be closed and dressed in the appropriate manner using adhesive skin closure or suture and dressing, making sure the two sides of the incision are closed together. Adhesive patches, included with the Eversense insertion tools pack, have an adhesive side that attaches to the back of the smart transmitter and a silicone adhesive side that attaches to the skin. It is important to inform the patient that adhesive patches should be replaced daily [22].

Even though, at first sight, the required minimally invasive procedure exceeds the area of experience for many diabetologists, the technique is easy to learn when performed with proper training, oversight, and attention to detail. Therefore, any physician interested in undertaking the procedure should be accompanied and consequently certified by the company's clinical training manager during the first several insertions and removals to ensure the required training to comply with the highest quality standards [20]. Recently in Poland, there are 32 doctors who have sufficient experience (3 training insertions of a sensor) to carry out of implementation procedure. Furthermore, 27 doctors carried out 3 or more insertions and removal of the Eversense system and thus obtained a full certificate. Above procedure can be carried out in one of 30 clinics or facilities, especially in large cities.

Clinical experience

Long-term accuracy and clinical utility of Eversense system have been validated in prospective clinical trials (Table 2). In the multinational, multicenter European PRECISE trial, the safety and accuracy of the Eversense CGM system was investigated. It also assessed sensor lifetime, system wearout time, participant reported outcome measures, and parameters of glycemic control. The Eversense system was studied in 71 participants aged 18 years and older with type 1 and type 2 diabetes during 180 days. CGM accuracy was assessed during eight in-clinic visits with the mean absolute relative difference (MARD) for venous reference glucose values > 4.2 mmol/L. The MARD value was 11.1% and 81% of hypoglycemic events were detected by the CGM system within 30 min. HbA_{1c} improved in the study group from 7.54% (59 mmol/mol) at baseline to 7.19% (55 mmol/mol) at the end of study. No device-related serious adverse events occurred during the study. The results from this study indicated that the use of a long-term Eversense CGM sensor is both effective and safe and provides specific usability benefits [23].

Barnard et al. determined acceptability and impact of an Eversense CGM sensor (as part of the PRECISE trial). Fifty-one participants took part in the study. Quantitative psychosocial assessments were administered at 90 days to participants to explore patient-reported outcomes associated with an implanted sensor.

Table 2. Parameters characterizing the accuracy and safety of the Eversense CGM (based on results of clinical trials)

Study	HbA_{1c} reduction Overall HbA_{1c} level	Accuracy (MARD) Overall (%) 40–400 mg/dl	Adherence Hours per day	Time in target Overall (%) 70–180 mg/dl	Hypoglycemic detection Overall (%) 70 mg/dl
PRECISE 1	0.4%	11.6%	23.5	59.9%	81%
PRECISE 2	0.5%	8.5%	23.4	57.6%	93%
PRECISION	0.3%	9.6%	23.4	59.0%	95%

Key issues included impact of device on perceptions of diabetes self-management and diabetes control, usability, safety, social relationships, and fear of hypoglycemia. The system was rated highly on ease of use, convenience, and comfort. CGM Impact Scale results showed that 86% of participants reported feeling better about their diabetes control. Furthermore, 73% felt safer while sleeping and 78% more confident about avoiding serious hypoglycemia. It was concluded that the Eversense CGM sensor was acceptable to participants and use of the system was associated with minimized burden of diabetes [24].

In 2018, there were also results of PRECISE II trial published. It was a nonrandomized, blinded, prospective, single-arm, multicenter study that evaluated the accuracy and safety of the Eversense CGM system among adult participants with T1D and T2D. Ninety participants were enrolled and each received the CGM system. The primary endpoint was the MARD between paired Eversense and reference measurements through 90 days post insertion. The overall MARD value against reference glucose values was 8.8%, which was significantly lower than the prespecified 20% performance goal for accuracy. The system had a favorable safety profile for its intended use. Clinicians with limited to no surgical experience were able to insert and remove the sensor without difficulty after appropriate training. The results of PRECISE II trial demonstrated that the use of Eversense sensor for a long-term is accurate and safe [25].

Aronson et al. conducted a prospective, single-centre, single-arm, 180-day study to evaluate the effectiveness and safety of the implantable CGM system in adolescent and adult subjects with T1D. Accuracy measures included mean absolute relative difference (MARD), 15/15% agreement between CGM glucose and blood glucose measured by SMBG and surveillance error grid analysis. Overall MARD was 9.4%. CGM system agreement at 15/15% through 60, 120 and 180 days was 82.9%, 83.6% and 83.4%, respectively. Surveillance error grid analysis showed 98.4% of paired values in clinically acceptable error zones. No insertion/removal or device-related serious adverse events were reported. Results of above study confirmed that the Eversense XL CGM system is safe and accurate through 180 days in a primarily adolescent population of subjects with T1D [26].

Clinical experience in Poland

We have also collected information on experience with Eversense CGM in various clinical centers in Poland.

First implantation of the Everense sensor was made on 22 October 2017 in Raszeja Hospital in Poznan (Department of Internal Medicine and Diabetology Poznań University of Medical Sciences). The implantation/reimplantation procedure is performed in an outpatient clinic. An earlier qualifying visit is indicated during which the patient's expectations and possible contraindications to the procedure are discussed. The procedure along with the preparation of the patient takes about 20–30 minutes. The implanting time usually does not exceed 15 minutes. The removal of the sensor is less predictable in time. It can last very short, but it can also be extended up to 30 minutes, especially when it is necessary to locate the sensor using an ultrasound scanner. Performing over 80 procedures of implantation and reimplantation, no complications were found. In no case did the wound require sewing. There was no infection, hematoma, non-healing wound, sensory disturbances, skin changes, allergic reaction. During one removal operation, ultrasound was used to find the sensor. Some patients were examined by physician 2 days after surgery to evaluate and change the dressing. In one case it was required to replace the steri-strips. Most of the patients stayed in contact with their doctors via telephone. Steri-strip were removed by patients themselves on the 6–7th day after the procedure. Regardless of whether the patients used insulin pen or a personal insulin pump, everyone was satisfied with Eversense. The majority, despite emphasizing the discomfort of the implantation procedure, had it carried out several times. Patients who resigned from the next implantation usually did so for financial reasons.

In the Central Clinical Hospital of the Medical University of Lodz the Eversense sensor implementation procedure was introduced in January 2018. So far, over 80 procedures of implantation, removal and

reimplantation of sensors have been performed. This CGM system was offered mainly to patients who, due to their profession and sports discipline, could not or did not want to use traditional transcutaneous sensors. Further groups were patients with skin reactions associated with the use of traditional sensors or patients who perceive frequent sensor replacement as a burden or benefited from on-body vibration alerts. 17 patients are now using the third sensor. The main reason for not continuing this CGM system is the price of the device. In the opinion of doctors from this centre, the sensor implementation was very simple and not a time consuming procedure. Removal of the sensor was usually an easy and short procedure. Problems while removing the sensor occurred only in two patients. Both patients required more lignocaine for anaesthesia and both needed ultrasonography to locate the sensor.

The next clinical experiences came from Clinical Diabetology Center in Krakow. The center has been implanting the sensors since August 2018 in patients with diabetes coming to the Clinic or directed by doctors from the Malopolska province. So far, 25 successful implantations and 13 sensor exchanges have been carried out. There were no complications both for implantation and replacement. The subcutaneous session implantation procedure with the provided tools proved to be simple and uncomplicated. Also, there were no difficulties in attaching the sensor, it is easy to feel the sensor by palpation. Re-establishment of the sensor is carried out on the other arm. Patients were eager to use the CGM system. Eversense CGM system was mostly indicated for patients with a fear of hypoglycemia, glycemic instability and aversion to frequent glycemic measurements in self-control, poor tolerance of traditional transcutaneous sensors or for patients practicing competitive sports.

In all centers, no permanent complications were found during the implementation or removal of the sensors. In two patients a transient slight lipodystrophy was observed after sensor removal. In one case, the wound was sewn together at the request of a very sport active patient.

In the opinion of doctors from all centres, the use of Eversense contributed to the improvement of the effects of therapy, expressed as the time spent in the target blood glucose values. They emphasize the comfort and usefulness for the patients practicing sports.

Patients experience in Poland

Roche Diabetes Care with the cooperation of Biostat, conducted a study to investigate the patients' opinion about Eversense CGM system [27]. The study was aimed at gathering information about the Eversense system and the impact of CGM on the life of a patient with diabetes. Advantages and disadvantages of the system and its individual components have also been evaluated. There were 86 enrolled patients who used the Eversense system for three months and then answered questions in the questionnaire. The study included almost as many men as women (48.8% *vs*. 51.2%). Among the respondents, the largest group were patients aged 30–39 (27.9%). About 22.1% of the respondents were between 18 and 29 years old. The smallest group of patients were people aged 50–59 (5.8%). About half of the patients (51.2%) had prior experience with CGM system.

Patients were also asked to rate the system components. In one of the questions, patients had to evaluate the sensor's activity time. Every third respondent (33.7%) rated the sensor activity very well, and 39.5% assessed it well. According to 16.3% of respondents, the sensor activity time was medium, and respectively 7% and 2.3% of the respondents considered this element of the system to be rather weak and weak. However, it should be emphasized that patients used a previous version of sensor which was implanted for 3 months. Over three fourth of the respondents rated the alarms positively: 37.2% very well and 41.9% good. About 11% of patients considered it as medium, 4.6% as rather weak, and 3.5% as weak.

One of the reasons for traditional CGMs limited use include a perceived burden of frequent insertions, fear of pain or discomfort during an implantation procedure. Therefore, patients were asked to evaluate a method for inserting sensor. Over half of the respondents considered the insertion of the sensor as very good (54.6%), moreover, according to 30.2% people, this method was good. About 8.1% of patients assessed the insertion of the sensor on medium rate, 5.8% as rather poorly and only 1.2% poorly.

Education is the core to fully understanding and integrating a CGM into daily practice and patients' lifestyles. Training on CGM is important to ensure that patients know how to use the device and set the appropriate expectations. The educational training in the use of system operation received particularly positive marks. As many as 83.7% of people evaluated the training very well, and 15.1% of people — well. Only one person (1.2%) considered the training to be poor.

Patients were also asked to answer the question: "What benefits do you see in using the Eversense system for therapy?". More than 34% of respondents indicated the possibility of continuous control and access to blood glucose level measurements. About 17.9% of patients indicated benefits related to warning alarms. Other significant benefits of the system in the respond-

ents' opinion were: displaying glucose trends (10.1%), limited number of SMBG measurements (7.7%), user-friendly application or the possibility of glycemic control during physical activities or sleep (5.4%).

However, most importantly, 87.2% of respondents confirmed that the use of the Eversense system had a positive impact on their daily life with diabetes. Respondents indicated that the use of the Eversense CGM system allows for easy glycemic monitoring, better maintaining of target glucose level and improvement of the quality of life (each of these answers were indicated by 15.4% of patients). The reduction in the number of finger pricks was indicated by 12.5%. About 5% of patients appreciated the usefulness of Eversense system in the prevention of hypo- and hyperglycemia and assistance in the selection of meals. Almost 4% of people indicated safety, psychological comfort, more precise adjustment of insulin doses and the ability to monitor blood glucose even at night.

Despite good ratings of the Eversense CGM system, only 39.5% of patients declared that they intend to continue using the Eversense system. Patients were asked why they do not intend to use Eversense system. The vast majority of patients (65.1%) indicated a too high price and lack of reimbursement.

Global reimbursement of CGM

Clinical studies demonstrated that the use of CGM could reduce hyperglycemia and hypoglycemia episodes by providing the patients information about blood glucose levels as well as the rate and direction of glycemia changes. Randomized controlled trials have shown that these electronic devices can be also helpful in lowering the HbA_{1c} level without increasing the risk of hypoglycemia in patients with T1DM. Despite the fact that the evidence base for CGM clinical efficacy has grown, coverage by global reimbursement authorities is still limited. It is associated with a lack of independent, robust, randomized clinical trials demonstrating both improved outcomes for hyperglycemia and hypoglycemia in specific patient populations. Moreover, there is only limited data on cost-effectiveness of CGM systems published. Reimbursement by payers is critical for the uptake and use of new diabetes technologies which can decrease the risk of acute and chronic complications of diabetes. Assuming that the daily costs for CGM usage are of approximately $5–10 per day, this account for $3,000 per year per patient. In some European countries the cost is around 4,000€ per year [28, 29].

However, recently CGM is reimbursed in the United States. In the US, national health insurance program (Medicare) covers CGM and related supplies instead of blood glucose meters for making diabetes treatment decisions. Coverage criteria include intensively insulin treated patients who perform four or more SMBG/day, are taking multiple insulin injections or are using an insulin pump, and will require frequent adjustment of insulin dose based on the reading from CGM [29].

CGM sensors are also reimbursed in many European countries. In England, National Institute for Health and Care Excellence (NICE) published clinical guidelines covering the management of type 1 diabetes in adults and children. It was recommended that CGM should be offered to people with challenging or severe hypoglycemia, to those with impaired awareness or fear of hypoglycemia, and to those for whom self-monitoring of blood glucose has failed to achieve optimum results. Recently, in Germany, real time CGM systems are reimbursed for T1DM and T2DM treated with insulin. In a few European countries (e.g., Slovenia, Spain), reimbursement of CGM is limited to pediatric population [29].

CGM reimbursement in Poland

Reimbursement of CGM in Poland is still limited. The National Health Fund covers the rtCGM for children and adults under 26 years old with T1DM, treated with insulin pump. Coverage criteria only include patients with hypoglycemia unawareness (lack of prodromal symptoms of hypoglycemia after alcohol consumption was excluded) [30].

Unfortunately, current regulations prevent patients from accessing the Eversense CGM system in this patient group. This provision stipulates that the limit for sensors is PLN 600 per month, up to 4 pieces per month and a patient surcharge of 30% of the limit value. In the current wording, both the description of the medical device, the limit of financing from public funds and the period of use have been formulated without the fact that other CGM systems with a period of use longer than a week are already available on the Polish market. This provision prevents the reimbursement concerning Eversense CGM from being carried out and settled by the National Health Fund.

FGM is reimbursed only for children from 4 to 18 years old with type 1 diabetes mellitus with very well monitored blood glucose, i.e. at least 8 blood glucose measurements per day [31].

It should be emphasized that huge proportions of the adult T1DM population are identified with persistent poor glycemic control (glucose level fluctuations, hypoglycemia episodes). Limited reimbursement influences the limited access of patients with diabetes to these devices and could worsen their prognosis.

Conclusions

Continuous glucose monitoring systems have revolutionized the treatment and monitoring of diabetes. These devices are especially recommended for diabetic patients treated with insulin. According to Diabetes Poland guidelines, use of CGM is indicated in patients with recurrent episodes of severe hypoglycemia or large circadian variation of blood glucose levels. CGM systems provide patients detailed information about the level of glucose and thus can reduce the risk of hypoglycemia and improve patient quality of life.

Eversense CGM is the only one system which is approved for long-term use (up to 180 days). The subcutaneous session implantation procedure proved to be simple and uncomplicated. This CGM system can be recommended in particular to patients who, due to their profession and sports discipline, cannot or do not want to use traditional transcutaneous sensors. Further groups are the patients with skin reactions associated with the use of traditional sensors or people who perceive frequent sensor replacement as a burden or benefited from on-body vibration alerts.

Authors' contributions

The authors are members of the expert panel of Roche Diabetes Care. The expert group was established by Roche Diabetes Care to provide opinion about the Eversense CGM system as a part of the meetings of advisory committee, which took place in Warsaw on October 27, 2017; December 12, 2017 and on March 09, 2018. This article describes the clinical practice of the authors, which was independent of any influence by the manufacturer and Roche Diabetes Care. The writing of the article was supported by Roche Diabetes Care, and certain information was provided by the manufacturer, Senseonics, Inc., while the authors were primarily responsible for the substance of the article and maintained full control over the final content. All authors and Roche reviewed and approved the article.

Conflict of Interest

A.Sz., B.W., A.G., M.M.: Advisory Board for: Abbott, Medtronic, Roche; founded Research for: Roche; sponsored lectures/seminars for: Medtronic, Roche, Abbott, D.Z.Z.: Advisory Board for: Abbott, Medtronic, Roche, sponsored lectures/seminars for: Medtronic, Roche, Abbott. K.C.: Advisory Board for: Medtronic, Roche; founded Research for: Medtronic, Roche; sponsored lectures/seminars for: Medtronic, Roche. MW declared no conflict of interest.

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