Epidemiological features of glycated haemoglobin A_{1c}-distribution in a healthy population

The Telecom study

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Summary. HbA_{1c} was measured in 3240 healthy non-diabetic adult individuals in a working population. There was no difference in HbA_{1c} between sexes. The distribution of HbA_{1c} was approximately normal with a slight difference between mean and median values at all ages in both sexes. HbA_{1c} increased with deterioration of glucose tolerance and with all the known risk factors for diabetes (age, obesity, family history of diabetes, history of a large newborn delivery); age but not body mass index appeared as a factor influencing HbA_{1c} independently. In women, HbA_{1c} levels rose particularly at the age of menopause but the use of oral contraceptives or oestrogens made no difference. In both sexes, HbA_{1c} was

higher in smokers than in non-smokers. No consistent seasonal variation was observed. Haematologic factors had a negligible influence on HbA_{1c} level. HbA_{1c} was more highly correlated with fasting plasma glucose than with 2 h-plasma/glucose ($r=0.20$ vs 0.11). In a stepwise multiple regression analysis, age followed by fasting plasma glucose were the only two significant factors associated with the level of HbA_{1c}. These data indicate that HbA_{1c} is influenced only by factors closely linked to diabetes.

Key words: Diabetes diagnosis, epidemiology, glycated haemoglobin.

The World Health Organization Study Group has recently suggested the evaluation of glycated haemoglobin [1] as an alternative method to plasma glucose measurement for screening of diabetes mellitus. At the present time, glycated haemoglobin measurement is considered as the best index of metabolic control in diabetic patients [2–5], providing a cumulative estimate of the mean blood glucose concentration over the preceding 2–3 months. However, its place in diabetes screening and diabetes diagnosis remains debatable. Different conclusions have been reached even between authors who have measured the major glycated haemoglobin, A_{1c} (HbA_{1c}) [6–10], the most reliable indicator of long-term blood glucose [11–13]. Consensus has been obtained on a single point: the need for conducting longitudinal surveys in order to validate glycated haemoglobin measurement as a tool for diabetes screening [6, 9, 10, 14, 15], by reproducing the epidemiological surveys which led to define the currently used plasma glucose criteria of diabetes [16–18]. As serotheques cannot be used for glycated haemoglobin

measurement, prospective studies on the relation of glycated haemoglobin to the development of specific complications of diabetes are needed. They will require lengthy follow-up.

An initial approach consists in determining the factors associated with the distribution of glycated haemoglobin in a healthy population. Plasma glucose is known to vary with age [19, 20], gender [20, 21] and season [22, 23]. In contrast, little epidemiological data are available concerning glycated haemoglobin. According to the most extensive reviews, glycated haemoglobin is considered to be influenced only by the erythrocyte life span and in diabetic patients by the blood glucose concentration [11, 12, 24, 25]. The study presented here has been designed to address the specific issue of the possibility of using HbA_{1c} as a screening tool for diabetes mellitus. This paper reports the distribution of HbA_{1c} in a large, healthy adult population and the effects of age, sex, season, tobacco and the classic risk factors for diabetes.

Fig. 1. Distribution of HbA_{1c} in the study population ($n=3240$)

Subjects and methods

From April 1985 to July 1987, 3240 consecutive subjects who voluntarily attended a centre for preventive medicine, were examined in a cross-sectional study; patients with haemoglobinopathies (1.5% of the total population), known diabetic patients and pregnant women were excluded. The subjects were "France Telecom" employees working in the Paris area. The study population consisted of adults, 40.2 ± 11.8 years old (mean $\pm$ SD), age range: 18 to 81 years, with 51.8% women. "France Telecom" is a national public company employing 38000 subjects in the Paris area (47.4% women), who retire between 60 and 65 years, depending on their occupation. Most of them are office workers and have little physical activity. They are invited to undergo a check-up, free of charge, every 5 years for those under 40 years of age and every 2 years for those over 40 years of age.

A self-questionnaire, filled out at home and reviewed by a secretary during the consultation, inquired about tobacco consumption, gynaecological and obstetrical history, present oral contraceptive (OC) use and family history of diabetes in the first degree relatives. A physical examination recorded height, weight, blood pressure and subscapular skinfold thickness. The Quetelet index [$\text{weight}(\text{kg})/\text{height}(\text{m})^2$] was used as a measure of body mass index (BMI). Biological investigations included a 0–2 h oral glucose tolerance test (OGTT) with a 75 g glucose load given after an overnight fast and HbA_{1c} measurement by a chromatographic technique derived from the Trivelli method [26]. In the same laboratory, a study of 32 diabetic patients, with samples measured in triplicate by this method, showed for HbA_{1c} a residual standard error of 0.22% with a range from 0.06 to 0.40% and an intra-assay coefficient of variation equal to 2.8%.

Table 1 describes the population of the study and compares each parameter between men and women. The data indicate that the studied population was in particularly good health. Concerning diabetes, its prevalence was low: 0.7% were newly discovered diabetic patients, defined by a 2 h-plasma glucose (2 h-PG) value ≥ 11.1 mmol/l.

Statistical analysis

Statistical analysis was performed by the χ^2 test for proportions, analysis of variance and correlation coefficients for quantitative variables and a stepwise multiple regression analysis by BMDP [27].

Results

Distribution of HbA_{1c}; role of sex and age

The distribution of HbA_{1c} in the whole population was approximately normal (Fig. 1). Some characteristics of the sex- and age-specific distributions of HbA_{1c} in the 3240 non-diabetic adults are shown in Table 2. There was no difference in HbA_{1c} according to gender: $5.03 \pm 0.53\%$ in men vs $5.07 \pm 0.55\%$ in women. Median values were the same in both sexes: 5.0%. A very slight difference existed between mean and median values: the greatest difference observed over all the age and sex groups was equal to 0.07%; this confirms the symmetry of the HbA_{1c} distribution. The dispersion of HbA_{1c} values, as reflected by the standard deviations, was remarkably constant over all the age groups.

As shown in Figure 2, HbA_{1c} increased linearly with age in both sexes ($p < 0.001$ for each sex). The percentile values were approximately similar in men and women (Table 2). However, surprisingly enough, HbA_{1c} decreased in men over 60. This age class showed the only sex difference for average HbA_{1c}: $5.05 \pm 0.57\%$ for men vs $5.32 \pm 0.63\%$ for women ($p < 0.05$).

Table 1. Description of the population studied

	Male subjects ($n=1561$)	Female subjects ($n=1679$)	<i>p</i> -value
Ethnic origin (%)			
Caucasian	90.2	88.9	
Black	9.4	10.7	
Asian	0.4	0.4	
Family history of diabetes (%)	11.3	13.2	
Smoker (%)	36.5	24.0	^b
Age (years)	38.6 ± 11.7	41.7 ± 11.8	^b
Body mass index (kg/m^2)	24.2 ± 2.9	23.0 ± 3.5	^b
Systolic blood pressure (mm Hg)	131 ± 15	122 ± 14	^b
Diastolic blood pressure (mm Hg)	78 ± 11	71 ± 11	^b
Fasting plasma glucose (mmol/l)	5.2 ± 0.5	4.9 ± 0.4	^b
2 h-plasma glucose (mmol/l)	5.5 ± 1.6	5.7 ± 1.4	^b
Fasting plasma insulin ($\mu\text{u}/\text{ml}$)	7.9 ± 3.3	7.5 ± 3.6	^a
Plasma total cholesterol (mmol/l)	5.9 ± 1.1	5.8 ± 1.1	
Plasma triglycerides (mmol/l)	1.1 ± 0.6	0.8 ± 0.5	^b
Erythrocyte count ($10^6/\text{ml}$)	4.9 ± 0.3	4.4 ± 0.3	^b
Mean corpuscular volume (μ^3)	90 ± 4	90 ± 5	^b
or γGT (U/l)	21 ± 20	14 ± 16	

For quantitative parameters: Mean $\pm$ SD. ^a $p < 0.01$; ^b $p < 0.001$

Influence of diabetic status

In the newly diagnosed diabetic patients, defined by a 2 h-PG value ≥ 11.1 mmol/l, HbA_{1c} was $5.76 \pm 1.19\%$ ($n=22$), while in subjects with impaired glucose tolerance (7.8 mmol/l $\leq$ 2 h-PG value < 11.1 mmol/l), HbA_{1c} was $5.14 \pm 0.64\%$ ($n=210$) and in normal subjects (2 h-PG value < 7.8 mmol/l) $5.03\% \pm 0.52\%$ ($n=2947$). Thus, there was a significant difference in HbA_{1c} between glucose tolerance groups, with an increasing trend in HbA_{1c} values as glucose tolerance deteriorates ($p < 0.001$). In the subjects with fasting plasma glucose (FPG) ≥ 7.8 mmol/l ($n=5$), HbA_{1c} was $6.54 \pm 1.62\%$.

Role of menopause and oral contraceptives

Among women, HbA_{1c} was higher in the post-menopausal, compared to the pre-menopausal group (Table 3) even after adjustment for BMI ($p < 0.001$); in this group, no significant difference was observed according to the use of oestrogen therapy. In women of child-bearing age, HbA_{1c} was lower in OC users (Table 3) ($p < 0.001$) but the difference was no longer significant after adjustment for age.

Influence of the other classic risk factors for diabetes

Among the 1136 mothers, the 143 who had delivered a newborn weighing more than 4 kg had a significantly higher HbA_{1c} than the others: $5.17 \pm 0.59\%$ vs $5.06 \pm 0.55\%$ ($p < 0.05$) while FPG and 2 h-PG were not different between these two groups: respectively 5.0 ± 0.4 vs 4.9 ± 0.5 mmol/l ($p = 0.11$) and 5.9 ± 1.5 vs 5.8 ± 1.4 mmol/l ($p = 0.75$).

In the whole population, HbA_{1c} was higher where there was a family history of diabetes: $5.15 \pm 0.57\%$ vs $5.03 \pm 0.53\%$ ($p < 0.001$).

Obesity, defined by a BMI over 28 kg/m^2 [28], was associated with a significantly higher level of HbA_{1c} in

Fig. 2. Variation of HbA_{1c} with age in each sex (mean $\pm$ SEM). $\square$ --- $\square$ Men; $\bullet$ $\bullet$ Women

Fig. 3. Seasonal variation of HbA_{1c} over a 2-year period (1985-1987) for each gender. (Su = summer, A = autumn, W = winter, Sp = spring). Men; ---- Women

men: $5.16 \pm 0.52\%$ vs $5.02 \pm 0.53\%$ ($p < 0.01$) and in women: $5.22 \pm 0.73\%$ vs $5.05 \pm 0.53\%$ ($p < 0.001$). By dividing the population into deciles for BMI in each sex, HbA_{1c} was shown to increase gradually with BMI in men ($p < 0.01$) and women ($p < 0.05$) but the difference was no longer significant in either sex after ad-

Table 2. HbA_{1c} (% of total Hb) distribution in adult men and women by five-year age groups

Age	Men										Women									
	No	Mean	SD	Median	Percentiles						No	Mean	SD	Median	Percentiles					
					1	5	10	90	95	99					1	5	10	90	95	99
<25	146	4.93	0.45	5.0	3.5	4.2	4.3	5.5	5.7	5.9	126	4.84	0.49	4.9	3.5	4.0	4.2	5.5	5.7	6.2
25-30	334	4.93	0.50	4.9	3.8	4.2	4.3	5.5	5.8	6.3	250	4.99	0.57	4.9	3.8	4.1	4.3	5.7	5.9	6.6
30-35	273	5.00	0.53	5.0	3.8	4.1	4.3	5.7	5.9	6.4	192	4.96	0.50	5.0	3.7	4.2	4.3	5.5	5.8	6.3
35-40	189	5.07	0.59	5.0	3.8	4.2	4.5	5.7	6.0	6.9	202	4.99	0.47	5.0	3.7	4.2	4.4	5.7	5.8	6.2
40-45	134	5.02	0.51	5.0	3.4	4.1	4.4	5.6	5.8	6.6	212	5.10	0.55	5.1	3.6	4.2	4.4	5.8	6.0	6.7
45-50	136	5.06	0.56	5.0	3.5	4.1	4.3	5.8	5.9	6.7	199	5.05	0.52	5.0	3.9	4.3	4.4	5.7	5.9	6.6
50-55	150	5.16	0.55	5.2	3.9	4.3	4.5	5.8	5.9	7.2	202	5.14	0.56	5.1	3.8	4.3	4.5	5.9	6.0	6.7
55-60	156	5.22	0.47	5.2	4.2	4.4	4.6	5.8	5.9	6.7	211	5.27	0.56	5.3	4.1	4.4	4.6	6.0	6.2	6.6
≥ 60	43	5.05	0.57	5.0	3.9	3.9	4.3	5.9	6.0	6.1	85	5.32	0.63	5.3	4.3	4.4	4.6	6.0	6.2	9.0
All	1561	5.03	0.53	5.0	3.8	4.2	4.4	5.7	5.9	6.4	1679	5.07	0.55	5.0	3.8	4.2	4.4	5.8	6.0	6.5

Table 3. HbA_{1c} levels in women according to menopause, oestrogen treatment and oral contraceptive (OC) use (number of cases in brackets)

Child bearing age (1195) 5.00 ± 0.53% (a)		Post-menopausal (453) 5.25 ± 0.57%	
OC use		Oestrogen treatment	
Yes (366)	No (829)	Yes (55)	No (398)
4.92 ± 0.53% (b)	5.03 ± 0.52%	5.26 ± 0.61% (c)	5.25 ± 0.56%

(a) Between post-menopausal and women of child-bearing age, significant difference even after adjustment for BMI ($p < 0.001$). (b) In women of child-bearing age, significant difference according to OC use ($p < 0.001$) which disappears after adjustment for age. (c) In the post-menopausal group, no significant difference according to oestrogen treatment even after adjustment for age and BMI

Table 4. Correlation coefficients between HbA_{1c} and metabolic and haematological parameters

Variable	<i>r</i>	<i>p</i> -value
Fasting plasma glucose	0.20	b
Plasma cholesterol	0.14	b
2 h-plasma glucose	0.11	b
Fasting plasma insulin	0.07	b
Mean corpuscular volume	0.04	a
Plasma triglycerides	0.01	
Erythrocyte count	-0.04	a

a = $p < 0.05$; b = $p < 0.001$

justment for age. On the contrary, the increase in HbA_{1c} with age remained significant after adjustment for BMI in men ($p < 0.01$) as in women ($p < 0.001$).

Influence of season and tobacco consumption

Seasonal variation of HbA_{1c} was approximately parallel for men and women but no consistent seasonal pattern was found in either sex across the 2-year period of investigation because of a difference between the HbA_{1c} values recorded in winter 1985 and in winter 1986 in men ($4.82 \pm 0.48\%$ vs $5.36 \pm 0.50\%$; $p < 0.001$) as in women ($4.85 \pm 0.50\%$ vs $5.42 \pm 0.47\%$; $p < 0.001$) (Fig. 3).

In both sexes, smokers were younger and leaner than non smokers. Nevertheless, male smokers had significantly higher values of HbA_{1c} than male non smokers: $5.11 \pm 0.51\%$ vs $4.99 \pm 0.54\%$ ($p < 0.001$). Female smokers had a slightly higher HbA_{1c} level than female non smokers ($5.09 \pm 0.54\%$ vs $5.06 \pm 0.56\%$) and the difference was significant after adjustment for age ($p < 0.001$).

Correlation coefficients of HbA_{1c} with the other variables likely to influence its level are shown in Table 4. In order to isolate the most powerful factors associated with the HbA_{1c} level, a stepwise multiple re-

gression analysis was performed including age, family history of diabetes, history of a large newborn delivery (for women), BMI, FPG, 2 h-PG, fasting plasma insulin, mean corpuscular volume, plasma cholesterol and or γ GT. When all these parameters were included in the model, the HbA_{1c} level was poorly explained: r^2 was equal to 0.03 in men and to 0.07 in women. However, in men and in women, age and then FPG were the only two factors which were significant.

Discussion

These cross-sectional data demonstrate that HbA_{1c} concentrations are unimodally distributed in this healthy population. Such a unimodal distribution probably exists in all populations, except in populations having an extraordinarily high prevalence of diabetes such as the Pima Indians [14]. The present study suggests that HbA_{1c} is influenced by very few factors. While FPG was higher and 2 h-PG was lower in men than women, as previously described [20, 21], no significant sex difference exists for HbA_{1c}, except for the group over sixty years of age, in which men had lower levels than women. A selection bias is the most probable explanation, with a healthy worker effect in this group of older men who were still working and volunteered for this medical examination. This hypothesis is supported by testosterone measurements: in this male adult population, the general trend of plasma testosterone was to decrease with age, as is usual [29], but in the over-sixty group it increased [30]. Moreover, the sample size for men is approximately half of that for women in this age group, suggesting selection biases and a survivor effect. The lack of sex difference for HbA_{1c} in a healthy population, also demonstrated for HbA₁ [31, 32], obviates the need for sex-specific reference standards. The increase of HbA_{1c} with ageing had been previously demonstrated in non-diabetic subjects between the ages of 50 and 89 years [33]. A controversial paper showing a lack of influence of ageing probably failed to show the role of age because of a lack of power due to small sample sizes [34]. The increase of glycated haemoglobin with abnormality of glucose tolerance had been demonstrated in several studies although the distributions of glycated haemoglobin values for the three glucose tolerance classes overlapped [6–8, 10]. The relation of HbA_{1c} with all the known risk factors for diabetes (age, obesity, family history of diabetes, history of large newborn delivery) must be underlined. In particular, the higher HbA_{1c} level in mothers having delivered large newborns, contrasts with the non significant differences observed in FPG and 2 h-PG values. The negligible influences of oestrogen replacement therapy in post-menopausal women and of OC use in women of child-bearing age upon HbA_{1c} level are parallel to the small hormonally induced modifications of FPG and 2 h-PG which have been observed in this population [35] as in

other healthy populations [36, 37]. The lack of consistent seasonal variation of HbA_{1c} in this study contrasts with data previously reported. In agreement with the known seasonal variations of FPG [22] and 2 h-PG [23], the highest values of glycated haemoglobin had been observed at the ends of autumn and winter and the lowest at the ends of spring and summer [38]. In our study, not only HbA_{1c} (Fig. 3), but also FPG and fasting plasma insulin were significantly increased in winter 1986 compared to winter 1985 (respectively 5.1 ± 0.5 vs 4.9 ± 0.7 mmol/l, $p < 0.001$ and 7.5 ± 3.2 vs 7.0 ± 3.3 μ u/ml, $p < 0.01$). Unusually mild weather in winter 1985 could have prevented the known increase of glycated haemoglobin [38], FPG [22, 39] and plasma insulin [40] with the cold. The data from 1986 are probably more representative of the usual seasonal pattern of HbA_{1c} and are in good agreement with the reported seasonal pattern of FPG, 2 h-PG and glycated haemoglobin [22, 23, 38]. This study shows an increase in HbA_{1c} in smokers, as recently reported for HbA₁ [32]; this shift was more evident in men, heavier smokers than women (14.5 g vs 11.2 g of daily tobacco consumption). However, the correlation coefficient between HbA_{1c} and tobacco consumption was not significant ($r = 0.08$ in either sex). So, the difference in consumption cannot explain the more important effect of tobacco upon HbA_{1c} in men. Nevertheless, the increase in HbA_{1c} in smokers contrasts with a lower level of FPG and 2 h-PG. By our method of HbA_{1c} measurement, the quantity is not affected by the presence of carboxyhaemoglobin; therefore the higher HbA_{1c} level in smokers cannot be due to an artefact. Considering that reactivity of the glucose molecules with the N-terminus of the β -chain has been demonstrated to increase in the presence of carbon monoxide in vitro [41, 42], it can be proposed as an hypothesis that this biochemical mechanism exists also in vivo and could explain the increased HbA_{1c} in smokers. So, if we admit that glycosylation of Hb could be a marker of the development of diabetic complications [43], these data provide an additional argument to recommend that diabetic patients not smoke.

All of the relationships between HbA_{1c} and the factors measured appear weak, even when the link is found to be statistically significant due to the power of analysis provided by the large size of the studied population. In particular, with regard to the known influence of haematologic disorders upon glycated haemoglobin level [11, 25], the low correlation coefficients observed here between HbA_{1c} and mean corpuscular volume, as well as with erythrocyte count, indicate the negligible role of haematologic factors when HbA_{1c} is measured by a reliable technique in a healthy population. It must be remembered that an haemoglobinopathy was discovered in 1.5% of subjects, preventing accurate measurement of HbA_{1c}. This percentage would have been close to zero if we had selected Caucasians as most of the excluded people were native from the West In-

dies. Between HbA_{1c} and plasma glucose values, the correlation coefficients appear strikingly low. Similar results, including an even weaker relationship with 2 h-PG than with FPG, have been previously observed in other healthy populations, for HbA₁ [32] as for HbA_{1c} [10]. The studied population had a low prevalence of diabetes (0.7%); a previous study in a diabetes screening centre in a high risk population with a high prevalence of diabetes (7.5%) found a correlation coefficient between HbA_{1c} and FPG equal to 0.52 and between HbA_{1c} and 2 h-PG equal to 0.51 [9], approaching the values observed in diabetic populations [44]. The discrepancy observed in the association between plasma glucose values and the HbA_{1c} level indicates that, as soon as plasma glucose becomes abnormal – as in diabetic populations – HbA_{1c} is more closely correlated with plasma glucose but, in a healthy population, HbA_{1c} appears as a very independent parameter, influenced only by factors closely linked to diabetes. Even if use of HbA_{1c} measurement in population studies has obvious advantages such as the ability to use non-fasting specimens, and no need for a glucose challenge, further prospective studies are needed in order to determine whether HbA_{1c} could be preferable to OGTT for diabetes screening because of prognostic as well as logistic considerations [10, 45].

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