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ASSESSMENT OF HAEMOGLOBIN A1C EVOLUTION USING TWO STATISTICAL APPROACHES (SURVIVAL ANALYSIS AND LINEAR REGRESSION) IN PERSONS WITH DIABETES MELLITUS

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Background: Intensive selfmonitoring is an important and cost-demanding part of diabetes treatment. Continuous glucose monitoring (CGM) using transcutaneous sensors offers "real time" information on glycemia. In the present study, we assessed the therapeutic efficacy of CGM on metabolic control using two different statistical methods: linear regression and "survival analysis".

Objectives: (1) to assess the therapeutic efficacy of CGM on metabolic control using two different statistical methods: linear regression and survival analysis; (2) to demonstrate the particular advantages of each statistical method.

Methods: A total of 42 persons with diabetes mellitus treated by means of an insulin pump participated in this study. According to the means of selfmonitoring persons with diabetes were divided into two groups: 1. intervention group of 17 persons using CGM, 2. control group of 25 persons using a glucometer. Each person was followed for a period of three months. At the beginning of the study and at the end of each month HbA1c was determined.

Results: Both the regression analysis and survival analysis brought evidence of significant changes of the HbA1c in either of the groups. The method of linear regression enables to analyse the evolution of HbA1c in each individual person followed by comparison of the groups. The survival analysis demonstrated that the probability of HbA1c decrease to the predefined level as well as its further maintaining at this level was higher in the CGM group. The mean time interval necessary to HbA1c decrease was shorter in the CGM group.

Conclusions: The efficacy of CGM was demonstrated. In addition to linear regression, survival analysis appears to be an useful complementary method in the statistical evaluation of the treatment efficacy.

INTRODUCTION

Intensive selfmonitoring is an important and costly part of diabetes treatment[5, 17, 19, 29], particularly in persons using insulin pumps[27]. In recent years, continuous glucose monitoring (CGM) with transcutaneous sensors, transmitters and monitors has become a sophisticated approach offering "real time" information on glycemia. Several studies have shown the effectiveness of CGM[2, 6]. However, the benefits, hazards, accuracy, reliability and clinical applicability of CGM[6, 12, 13] need to be re-established using both case reports and appropriate statistical methods even though recent trials demonstrate that interstinal fluid glucose and blood glucose concentrations could be made identical by resorting to algorithmus based on concurrent blood glucose levels alone[20].

Since 2002 we have done statistical analyses for a number of clinical studies on diabetes [7-13, 21, 23-26]. The glycaemic profiles and haemoglobin A1c were evaluated as parameters of diabetes control indicating the success of treatment. Concentration of haemoglobin A1c highly correlates with the mean plasma glucose concentration. Various statistical methods but survival analysis were applied according to the analyzed data and objectives of the respective study.

Linear regression is a form of regression analysis in which the relationship between one or more independent variables and another variable (dependent variable), is modelled by a special function, namely, linear regression equation[1].

Survival analysis is a set of statistical methods which evaluate the time interval from the beginning of the observation until the occurrence of a certain event. Generally, this time interval is called the survival interval (although it does not need to identify the survival of a patient). The survival interval identifies the number of years, months, weeks or days from the beginning of the observation until the occurrence of a defined event.

Most studies are complete before the observed event occurs for all subjects. This situation is in survival analysis described as "censoring".

An example of such event would be the achievement of a certain level of diabetes compensation. Survival analysis was first described by Kaplan and Meier

in 1958[14, 15] and was used to evaluate the survival time of oncological patients. This statistical method has been mostly used in epidemiological studies[3, 4, 16, 22, 28].

In the present study, in order to describe the therapeutic effects of continuous glucose monitoring on metabolic control (i.e. mean plasma glucose concentrations over the last 2–3 months represented by HbA1c concentrations) two different statistical methods were applied: 1) the widely used linear regression analysis and 2) the survival analysis of data which has not been routinely used for this purpose[26].

The objective of this study was (1) to assess the therapeutic efficacy of CGM on metabolic control using two different statistical methods: linear regression and survival analysis, and (2) to demonstrate the particular advantages of each statistical method applied.

METHODS

The data in this statistical analysis were gathered at The Faculty of Medicine and Dentistry, Palacky University Olomouc and the University Hospital Olomouc since the year 2006 until the year 2008. Each subject was followed in the outpatient clinic for a period of three months. HbA1c was assesed at the beginning $HbA_{1c}1$and at the end of each month ($HbA_{1c}2$, $HbA_{1c}3$, $HbA_{1c}4$).

Study subjects

Two independent groups of persons with diabetes mellitus were followed (Table 1):

1. An intervention group of 17 persons with diabetes using transcutaneous sensors, 11 men and 6 women, aged 19–69 years, (mean 44.9 years, SE 4.0).
2. A control group of 25 persons with diabetes using a glucometer, 13 men and 12 women, aged 24-66 years (mean 44.9 years, SE 2.9).

Determination of Haemoglobin A1c

The HbA1c concentration in blood was determined using the sophisticated HPLC procedure in the Department of Clinical Biochemistry, University Hospital Olomouc (Table 2).

Principles of the HbA1c estimation. The analyzer PDQ Plus employs the principles of boronate affinity and high-performance liquid chromatography (HPLC). Glycated proteins (haemoglobins and plasma proteins) differ from non-glycated proteins by the attachment of sugar moiety to the former at various binding sites by means of a ketoamine bond. GHb and GPP thus contain 1,2-cis-diol groups not found in non-glycated proteins. These diol groups provide the basis for separation of glycated and non-glycated components by boronate afinity chromatography. In this analytical technique, a boronate is bonded to the surface of the column support. When a solution of proteins is passed through the column, the glycated component is retained by the complexing of its diol groups with the boronate. After the unretained non-glycated component elutes from the column, the glycated component is eluted from the column with a reagent that displaces it from the boronate. Both components are detected spectrofotometrically at 413 ± 2 nm.

Parameters of reliability. Limit of quantitation: 3.0 %, Linearity: up to 19.5 %

Repeatability (within-run imprecision): 1.4 %. Reproducibility (between-run imprecision): 1.4 %. Reference range for normal population: 2.8 to 4.0 %.

Table 1. Characteristics of the intervention (CGM) and of the control group.

Group	CGM	Control	Significance (*P*)
N	17	25	
Male/Female	11/6	13/12	0.414
Age (mean ± SE) [years]	44.9 ± 4.0	44.9 ± 2.6	0.996
Age range [years]	19–69	24–66	
Duration of diabetes (mean ± SE) [years]	17.8 ± 2.9	15.4 ± 2.0	0.482
Duration of diabetes range [years]	1–45	2–43	

Table 2. Evaluation of metabolic control in persons with diabetes according to HbA1c concentration in blood.

Metabolic control in diabetes	Calibration according to DCCT (valid before 1. 1. 2004)	Calibration according to IFCC (valid since 1. 1. 2004)
Excellent	< 6.5 %	< 4.5 %
Satisfactory	6.5 – 7.5 %	4.5 – 6.0 %
Unsatisfactory	> 7.5 %	> 6.0 %

Statistical Analysis

The Program SPSS v.15,0, SPSS, Inc., Chicago, IL, USA was used in the statistical analysis.

First, the method of regression analysis was used to evaluate the development of the HbA1c values in the course of three months. In each subject, regression coefficient was calculated in order to describe the evolution of HbA1c (see Table 3a and Table 3b). Two samples t-test was applied to compare the regression coefficients of subjects in the intervention and in the control group. Second, the method of survival analysis was applied to evaluate the same data. The survival analysis was aimed at the occurrence of the followed events in time in subjects in both of the groups.

A total of 8 various events was empirically defined, and analyzed:

1. first decrease of HbA1c below the 5 % value determined in the laboratory,
2. maintained decrease of HbA1c below the 5 % laboratory value in two consecutive controls,
3. first decrease of HbA1c by at least 3 % from the baseline (this event was defined considering the reliability, repeatibility and reproducibility of HbA1c determination),
4. maintained decrease of HbA1c by at least 3 % from the baseline in two consecutive controls,
5. first decrease of HbA1c by at least 5 % from the baseline (this event was defined empirically),
6. maintained decrease of HbA1c by at least 5 % from the baseline in two consecutive controls,
7. first decrease of HbA1c by at least 10 % from the baseline (this event was defined empirically),
8. maintained decrease of HbA1c by at least 10 % from the baseline in two consecutive controls.

The aim of the survival analysis was to determine the probability of HbA1c decrease below an empirically defined value over time. For the graphic representation of the probability of the HbA1c decrease below the defined value the Kaplan-Meier curve presenting one minus "survival function" was used. The final curve is of ascending character which is the optic equivalent for increasing probability in the course of time.

The statistical significance of differences between survival curves in the control and intervention group was evaluated by means of log-rank test. $P < 0.05$ was considered significant.

RESULTS

Linear regression analysis (Fig. 1)

In the intervention group, the mean regression coefficient was – 0.246 (the negative value demonstrates a decrease of HbA1c in the course of the observational period), SD 0.395, range from – 1.24 to 0.47. In the control group the mean regression coefficient was 0.138 (positive value demonstrates an increase of HbA1c in the course of the observational period), SD 0.248, range from -0.46 to 0.72. Two samples t-test revealed a significant difference between the mean regression coefficients (p = 0.001). See box graph in Fig. 1.

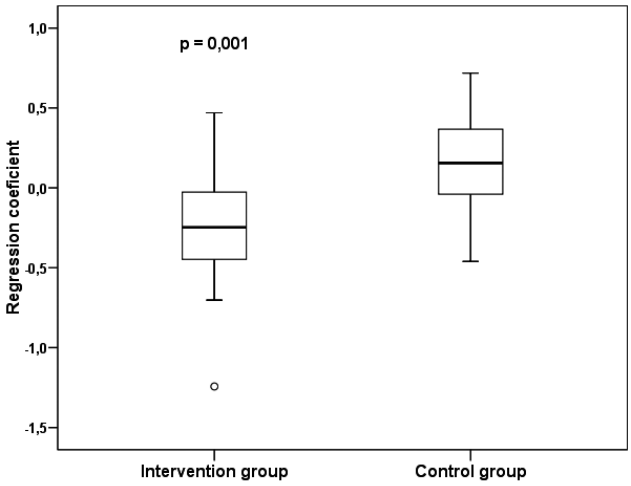

Fig. 1. Regression analysis: Distribution of regression coefficients in the intervention (n = 17) and control group (n = 25). P-significance of difference (two samples t-test between both groups).

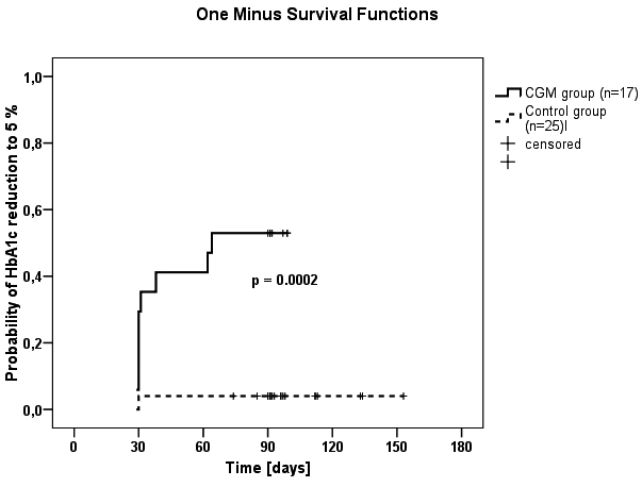

Fig. 2. Survival analysis: Kaplan-Meier curve showing the probability of the first decrease of HbA1c below the 5 % value determined in the laboratory in the intervention (CGM) and control group. *P* – significance of difference (log-rank test).

Survival analysis (Fig. 2, Fig. 3 and Table 4).

The probability of HbA1c decrease below the defined value (5 %) and the probability of maintaining the decrease in at least two consecutive controls is shown in Kaplan – Meier curves in Fig. 2–3. The estimates of mean time interval until the decrease below the defined value for both intervention and control group and the significance of the log-rank test are shown in Table 4. A significant difference between the intervention and the control group was shown in all defined events. The mean time interval until the HbA1c decrease was proved to be significantly shorter in the intervention group.

DISCUSSION

Linear regression analysis and survival analysis are two methods evaluating the development of change in an independent manner. While linear regression enables the assesment of change in a complete time interval, survival analysis expresses the probability of occurrence of an expected event. Currently, survival analysis is mainly used in epidemiological studies which follow mortality. Recently the role of HbA1c as a risk factor for heart failure in persons with diabetes was assessed using the survival function[22]. Our aim was to point out the possibility of survival analysis application in clinical studies which follow therapeutic efficiency.

In our study, in the intervention group the negative value of regression coefficient appeared in 13 of 17 persons with diabetes (76 %) showing a decrease of HbA1c. On the other hand, in the control group the negative value of regression coefficient appeared only in 8 of 25 persons (32 %). So, using two samples t-test a significant diference between the distribution of regression coefficients in both groups was demonstrated.

Survival analysis shows the decrease of HbA1c below the defined values in the intervention group using CGM in the course of three months. Fig. 2 and 3 shows that

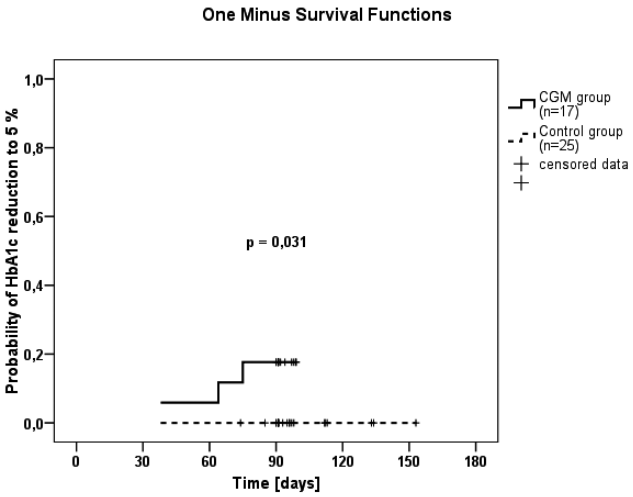

Fig. 3. Survival analysis: Kaplan-Meier curve showing the probability of maintained decrease of HbA1c below the 5 % laboratory value in two consecutive controls in the intervention (CGM) and control group. *P* – significance of difference (log-rank test).

Table 3a. Intervention group: Evoluation of HbA1c from the introduction of CGM (HbA1c 1) to the end 3rd month (HbA1c 4).

Patient No	Age [y]	Diabetes duration [y]	HbA1c 1 [%]	HbA1c 2 [%]	HbA1c 3 [%]	HbA1c 4 [%]	Regression coefficient
1	31	19	8.7	5.9	5.3	6.6	-0.70
2	27	10	8.6	6.4	7.5	7.2	-0.25
3	64	37	6.1	4.8	5.5	4.7	-0.31
4	62	1	5.8	3.9	3.6	3.7	-0.49
5	53	18	6.7	10.1	6.5	6.2	-0.45
6	48	26	6.5	5.4	5.2	5.6	-0.28
7	65	12	5.7	5	5.1	5.5	-0.05
8	32	23	6.5	6.2	5.1	5.6	-0.38
9	60	1	5.9	5.2	4.7	6	-0.03
10	35	26	5.2	4.2	5.4	5.4	0.18
11	51	19	6.4	4.8	6	5.6	-0.10
12	40	26	11.9	10	9.4	7.9	-1.24
13	69	10	9.3	8.4	9.9	9.8	0.09
14	24	12	5.2	4.8	5.6	7.2	0.47
15	27	11	5.6	4.7	4.2	5.3	-0.12
16	57	45	6.3	5.1	3.9	4.6	-0.62
17	19	7	8.3	6.8	8.5	8	0.08
mean ± SE	44.9 ± 16.5	17.8 ± 11.9	7.0 ± 1.8	6.0 ± 1.9	6.0 ± 1.8	6.2 ± 1.5	-0.25 ± 0.40

Table 3b. Control group: Evoluation of HbA1c without CGM from beginning of the study (HbA1c 1) to the end 3rd month (HbA1c 4).

Patient No	Age [y]	Diabetes duration [y]	HbA1c 1 [%]	HbA1c 2 [%]	HbA1c 3 [%]	HbA1c 4 [%]	Regression coefficient
1	27	6	5.8	4.2	5.4	5.2	-0.04
2	53	6	6.9	5.7	5.6	5.7	-0.34
3	42	32	7.5	6.5	7.6	8.6	0.37
4	53	17	6.2	5.6	6.9	6.3	0.16
5	58	6	11.4	10.2	12.3	12	0.39
6	48	27	6.3	5.8	6.5	6.7	0.19
7	48	3	6.8	6.9	9	7.9	0.53
8	58	5	7.1	5.5	6.9	7.1	0.16
9	46	12	9	11	9.8	9.6	0.07
10	27	10	6.6	8	6.8	7.2	0.06
11	66	13	10.1	12.7	12.6	12.6	0.72
12	24	22	8.8		7.5	7.5	-0.46
13	41	8	10.2	10.4	10.9	11.4	0.40
14	35	11	6.2		6.2	6.8	0.16
15	34	20	6.4	7.2	7.3	6.8	0.13
16	55	20	6.3	7.6	8	7.8	0.41
17	62	14	6.2	7.4	7.7	5.6	-0.17
18	56	17	6.5	8.3	8.6	8.1	0.50
19	43	17	7.1	7.6	7.4	7.1	0.01
20	31	22	6.6	5.8	6	6.4	-0.03
21	54	2	5.9	6	5.6	5.3	-0.21
22	24	10	5	5.5	5.5	6.3	0.26
23	33	10	8.5	7.5	8.7		-0.05
24	63	43	6.6	6.4	6.3	6.4	-0.10
25	42	32	7.5	6.5	7.6	8.6	0.37
mean ± SE	44.9 ± 12.9	15.4 ± 10.1	7.3 ± 1.6	7.3 ± 2.1	7.7 ± 2.0	7.6 ± 2.0	0.14 ± 0.28

the probability of this change is significantly higher in the intervention group than in the control group. The same applies on all other criteria of HbA1c decrease which we have defined pragmatically. It is evident that the probability of maintaining a longer lasting HbA1c decrease is below the defined value for at least until the next control (one month) is smaller than the probability of achieving at least one HbA1c value below the defined value (Fig. 2 vs. Fig. 3). Table 4 shows that the HbA1c decrease is achieved significantly sooner in the intervention group in comparison to the control group. The results of survival analysis show (similarly as the slopes-analysis in individual subjects using regression analysis) the positive effect of CGM on HbA1c values which is in accordance with conclusions of various studies using other statistical methods such as paired t-test etc.

CONCLUSIONS

We can conclude that CGM is an effective tool to improve diabetes control. In addition to linear regression survival analysis has proven to be an useful method complementing other statistical methods used for evaluation of the treatment efficacy for diabetes. It may be applied in situations with fluctuating therapeutic outcomes with alternative remissions and relapses.

Table 4. Mean time elapsed (± standard error) until the HbA1c reduction to the defined target level.

Defined target level of HbA_1c	CGM group [days]	Control group [days]	Significance P (log-rank test)
<= 5 % at first occasion	66.8 ± 7.7	148.1 ± 4.8	0.0002
<= 5 % at two sequential occasion	91.9 ± 4.0	*	0.031
<= 3 % of the start value at first occasion	38.2 ± 4.5	88.1 ± 11.2	0.002
<= 3 % of the start value at two sequential occasions	55.9 ± 10.0	120.8 ± 10.3	0.0004
<= 5 % of the start value at first occasion	39.8 ± 4.7	88.1 ± 11.2	0.002
<= 5 % of the start value at two sequential occasions	70.3 ± 12.2	129.0 ± 9.6	0.001
<= 10 % of the start value at first occasion	43.3 ± 4.7	96.3 ±11.8	0.005
<= 10 % of the start value at two sequential occasions	83.9 ± 12.9	144.4 ± 5.8	0.0002

* No statistics are computed because all cases are censored.

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