Ketoacidosis at diagnosis in childhood-onset diabetes and the risk of retinopathy 20years later

KETOACIDOSIS AT DIAGNOSIS IN CHILDHOOD-ONSET DIABETES AND THE RISK OF RETINOPATHY 20 YEARS LATER

Short running title: Ketoacidosis at diagnosis and diabetic retinopathy

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ABSTRACT

Aims : To investigate on the relationship between the severity of ketoacidosis, an important risk factor for C-peptide preservation, and long-term microvascular complications in childhood-onset type 1 diabetes mellitus (T1DM).

Methods: 230 childhood-onset diabetic patients (177 pre-pubertal), aged 7.0±3.8 years followed for at least 15 yrs after their diagnosis, were enrolled. Clinical and laboratory data at diagnosis, and C-peptide levels in a subset of patients, were compared with the severity of retinopathy and nephropathy, after a mean of 19.6±3.8 years of disease. Digital retinal photographs were taken in all patients, and centrally graded. Repeated measurements of HbA1c and microalbuminuria for the whole duration of diabetes were collected in over half of the cases.

Results: Out of 230 patients, those with the lowest age at diagnosis had the most severe DKA and clinical conditions ($p<0.05$), and lower C-peptide levels ($p<0.0001$) at diagnosis. There was a significant relationship between pH and clinical severity (($r= - 0.783$, $p<0.0001$), and between pH and C-peptide levels ($r= 0.278$, $p<0.05$). The severity of ketoacidosis had no relationship with subsequent lifetime HbA1c values and long-term microvascular complications. In logistic regression analysis, the only variables that independently influenced severity of retinopathy were lifetime HbA1c ($B=0.838$, $p<0.001$), duration of disease ($B= 0.208$, $p<0.005$) and age at diagnosis ($B=0.116$, $p<0.05$).

Conclusions: The degree of metabolic derangement at diagnosis is not associated with retinopathy and nephropathy in childhood-onset T1DM. Age at diagnosis seems to be an important variable to be considered when evaluating the long-term effects of residual beta-cell function.

Keywords: ketoacidosis, childhood-onset diabetes, retinopathy, nephropathy, C-peptide, HbA1c

1. INTRODUCTION

The occurrence of DKA in children with newly diagnosed type 1 diabetes mellitus (T1DM) is still high, especially in younger children [1-3] and has not significantly changed over time sometimes despite the efforts of information programs [4] . Younger age, lack of private health insurance, ethnic minority status and no family history of T1DM are independently predictive of DKA [3,5]. While there are no doubt on the fact that DKA is acute life-threatening complication, data about its relationships with future long-term complications are lacking. The identification of this relationship may be relevant, since the degree of metabolic derangement at diagnosis may partly reflect the degree of the residual beta-cell function [1,6,7] that, according to some authors [8-10], is, in turn, associated with the risk of late complications. In contrast, other authors refute the hypothesis of this link [11,12], or, at most, its effects would be indirectly mediated by improved metabolic control [13,14]. Even more uncertain is the future of the patients with younger age at diagnosis and low levels of C-peptide [2,15], since they are little represented in the various studies, despite the fact that TIDM in this age range is increasing [16] . In a previous study of our group [17] we reported that, if diabetes is diagnosed in infant or toddlers and the prepubertal duration is the longest, the patients seem to be protected against the risk of microvascular complications.

In the present study we aimed to verify whether the severity of metabolic derangement at diagnosis in childhood-onset T1DM is predictive of long-term micro-vascular complications 20 years later. The majority of cases included in the study were prepubertal children and to our knowledge, a long-term outcome in a higher number of very young children has not been reported in other studies.

2. MATERIALS AND METHODS

This is a multicenter retrospective cohort study involving 11 pediatric units in Italy with current cross-sectional data on retinal and renal complications.

The patients were recruited among those who were diagnosed with T1DM as children between 1981 and 1992 and were included in the study according these criteria: each participating centre has attempted to trace all cases of T1DM who had been diagnosed between 1981 and 1992, i.e., with at least 15 years of disease, and who had been transferred to adult care at the time of study. If year by year at least 60% of the original cohort was tracked down, then all tracked patients , with diagnosis in that year, were included in the study. Otherwise, if the percentage of the tracked patients was lower, all the participants with onset in that year, tracked from that centre, were excluded.

In total, 230 caucasian patients (115 males/115 females, 177 prepubertal (77%) defined by Tanner stage), aged 7.0 ±3.8 years at diagnosis (range 0.8–14.9 yrs; n.84 aged <5 yrs) were enrolled. All

patients but six showed at least one autoantibody at diagnosis among ICA, and anti-insulin antibodies, and the diagnosis was confirmed during follow-up by the clinical course of the patient and the insulin requirement leading to total insulin dependence within 2 years. The few cases without autoantibodies or with wide fluctuations in the need for insulin were screened for possible mutation in the glucokinase, HNF1A and HNF4A genes, and found negative. Ninety-nine patients were diagnosed between 1982 and 1989 and 131 between 1990-1992. Over this time period most patients changed insulin regimens switching from 2 to 3 or more daily insulin injections and from human insulin to analog insulins starting from 1984. Mean diabetes duration was 19.6 ±3.8 (range 15–28.5), being 20 years or more in 80 cases. Patients were recalled between 2007 and 2009 to perform retinal photography and to retrieve clinical and laboratory data at diagnosis and during follow-up from existing clinical records. . A subset of this cohort has been studied in our recent study [17] .

2.1 At diagnosis

Severity of disease at onset was categorized according to pH levels and clinical presentation (Table 1): grade 1 (n=22) asymptomatic and/or serendipitous diagnosis; grade 2 (n=102) polyuria and polydipsia together with good general clinical conditions; grade 3 (n=71) severely compromised clinical conditions with Kussmaul' respiration and manifest signs of dehydration; grade 4 (n=34) impaired consciousness to coma. Ketoacidosis was defined as capillary pH ≤7.30. Glycemic values were available in all but 11 patients, capillary pH value in 178 patients, basal fasting C-peptide levels in 117 and C-peptide levels after glucagon or test meal stimulation in 70 of them, performed only in some centers. The cases who had C-peptide measurements were not different from those who did not have, as regards the clinical and laboratory features at diagnosis. C-peptide measurements was performed during the first admission between the 3rd and the 7th day after the diagnosis, once the acute metabolic derangement was resolved. C-peptide was measured by radioimmunoassay using kits (Bio-Rad, Richmond, CA and Technogenetics, Lisophase, Milan, Italy) with lower limits of detection varying between 0.03 and 0.10 nmol/L. To compare data from different laboratories, we arbitrarily assigned scores to the C-peptide levels reported (Table 1): score 1 (n=23) undetectable or below 0.03 nmol/L, the lowest limit of detection of the kits, and also of the C-peptide RIA used in the DCCT; score 2 (n=32) between 0.03 and 0.10 nmol/L, i.e. under the lower limit of detection of some other kits; score 3 (n=29 cases) minimal secretion between 0.11 and 0.20 nmol//L; score 4 (n=33 cases) moderate secretion, above 0.20 nmol/L, as used in the DCCT [5] as cut off for stimulated C-peptide.

2.2 Follow-up

Repeated measurements of HbA1c for the whole duration of diabetes were available for 135 patients (Table 2). HbA1c had been measured by different methods (Bio-Rad minicolumn, high-performance liquid chromatography, or DCA 2000 analyzer). To compare results from different laboratories, the values were transformed into percentages of HbA1c above the upper normal reference value of each laboratory. Values were averaged throughout the entire duration of disease (excluding the value at diagnosis) and also in separate clusters for the years 0–5, 5–10,10–15, 15–20, and 20–25.

Digital retinal photographs were taken in mydriasis of two 50° fields per eye, one centered on to the macula and the other nasally to the disc, according to the EURODIAB protocol [18] . The pictures were centrally graded in the Diabetic Retinopathy Centre of the Department of Medical Sciences at Turin University by a trained reader. The pictures were graded according to a 5 degree severity scale based upon the American Academy of Ophthalmology simplified classification [19], from no diabetic retinopathy (DR) (Grade 1), to mild nonproliferative DR (2), moderate nonproliferative DR (3), severe nonproliferative DR (4), and proliferative DR (5). For statistical purposes, all patients with grades 3, 4 and 5 were grouped together as moderate-to-severe DR.

Data on repeated measurement of urinary albumin excretion (UAE) during follow-up and at the time of retinal photography were available in 168 patients. Microalbuminuria was defined as UAE between 30 and 300 mg/24 h or as albumin excretion rate (AER) ≥20 ug/min; macroalbuminuria as UAE >300mg/day or AER >150µg/min.

This study was performed in accordance with the Declaration of Helsinki as revised in the year 2000 and approved in the participating centers by an Institutional Review Board regulating non-interventional studies. Written informed consent was obtained from each patient or parent.

2.3 Statistical analysis

All statistical analyses were carried out using the SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) for Windows software program version 14.0.1. Data distribution was analyzed by skewness and kurtosis coefficients and the Kolmogorov-Smirnov test. Where applicable, normal distribution was obtained after logarithmic or square root transformation. For normally distributed data statistical significance was assessed using one-way analysis of variance for multiple comparisons (Bonferroni test), Student's unpaired t-test and Pearson's correlation index. The multifactorial model was evaluated using stepwise logistic regression analysis. For non-normally distributed data Kruskal-Wallis and Mann-Whitney tests and Spearman correlation index computed

on the ranks were used. Pearson chi-square, Yates' continuity correction and Fisher's exact tests for nominal variables were used. A *p* less than 0.05 was considered significant.

3. RESULTS

Out of 230 patients, 46% were in severe clinical conditions (grade 3-4) at diagnosis and 15% were in a state of impaired consciousness or in a coma; 56% had any form of ketoacidosis and 21% had a severe DKA with a blood pH value <7.10 (Table 1). There was an high correlation between the severity score and pH (r= - 0.783, p<0.0001). In the patients <5 years the occurrence of a more severe clinical decompensation and severe DKA was significantly (p< 0.05) higher than that of patients >5yrs (24% vs 9%, p<0.025, and 29% vs 16%, p<0.05, respectively). The younger patients , also , showed lower basal C-peptide levels at diagnosis (r=0.361, p<0.0001). Among the patients <3yrs, 4% belonged to the category with highest C-peptide levels, compared to 85% in the lowest categories 1 and 2: the difference was significant (p<0.0001). There was a significant correlation between basal C-peptide levels and pH (r=0.279, p<0.05), clinical score (r= -0.354, p<0.0001), and stimulated C-peptide values (r=0.69, p<0.0001) at diagnosis. The duration of disease and mean levels of HbA1c at 5 years and lifetime were similar for all gravity scores and C-peptide groups. The degree of metabolic decompensation and C-peptide levels at diagnosis were not significantly related to HbA1c values, both lifetime and subdivided by five-years groups.

After almost 20 year duration, the prevalence of any DR was 55% (n=127 out of 230). Of these, 93 patients (40%) had grade 2 DR; 20 (8.7%) had grade 3; 8 (3.5%) had grade 4 and 6 (2.6%) had grade 5 (Table 2). Among the 34 patients with moderate to severe DR five (15%) were aged <3 years, and 15 (44%) >9 yrs at diagnosis. Table 3 show the relationships between DR and all the main variables. Severity of DR increased with age at diagnosis (p<0.025), longer duration of disease (p<0.0001) and higher HbA1c, either lifetime (p<0.025) or within each 5 year time interval. On the contrary, the presence and severity of DR were not related to the variables at diagnosis, such as blood pH values, clinical severity score, basal C-peptide levels (Table 3), C-peptide levels after stimulation, glycaemia and base excess. No relationships were found even when adjusted for different age-groups. Furthermore, the 34 patients with the best clinical conditions at diagnosis (absence of symptoms or ketoacidosis and presence of detectable C-peptide) did not differ from the 30 worst ones at diagnosis (coma or impaired consciousness, severe ketoacidosis and undetectable C-peptide levels) by DR severity after 20 years, patients without DR being 47% vs 57% and those with severe DR being 15% vs 7%, respectively. In logistic regression analysis, the only variables that independently influenced retinopathy were lifetime HbA1c (B= 0.838, p<0.001), duration of disease (B= 0.208, p< 0.005) and age at diagnosis (B= 0.116, p<0.05).

Twelve (7.2%) out of the patients studied for renal function had abnormal value: 8 had microalbuminuria (4.8%), 3 had macroalbuminuria (1.8%) and one had end-stage disease with renal transplant (0.6%). Three out of these last 4 patients with overt nephropathy had more than 9 years at diagnosis. Proliferative DR was present in 3 of them (1 with microalbuminuria, 1 with macro and 1 with renal transplant). Five patients with microalbuminuria had no DR, 2 with micro and 1 with macroalbuminuria had DR grade 2 and 1 with macroalbuminuria had DR grade 3.

Patients with nephropathy did not differ significantly from all others by age at diagnosis, duration of disease, severity of ketoacidosis, clinical conditions, and basal C-peptide at diagnosis, or lifetime HbA1c.

4. DISCUSSION

The main finding of this study is that the severity of metabolic derangement at clinical onset of diabetes in childhood predict neither presence nor severity of retinopathy or nephropathy, as assessed 20 years later.

The 56% prevalence of all forms of DKA at diagnosis in the present study results higher than that reported in other studies [3,4,20], but this difference may partly be due to variability of some parameters considered in the studies, as calendar years of diagnosis, age range, definitions of DKA, as pH <7.25, <7.30, ≤7.30, from venous, arterial, or capillary blood. The prevalence of 15% of severe DKA with impaired consciousness or coma, is, instead, more similar to the findings of other studies, according to which this percentage amounted between one-sixth and one-fifth of all patients and is not falling significantly over the years [5,20,21]. Our data confirm well-known findings that younger children are characterized by more severe metabolic derangement at diagnosis [1, 3-5, 22] and lower C-peptide levels [2,15]. Younger age and ketoacidosis at diagnosis have been reported, in turn, as the stronger predictors of loss of residual beta-cell function at 12 [1,7, 15] and 24 months [6], so that, has been hypothesized [7] that the high occurrence of DKA and the low rate of partial remission in youngest children may reflect more aggressive beta-cell destruction in these patients. Furthermore, beta-cell function has been related in adult or adolescent patients, on later glycemic control [8,23], even if is not well clear whether sustained C-peptide levels are the cause or the consequence of good control, since high blood glucose is toxic to beta cells [24]. We have failed to find an predictive association between the degree of metabolic decompensation or C-peptide levels at diagnosis and subsequent HbA1c values. This is in agreement with recent studies [1,25] examining, as our study, only pediatric patients and evaluating C-peptide levels at or near diagnosis.

The prevalence of any DR (55%) and severe DR (6%) is comparable with European studies [26] but lower than that found in American studies [27] in cases with similar age at diagnosis and duration of disease. Our findings, summarized in Table 3, confirm that long disease duration, higher age at diagnosis [13,28,29] and lifetime metabolic control [23,30] play a significant role in the risk of micro-vascular complications, whereas, similar to previous reports [11,12], severity of metabolic derangement or C-peptide levels at diagnosis do not play any role. This result is supported by the finding that the two subsets of patients with the best and worst conditions at diagnosis, have a similar long-term outcome, and although the number of these selected patients is limited, the result seems noteworthy. Additional evidences for the lack of association between DKA at diagnosis and microvascular complications may be found in the finding that the patients with higher age at diagnosis had both best clinical conditions along with higher C-peptide levels at diagnosis and higher risk for retinopathy, while those with younger age had higher incidence of ketoacidosis, along with lower C-peptide levels and lower prevalence of DR. Other studies, on the contrary, reported opposite results, supporting a link between beta-cell function and long-term complications [9,10], which may be exerted indirectly, through better metabolic control [9,13]. DCCT study [23] seems confirm this latter hypothesis, since the significant difference in the risk of complications between C-peptide groups disappeared after adjusting for hemoglobin A1c value. However, these studies involved adult or adolescent patients, while our cohort is strictly pediatric, consisting in large part of very young children, 84 cases below 5 years of age. The difference in age could be the main cause of these discordant results, as residual beta-cell secretion in children is typically small and rapidly exhausted over the first years of diabetes [15,31,32], probably due to lower beta-cell mass than adults [33]. In adults and adolescents, in contrast, C-peptide levels are easily detectable also after tens of years of disease [10,34], and in fact Nakanishi et al. [13] suggested that prolonged preservation of detectable C-peptide for at least 10 years is needed to confer clinically meaningful benefits on risk of DR, and such a length is unimaginable in the most cases of very young children.

Our data confirm that nephropathy is less frequent than DR. The prevalence in our cases is the same, 7%, as in our previous report including about half this cohort [17] but lower than reported in other studies [28]. The patients with nephropathy did not differ significantly from all others for any of the variables considered, although the most severe cases were older at diagnosis, according to what we reported in our previous study [35]. However statistical power deriving from their low numbers does not allow to speculate.

Strengths of this study include 1) availability of long-term follow-up of the largest cohort (177 cases) of prepubertal children at diagnosis, 53 of whom <3 years, compared to previous studies, 2)

homogeneity for calendar period of onset and disease duration, 3) information on biochemical and/or clinical conditions at diagnosis in all participants, 4) assessment of retinal photography in all tracked patients along with centralized grading of the pictures by a single independent observer.

Limits include the following: 1) availability of basal C-peptide determinations in only about half of the subjects and of stimulated values in just over half of these, and the lack of follow-up C-peptide measurements. Actually, a single assessment of C-peptide status at the time of diagnosis may not represent a true reflection of patients who will versus those who will not go through the partial remission phase, and the degree of preservation of residual beta-cell function well beyond diagnosis may be more important. We care caution to consider the severity of metabolic derangement at diagnosis as synonymous of the level of residual beta-cell function. However, the finding reported in literature [1,2,6], that DKA is an important risk factor for C- peptide preservation at 12 and 24 months, a doubt on the existence of this link may be raised; 2) the majority of our patients were prepubertal and the effects of age could mask the beneficial effect of basal C-peptide, as the younger age at diagnosis is independently associated with a protective effect on DR. However, even when the influence of age is excluded, considering separately each age group, the prevalence of DR remain similar in each clinical severity and C-peptide subgroup; 3) missing HbA1c might be argued as selection bias in contributing with patients with lower HbA1c. However, the missing values are mainly due to the difficulties in some centers to collect data from old clinical chart for unselected reasons. Furthermore, our rather good mean values are not unusual in our Regions and are fully consistent with other Italian [36] or international multicenter studies [37] in which Italian HbA1c levels were among the lowest.

5. CONCLUSIONS

Even if these results cannot be generalized to other ethnic groups and to different cohorts of patients with worse metabolic control than ours, this study provides evidence that the severity of metabolic decompensation at diagnosis is not associated with the presence and severity of long-term complications in childhood-onset diabetes, at least in a population of very young patients diagnosed 20-30 years ago. Although this is not necessarily applicable to the decline in residual beta-cell function after diagnosis, our data seem to suggest that the long-term effects of residual beta-cell function can be very different in patients with young age at diagnosis. By contrast, metabolic control during the whole disease duration, seems fundamental in determining microvascular complications also in children. It will be interesting to verify in the next years whether these findings are confirmed also in a cohort of patients diagnosed in the last 10-15 years, i.e. after the awareness of the lesson from DCCT and widespread use of analog insulins..

ACKNOWLEGMENTS

The authors thank Stefano Gualandi, PhD, University of Bologna, Bologna, Italy, for help with statistical analysis

DECLARATION OF INTEREST

The Authors have no conflict of interest to disclose. No funding was secured for this study. No financial relationships relevant to this article to disclose

CONTRIBUTORS' STATEMENT

S.S. and M.P. conceptualized and designed the study and drafted the initial manuscript. M.P.and S. R. graded the digital retinal photographs. G.M. designed the data collection instruments, coordinated and supervised data collection and reviewed the manuscript. F.C. participated in study supervision. S. Z. searched published data, contributed in interpretation of data and revised the manuscript. D. I., S. T., V. C., F. C., G. d'A., S.T., A. S., M. A. Z., R. S. recruited the patients, took the retinal pictures, collected data in each centre and reviewed the manuscript. All authors approved the final version for submission.

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Table 1. Patients' characteristics at diagnosis*

Patients n. 230 (115 m/115f)	*Prepubertal* n. 177	*Pubescent* 37	*Pubertal*** 15
Age (yrs) n. 230	7.0 ± 3.8 (0.8-14.9)		
	< 3yrs n. 53	*3-9 yrs* 104	*>9 yrs* 73
Calendar year	1981-1992		
	1981-1989 n. 99	*1990-1992* n. 131	

Clinical severity score n. 230	*grade 1*	*grade 2*	*grade 3*	*grade 4*
	n. 23	102	71	34
	% 10.0 (6.2-13.8)	44.3 (37.9-50.6)	30.9 (25.0-36.9)	14.8 (19.6-19.7)

Blood glucose n.219 (mg/dl)	409 ± 184 (110- 1025)
Base excess n.167 (mmol/L)	- 10.7 ± 9.2 (-31/+ 8)
pH n. 178	7.23 ± 0.16 (6.71 – 7.48)

	<7	*7 - 7.10*	*7.10 - 7.20*	*7.20 - ≤7.30*	*> 7.30*
n.	14	24	21	40	79
%	7.9 (4.5-12.4)	13.5 (9.0-18.9)	11.8 (7.6-17.0)	22.5 (16.7-28.8)	44.3 (37.0-51.4)

Basal C-peptide n.117 (nmol/L)	0.55 ± 0.57 (0.02 – 2.65)			
	≤ 0.03 (group 1)	*0.04 – 0.10 (group 2)*	*0.11 – 0.20 (group 3)*	*> 0.20 (group 4)*
n.	23	31	30	33
%	19.7 (13.1-27.3)	26.5 (19.0-34.7)	25.6 (18.2-33.8)	28.2 (20.5-36.5)

Stimulated C-peptide n.70 (nmol/L)	0.9 ± 0.8 (0.02 – 3.78)	
	≤ 0.20	*> 0.20*
n.	32	38
%	45.7 (33.9-56.6)	54.3 (42.0-64.8)

*Data are expressed as mean ± SD (range) or as number of cases and percentage with 95% confidence intervals in brackets

** Tanner stages 2 and 5, respectively, are the thresholds for the definition of pubescent and pubertal

Table 2. Patients characteristics at the time of retinal photography*

Calendar year				2007 – 2009
Attained age (yrs) (n=230)				26.6 ± 5.4 (16 – 40.3)
Duration of disease (yrs) (n=230)				19.6 ± 3.8 (15 – 28.5)
Last HbA1c value (%) (n=172) (percentage above the upper normal range)				7.9 ± 1.2 (5.3 – 12.8) 132.6 ± 22.4 (85 – 213)
Lifetime HbA1c (%) (n=132) (percentage above the upper normal range)				7.9 ± 0.8 (6.2 – 9.8) 134.2 ± 14.2 (102- 165)
HbA1c values in the first 5 years of disease (n=153) (percentage above the upper normal range)				8.0 ± 1.0 (6.0 – 13.1) 134.1 ± 17.1 (100- 226)
HbA1c values 5-10 years of disease (n=150) (percentage above the upper normal range)				8.2 ± 1.1 (5.7 – 12.4) 136.7 ± 24.7 (98- 214)
HbA1c values 10-15 years of disease (n=165) (percentage above the upper normal range)				8.1 ± 1.1 (5.9 – 13.1) 137.7 ± 19.7 (97- 226)
HbA1c values 15-20 years of disease (n=127) (percentage above the upper normal range)				7.9 ± 1.2 (5.9 – 13.6) 133.1 ± 19.9 (101- 232)
HbA1c values 20-25 years of disease (n=36) (percentage above the upper normal range)				7.8 ± 1.0 (5.9 – 9.6) 117.5 ± 38.4 (100- 153)
Patients with diabetic retinopathy	*n.* 127/230 *%* 55.2 (48.5- 61.3)			
	grade 2 *n.* 93 *%* 40.4 (34.1-46.7)	*grade 3* *20* 8.7 (5.5-12.8)	*grade 4* 8 3.5 (1.6-6.4)	*grade 5* 6 2.6 (1.1-5.3)
n. Patients with abnormal urinary albumin excretion			*n.* 12/168 *%* 7.1 (3.9-11.7)	

*Data as mean ± SD (range) or as number of cases and percentage with 95% confidence intervals in brackets

Table 3. Distribution of the variables, according to presence and severity of diabetic retinopathy (DR)*

	At diagnosis										Duration of disease (yrs)	Lifetime HbA1c (% upper normal reference value)
	pH		Grade of clinical severity				Basal C-peptide group			Age (yrs)		
	≤7.30	> 7.30	1	2	3	4	1+2	3	4			
Without DR n.103	59	41	11	44	28	17	51	18	31	6.1±3.6	18.5 ±3.1	130.8±13.3
mild DR grade 2 n.93	56	44	9	44	36	11	44	29	27	7.4±3.9	20.1±4.0	135.4±13.7
severe DR grade 3+4+5 n.34	48	52	9	48	24	18	41	35	24	8.1±3.9	21.9±4.1	140.9±16.1
	NS		NS				NS			p< 0.025	p<0.0001	p<0.025

*Data as percentages or means ± SD