HbE dan Diabetis Melitus
Lapuran Akhir Projek Jangka pendek USM

Penyelidik

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Penghargaan

Kami ingin mengucapkan ribuan terima kasih kepada mereka yang namanya tertera di bawah ini kerana telah sama-sama menjayakan penyelidikan ini.

Abstract

Glycated hemoglobins (HbA1/HbA1c) serves as an indicator of diabetic control and hence the effectiveness of treatment. Studies on the methods available have shown that factors such as hemoglobin concentration and the presence of high lipid in the blood may affect the validity of the results. Atypical hemoglobins such as HbS and HbC have also been shown to affect the determination of glycated hemoglobins. However no study has been done on the effect of HbE , the hemoglobin variant that is present in the South East Asian population. Thus this study was undertaken to determine the effect of HbE on the determination of HbA1/HbA1c by 4 commercial kits and also to determine its prevalence among diabetic subjects in Kelantan.

Blood was taken from 58 normal subjects (23 males and 35 females) and 63 HbE (31 males and 32 females) heterozygous subjects. All subjects had no history of diabetes mellitus. They had random blood glucose values < 7.8 mmol/l and hemoglobin (hb) values between 7 to 24 g/dl. The EDTA plasma collected were tested for HbA1 and HbA1c using 4 commercial kits i.e. Eagles Diagnostics, Boehringer Mannheim (BM), Diastat and Ames DCA 2000. The tests were done within one week and samples were stored at 4°C before analysis.

Results showed that the mean ± s.d HbA1 levels in normal vs HbE heterozygous subjects using Eagles Diagnostics, Boehringer Mannheim and Diastat kits were 6.9 ± 1.1% vs 9.5

± 3.9%; 7.1 ± 1.2% vs 14.8 ± 9.3% and 8.3 ± 3.8% vs 13.5 ± 10.8% respectively. All were significantly different (p<0.001). The mean ± s.d HbA1c levels for normal vs HbE heterozygous subjects determined using Ames DCA 2000 and Diastat kits were 5.1 ± 0.5% vs 5.3 ± 2.7% and 6.1 ± 1.9% vs 10.7 ± 8.7% respectively. The results measured using the Diastat kit were significantly different (p<0.001) while those measured using the Ames DCA 2000 kit were not significantly different (p = 0.5).

Therefore the cation-exchange chromatography based kits (Eagles Diagnostic, Boehringer Mannheim and Diastat) were affected by the presence of HbE. This interference was minimised by dilution. The use of specific antibody (Ames) did not have any influence on HbA1c determination.

The prevalence of HbE among diabetics were also studied. Blood was taken from 202 diabetic patients (121 females and 81 males; aged 46.2 ± 15.2 years) who were attending the outpatient diabetes clinic, Hospital USM. 28 patients (13.9%) were confirmed to carry HbE by hemoglobin electrophoresis. This consists of 4 males and 24 females. The finding is consistent with the prevalence of HbE heterozygotes in the general population.

In conclusion, this study showed that HbE affect the determination of HbA1/HbA1c by kits using cation exchange chromatography and that the interference may be diluted out. The prevalence of HbE among diabetes mellitus patients is similar to that of the general population.

1.0 INTRODUCTION

Glycated hemoglobins are formed during a non-enzymatic reaction when glucose attaches itself to the N-terminus of the β- chain of hemoglobin Ao(1). The level of glycated hemoglobins is proportional to the level of blood glucose over a period of two months (2). Thus glycated hemoglobins are accepted as an indicator of bood glucose level over the preceeding two months.

The commonly measured glycated hemoglobins for monitoring diabetic control are hemoglobin A1 (HbA1) or its subfraction hemoglobin A1c (HbA1c). Using these levels, treatment are adjusted to achieve good metabolic control in diabetic patients.

Most of the commercially available kits for the determination of HbA1 or HbA1c use cation-exchange chromatography technique - although the use of specific antibody against the β-chain has gained popularity. The use of cation-exchange chromatography based kits will still be popular locally as it is simple to perform and cheap, costing about a quarter to half the price of an immunoassay based kit.

Measurements of HbA1 or HbA1c involve separating these from the other hemoglobin fractions by either the use of electrophoresis, cation-exchange chromatography or specific antibody. The value is then reported as a percentage of the total hemoglobin.

Normal HbA1 levels for non-diabetic subjects had been reported as 6 - 8 % while for

HbA1c it is 3-6 % (3.4.5).

Thus the accuracy of the results obtained is affected by the total hemoglobin

concentration and the level of inaccuracies varies with each kit. The same is true

regarding the influence of hemoglobin variants. The manufacturers reported that atypical

hemoglobins such as HbS, HbC and HbF co-elute with HbA1 or HbA1c during cation-

exchange chromatography resulting in higher HbA1 or HbA1c values respectively

(6,7,8). The influence of these variant hemoglobins were not seen in methods employing

specific antibody (9). Although extensive studies were done on the effect of HbS, HbC

and HbF, no study was done to determine the influence of HbE on the validity of HbA1

or HbA1c measurements. HbE is an atypical hemoglobin, more commonly present in the

South East Asian population than the western population. In the North East region of

Peninsular Malaysia, 13 % of the population was observed to be HbE heterozygotes and

3% were clinically symptomatic homozygous (10).

The presence of HbE was observed to affect the total hemoglobin concentration by

masking anemia (10). Thus, because the HbA1 or HbA1c levels are reported as a

percentage of total hemoglobins, the presence of HbE might affect the validity of HbA1

or HbA1c results. Further, HbE might behave exactly like HbS or HbC by co-eluting with

HbA1 or HbA1c during cation-exchange chromatography.

Thus in this study, the effect of HbE on 4 commercial kits were studied. Three of the kits used employed cation-exchange chromatography technique while the other kit is the more expensive based on the use of specific antibody. This is necessary as the results obtained during this study will be used to choose the most suitable method to routinely monitor the diabetic control of our patients.

The prevalence of HbE among diabetes mellitus was also studied. This is necessary so that the extent of the problem in monitoring diabetes control via HbA1/HbA1c measurements could be gauged and appropriate actions be taken.

1.1 AIM

1. To study the effect of HbE on the determination of HbA1 and HbA1c by 4 commercial kits;

2. To determine ways to reduce the above effect;

3. To determine the prevalence of HbE among diabetes mellitus patients

2.0   MATERIALS AND METHODS

2.1   MATERIALS

I.   Diastat hemoglobin kit, cat. no. 210-0002, Bio-Rad Laboratories, USA.

- A fully automated low pressure cation exchange column chromatoraphy method using gradient elution to separate human hemoglobin subtypes and variants from hemolysed whole blood. Measures both HbA1 and HbA1c.

ii.   Hemoglobin A1 kit , cat. no. 575453, Boehringer Mannheim GmbH, Germany.

- A manual low pressure cation exchange column chromatography method. Measures HbA1.

iii.   Glycohemoglobin, cat no. GH-100, Eagles Diagnostics, USA.

A manual cation-exchange resin method. Measures HbA1.

iv.   Ames DCA 2000 HbA1c ragent kit, cat no 5036, Bayer Diagnostics, UK.

- A fully automated cartridge style kit using specific antibody to the glycated amino-terminal residues of the β-chain of HbA. Measures HbA1c.

v.   Accutrend glucose, cat no. 07786, Boehringer Mannheim, Germany.

vi.   Hb electrophoresis kit, cat. no. 441780, Beckmann, UK.

vii.   Anticoagulant K2 EDTA for 2.5ml blood, cat no 113337, LP, Italy.

viii.   Isolab hemocard hb FAE control, HE 1580.

ix.   Isolab Hb A/E control, HEC-901

x.   1.5ml tube with cap, Eppendorf

xi.   Sterile syringes and needles, 10 cc, 21Gx!1/2, Becton Dickinson, USA.

2.2. SUBJECTS

2.2.1. NORMAL SUBJECTS

Blood was taken from volunteers and tested immediately for random blood glucose (RBS) (Accutrend, Boehringer Mannheim, Germany) and full blood picture (FBP). All volunteer subjects have no history of diabetes mellitus. Only those with RBS values less than 7.8 mmol/l and normal FBP were included in this study.

Complete data were obtained from 58 subjects consisting of 23 males and 35 females. The mean age $\pm$ s.d. was 24 $\pm$ 8.2 years (range 17 - 47 years). Their random blood glucose level (RBS) was 5.1 $\pm$ 1.1 mmol/l (3.6 - 7.8 mmol/l) and hemoglobin (hb) was 13.8 $\pm$ 0.7 g/dl (10.6 - 16.6 g/dl).

2.2.2 THALASSAEMIC SUBJECTS

103 blood samples with 'thalassaemic FBP' profile were confirmed for the presence of HbE using Hb electrophoresis (Beckmann, USA). All patients had no history of diabetes mellitus. The RBS and hb were also measured and noted. Only those with RBS levels of <7.8 mmol/l and hb 7 - 24 g/dl were included in the study.

Complete data were obtained from 63 subjects (31 males and 32 females). Their mean ±

s.d. age was 23.6 ± 15.6 years (1- 64 years). Their RBS level was 4.3 ± 1.3 mmol/l (3.4 -

7.4 mmol/l) and the hb level was 10.8 ± 2.1 g/dl (7.2 - 15.6 g/dl).

2.2.3. DIABETIC SUBJECTS

284 diabetes mellitus patients who were attending the diabetes outpatient clinic at

Hospital USM were screened for urine sugar, RBS, FBP and HbA1 (Eagles Diagnostic,

USA). Those with 'thalassaemic FBP' profile were tested further with hb electrophoresis

to confirm the presence of HbE.

Complete data were obtained from 202 patients - 121 females and 81 males. The mean ±

s.d. age was 46.2 ± 15.2 years, (6 - 78 years), hb level was 12.6 ± 2.3 g/dl (7.4 - 17.4

g/dl) and RBS was 9.1 ± 5.6 mmol/l (3.2 - 25.4 mmol/l).

2.3 METHODS

2.3.1 EVALUATION OF KITS

The performance for each of the kits were evaluated in terms of its intra- and inter day

precisions using quality control samples at normal and elevated levels (Eagles

Diagnostics, USA).

2.3.2 EVALUATION OF STORAGE CONDITION FOR THE SAMPLES

Pooled plasma samples from normal (normal level) and diabetic subjects (elevated level) were analysed for HbA1 using Eagles Diagnostic kit (USA). The samples were then divided into aliquots of 200 ul and stored at 3 temperatures i.e. room temperature (25°C), 4°C and -20°C. Aliqouts were taken, thawed (if necessary) and analysed for HbA1 using Eagles Diagnostic kit (USA) daily until day 25.

2.3.3. EFFECT OF HbE ON THE DETERMINATION OF HbA1/HbA1c

Each EDTA plasma samples from normal subjects and HbE positive subjects were analysed for HbA1 and/or HbA1c using Diastat, Boehringer Mannheim, Eagles Diagnostic and Ames DCA 2000 kits. The plasma samples were stored at 4°C before analysis. All the HbA1/HbA1c determinations on each sample were done within 1 week.

The mean HbA1 and HbA1c levels ± s.d. were then calculated for the normal and the HbE heterozygous groups. The results were then statistically compared using Student's t-test (Microsoft Excel, USA).

2.3.3.1. Effect of diluting Hbe positive samples

EDTA plasma samples from normal subjects were pooled and the HbA1/HbA1c levels determined using the 4 commercial kits. This was then used to dilute HbE positive samples by 1:2 and 1:4 dilutions . The HbA1/HbA1c levels of the neat and the diluted

samples were then measured. Actual HbA1/HbA1c levels obtained by measurement were then compared with the calculated or expected values.

2.3.4. PREVALENCE OF HbE AMONG DIABETES MELLITUS PATIENTS

Blood taken from diabetes mellitus patients was tested for RBS and HbA1 (Eagles Diagnostics) and FBP (Beckman, USA) immediately. Those samples with 'thalassaemic FBP'profile were confirmed for HbE on hb electrophoresis. The number and percentage of those with HbE was then calculated.

3.   RESULTS AND DISCUSSION

3.1.   PERFORMANCE OF HbA1 AND HbA1c KITS

Table 1 shows the within-run precision of HbA1 determinations using Eagles Diagnostic, Boehringer Mannheim and Diastat kits and HbA1c determinations using Diastat and Ames DCA 2000 kits.

Table 1. Intra-day precision of HbA1 and HbA1c determinations using Eagles Diagnostic(Eagles), Boehringer Mannheim(BM),Diastat and Ames DCA 2000 (Ames) kits.

	EAGLES HbA1 (%)	BM HbA1 (%)	DIASTAT HbA1 (%)	DIASTAT HbA1c (%)	AMES HbA1c (%)
mean	7.8	7.9	7.9	5.7	5.2
s.d	0.2	0.2	0.2	0.1	0.1
cv	2.7%	2.5%	2.5%	1.8%	2.3%
n	10	6	6	6	6
mean	13.4	17.5	20.1	14.8	11.9
s.d	0.2	1.3	1.2	0.3	0.3
cv	1.7%	7.4%	6.0%	2.0%	2.8%
n	10	6	6	6	6

The coefficient of variations (cv) of the within-run precision for the normal level is below 3% for all the kits tested and agrees to the manufacturers reported range (6,7,8,9). However at elevated levels, the HbA1 values determined by Boehringer Mannheim and Diastat kits were high (c.v 7.4 and 6% respectively).

Table 2. Inter-run precision for HbA1 and HbA1c determinations using Eagles Diagnostic (Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

	Eagles HbA1(%)	BM HbA1(%)	Diastat HbA1(%)	Diastat HbA1c(%)	Ames HbA1c(%)
mean	7.2	8.4	7.8	5.8	5.1
s.d.	0.5	1.0	0.7	0.6	0.1
cv	6.8%	11.5%	9%	10.3%	1.2%
n	11	9	12	12	12
mean	14.3	17.9	19.3	14.2	11.8
s.d	0.7	1.5	0.9	1.7	0.1
cv	5%	8.4%	4.7%	12.0%	0.8%
n	12	9	12	13	12

Table 2 shows the interday precision of HbA1 and HbA1c using the 4 commercial kits tested in this study.

The Ames DCA 2000 kit gave reproducible results as is shown by the low standard deviation (s.d) and cv (tables 1 and 2). The worst cv was obtained using the BM and Diastat kits.

Good precisions were obtained using the Ames DCA 2000 kit. This is not surprising as this is a fully automated cartridge type procedure. Acceptable reproducibility was also obtained using the Eagles Diagnostic kit eventhough it is a manual method. However this method is used routinely in our laboratory and hence the technologists are skillful in performing this assay. The comparatively poor reproducibilities of BM kit may be due to lack of experience in handling this manual method. However what is surprising is the comparatively poor precision obtained with the fully automated cation-exchange column chromatography based Diastat kit. This could be due to the instability of the columns or the inherent variability and efficiency of the eluting system.

Thus the fully automated antibody based Ames DCA 2000 kit gave the best within - and inter- run precisions. For the cation-exchange chromatography based kits, experience in handling as well as the efficiency of the column and its eluting system is important in ensuring good reproducibility.

3.2   STABILITY OF SAMPLES

Figures 1 and 2 show the stability of samples stored as whole blood or hemolysate under various conditions for over 25 days. Figure 1 shows the stability of HbA1 at normal range (normal subjects) and Figure 2 shows the stability of HbA1 at elevated range (diabetic subjects).

As can be seen from the results, deterioration in the samples caused the results to be elevated.

From the results, whole blood samples stored as 4°C gave the best stability over 11 day period. Thus for this study, samples for HbA1 and HbA1c determinations were stored as whole blood at 4°C for a period not exceeding 7 days.

FIGURE 1: STABILITY OF HbA1 NORMAL SAMPLES STORED AS WHOLE BLOOD (NH) OR HEMOLYSATE (H) AT 25C, 4C AND -20C.
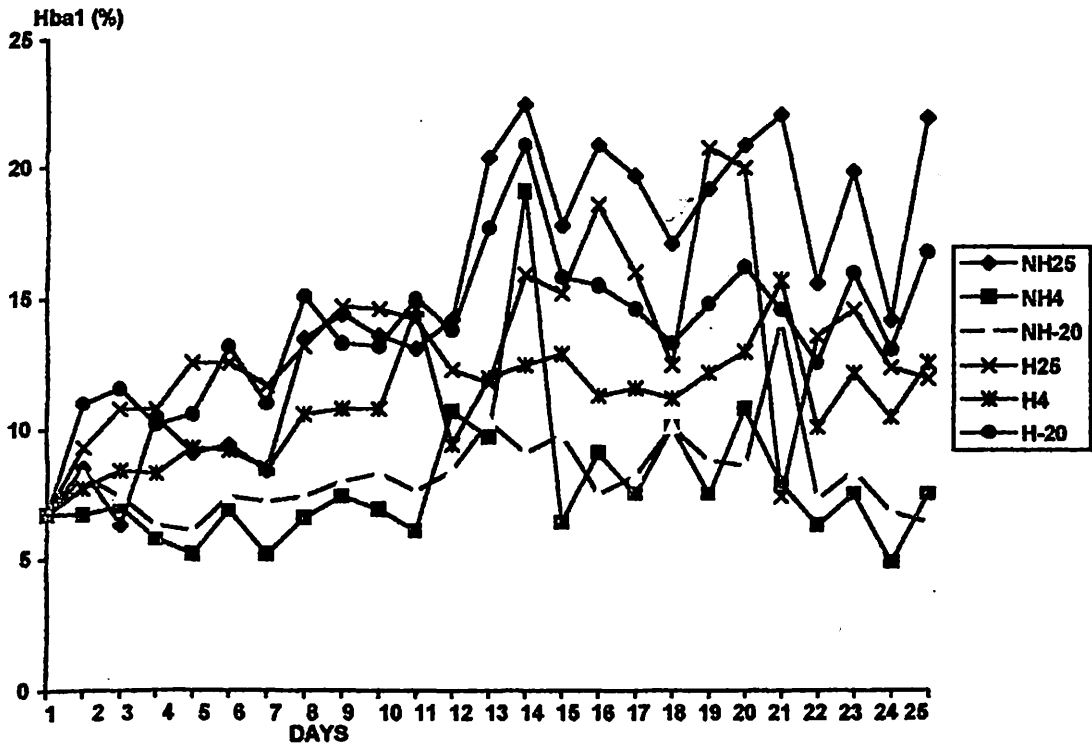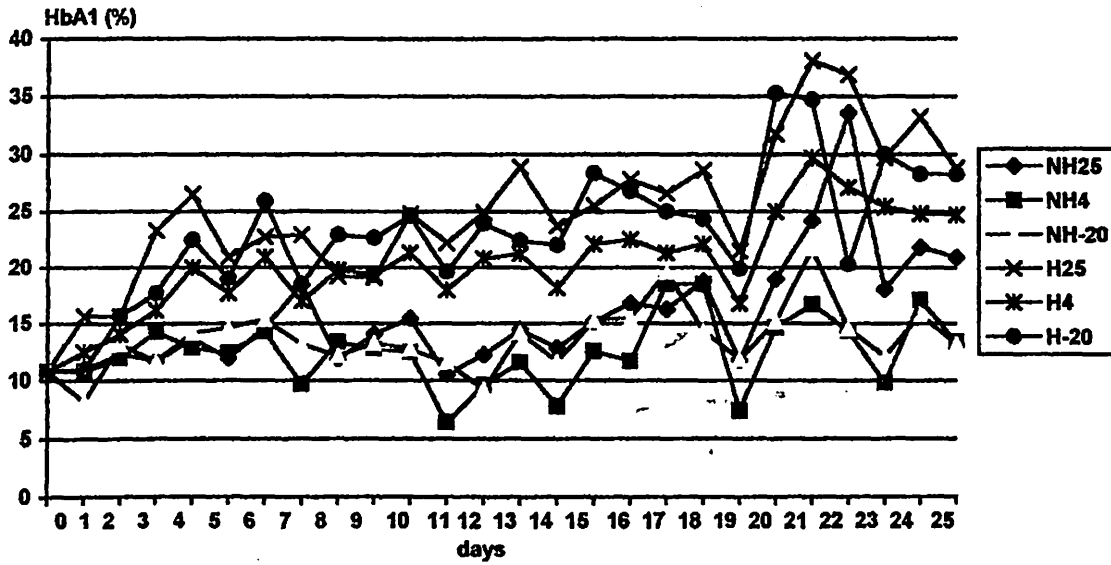
FIGURE 2: STABILITY OF HbA1 ELEVATED SAMPLES STORED AS WHOLE BLOOD (NH) OR HEMOLYSATE (H) AT 25C, 4C AND -20C.

3.3   NORMAL HbA1 AND HbA1c LEVELS

Table 3 shows the means ± s.d of HbA1 and HbA1c levels for the normal volunteers.

Table 3: Normal HbA1 and HbA1c levels in normal subjects as measured by Eagles Diagnostic (Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

	Eagles HbA1 (%)	BM HbA1 (%)	Diastat HbA1 (%)	Diastat HbA1c (%)	Ames HbA1c (%)
mean	6.9	7.1	8.3	6.1	5.1
s.d	1.1	1.2	3.8	1.9	0.5
n	58	58	58	58	58

The normal levels obtained in this study agreed with those published in the literature (normal HbA1 6 - 8% and HbA1c 3 -6 %). However results obtained with the Diastat kit gave a slightly higher value.

There was no significant difference in HbA1 and HbA1c levels between the sexes again agreeing to earlier observations.

3.4. EFFECT OF HbE ON THE DETERMINATION OF HbA1 AND HbA1c

Table 4 shows the comparison between the means ± s.d of HbA1 and HbA1c in normal versus HbE heterozygous subjects as determined by the 4 commercial kits tested in this study.

Table 4: Comparison of the means ± s.d HbA1 and HbA1c levels of normal and HbE heterozygous subjects as determined using Eagles Diagnostic (Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

	Eagles HbA1 (%)	BM HbA1 (%)	Diastat HbA1 (%)	Diastat HbA1c (%)	Ames HbA1c (%)
normals	6.9 ± 1.1	7.1 ± 1.2	8.3 ± 3.8	6.1 ± 1.9	5.1 ± 0.5
HbE	9.5 ± 3.9	14.8 ± 9.3	13.5 ± 10.8	10.7 ± 8.7	5.3 ± 2.7
p value	<0.001	<0.001	<0.001	<0.001	0.5

The results clearly show that there was no significant difference between the means ± s.d of HbA1c levels of normals and HbE subjects as determined by the Ames DCA 2000 kit. Ames DCA 2000 uses antibody that binds to the glycated terminus of the β-chain while HbE involves mutation at point 26. Thus the antibody is able to recognise HbE as well as HbA1or HbA1c.

The cation- exchange chromatography based kits were all affected by the presence of HbE (Table 4). HbE levels increased the HbA1 and HbA1c results determined using the Eagles Diagnostics, Boehringer Mannheim and Diastat kits (p<0.001). This conclusion can also be gathered from Figures 3,4,5,6 and 7 which show the dotplot diagrams of HbA1 and HbA1c levels in normal and HbE heterozygous subjects as measured by the 4 kits tested.

The interference HbE exerts on the above measurement could be due to 2 factors: HbE affecting the total hemoglobin determination or HbE co-elutes with the HbA1 and HbA1c fractions.HbE was observed earlier to affect the results of total Hb by giving a falsely raised value (10). If this is the main interference, then the HbA1 or HbA1c results measured would be low. However, we noticed an increased in the level of glycated hemoglobin measured using the cation-exchange chromatography based kits. Thus this could mean that the co-elution of HbE with the glycated hemoglobin is the predominant effect.

The co-elution with HbE on cation-exchange columns is not a strange phenomena. Similar observations were noted with other hemoglobin variants such as HbS and HbC resulting in the raised HbA1 or HbA1c levels (7). All three kits used low pressure chromatography. The use of high performance liquid chromatography or different columns such as PolyCAT A (11) might be able to resolve HbE from HbA1 or HbA1c.

Fig. 3: HbA1 Levels in Normal and HbE Heterozygous Subjects as Determined Using Eagles Diagnostic Kit.

HbA1(%)
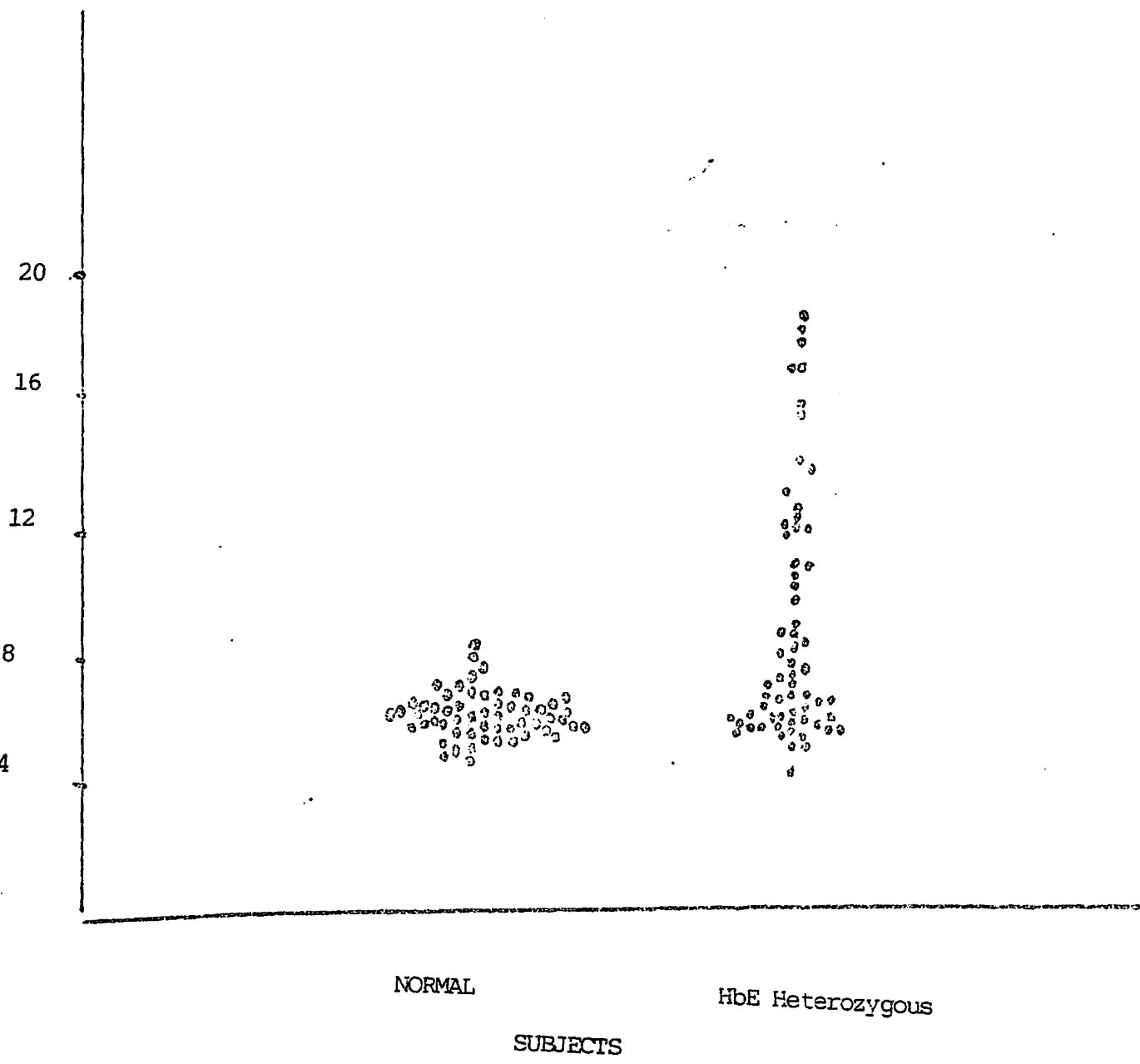

NORMAL   HbE Heterozygous

SUBJECTS

Fig. 4: HbA1 Levels in Normal and HbE Heterozygous Subjects as Determined using Boehringer Mannheim Kit.
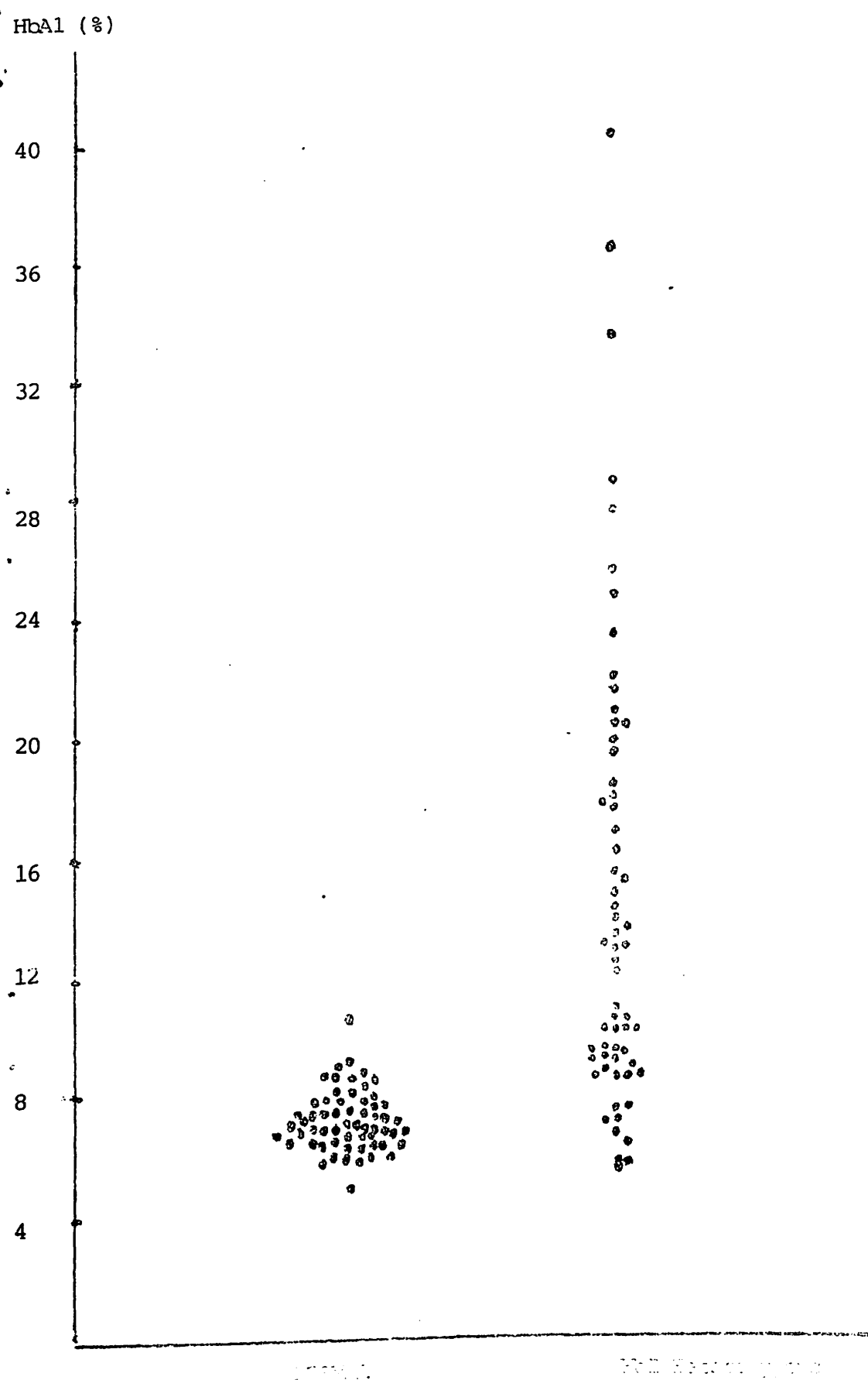
Fig 5: HbA1 Levels in Normal and HbE Heterzygous Subjects As Determined Using Diastat Kit.
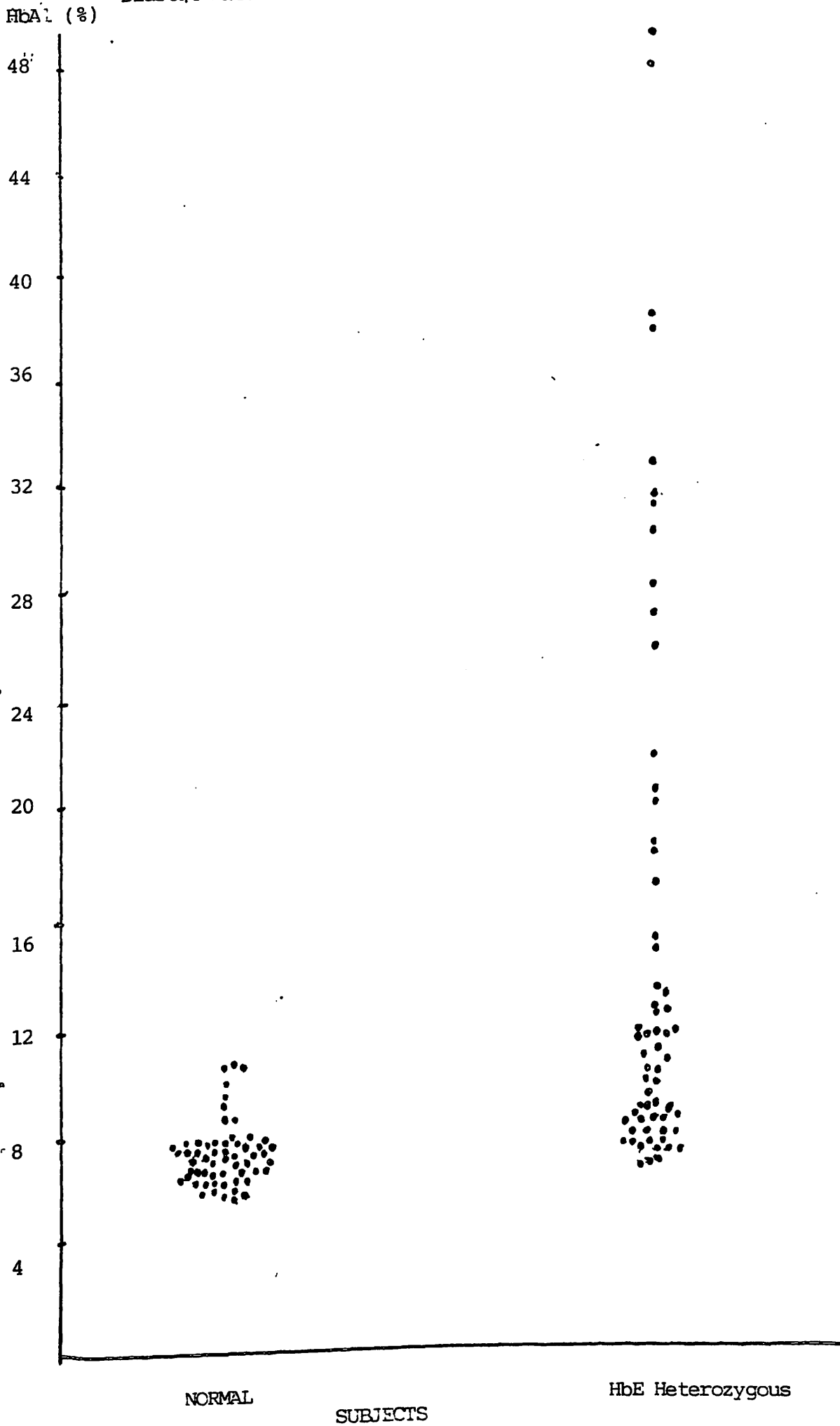
Fig 6:   HbA1c Levels in Normal and HbE Heterozygous Subjects as Determined
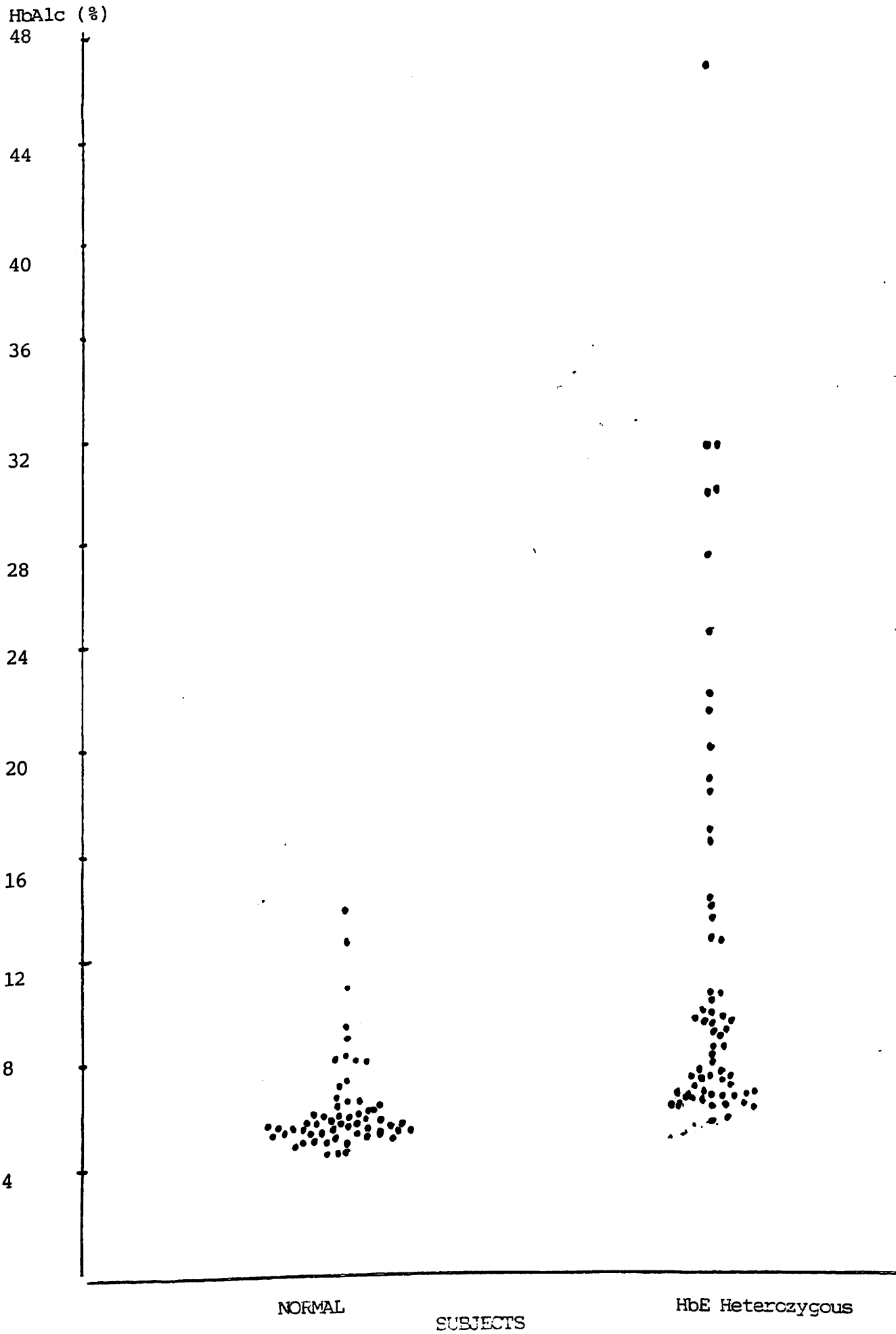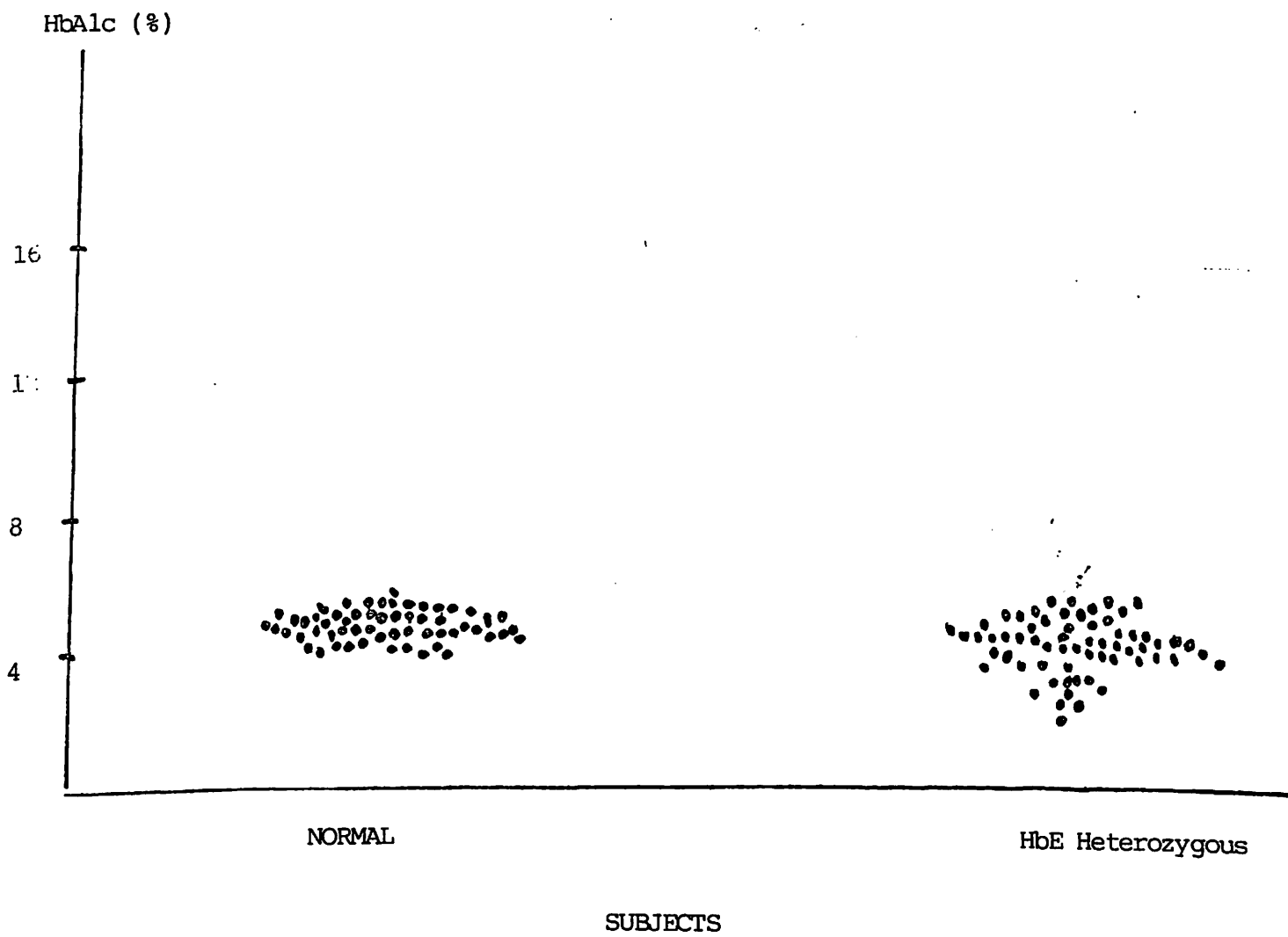Using Diastat kit.

Fig. 7: HbA1c Levels in Normal and HbE Heterozygous subjects as determined using Ames DCA 2000 kit

HbA1c (%)

16

1

8

4

NORMAL   HbE Heterozygous

SUBJECTS

Therefore this study shows that HbE influences the results of HbA1 and HbA1c determinations done on cation-exchange chromatography based methods.

3.4.1. Effect of dilution on the influence of HbE

Tables 5 and 6 show the results of the dilution studies on HbE samples.

Table 5: Dilution study on HbE samples: Expected and Actual HbA1 values after dilution with pooled normal non HbE samples as measured using Eagles Diagnostic (Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

Dilution	Eagles		BM		Diastat	
	Expected	Actual	Expected	Actual	Expected	Actual
sample A						
1:2	7.3	6.3	7.5	6.3	9.3	8.9
1:4	7.0	6.2	7.2	6.2	8.3	7.9
sample B						
1:2	7.9	6.9	8.1	6.6	8.1	6.5
1:4	7.4	6.1	7.4	6.2	7.6	6.7

Table 6: Dilution study of HbE samples: Expected and Actual HbA1 values after dilution with pooled normal non HbE samples as measured by Eagles Diagnostic (Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

Dilution	Ames (%)		Diastat (%)	
	Expected	Actual	Expected	Actual
sample A 1:2	5.2	5.2	6.5	6.0
1:4	5.2	5.2	5.8	5.4
sample B 1:2	5.2	5.1	5.9	5.0
1:4	5.2	5.1	5.7	5.0

The 'expected' results are the calculated values from the levels of the neat, undiluted samples. The 'actual' values are the results observed on measurement. In table 5, each of the actual values obtained was infact lower than the expected value. Thus the high HbA1 level present when measuring the neat sample was infact due to interference. This interference was abolished upon dilution. Hence the actual results obtained during dilution corresponds more to the normal non diabetic HbA1 levels.

Similar observations were seen with the HbA1c determination using the Diastat kit (Table 6). HbA1c measurements by the Ames DCA 2000 kit was not affected by the presence of HbE in the samples and this is shown by the similarities in the expected and actual values obtained after dilution.

Thus the interference by HbE on HbA1 and HbA1c determinations can be overcome by dilution of the samples.

Table 7 shows the percentage of HbE subjects having HbA1 or HbA1c values higher than the upper limit of normal (HbA1 = 8% and HbA1c = 6%)

Table 7: Percent HbE heterozygous subjects with elevated HbA1 or HbA1c values as determined by Eagles Diagnostic(Eagles), Boehringer Mannheim (BM), Diastat and Ames DCA 2000 (Ames) kits.

	Eagles HbA1	BM HbA1	Diastat HbA1	Diastat HbA1c	Ames HbA1c
percent HbE subjects with elevated HbA1 or HbA1c levels	38.1	82.0	58.7	74.6	1.6

The results show that considerable number of patients with elevated HbA1 and HbA1c levels as measured by the cation-exchange based methods. However the worst affected was the Boehringer Mannheim manual kit. The importance of the inherent quality of the separating system is shown by the fact that the fully automated Diastat system gave results worse than the manual Eagles Diagnostic system. Again, the results of Table 7 showed that the HbA1c levels measured using the Ames DCA 2000 kit were not affected by the presence of HbE. Thus it is recommended that samples from HbE heterozygotes patients should not be measured by cation exchange chromatography based methods. The antibody based procedures are more reliable in giving valid results under this condition. However if there is no other choice, then samples from patients with HbE should be diluted for the measurement of glycated hemoglobins using cation exchange based methods.

3.5 PREVALENCE OF HbE HETEROZYGOTES AMONG DIABETES MELLITUS PATIENTS

From this study, we found that 28 patients were carriers of HbE and this was confirmed by hb electrophoresis. They constituted 13.9% of the diabetes mellitus patients. Of these, 4 were males and 24 were females.

This observation agrees with the earlier observation on the prevalence of HbE among the general population (10) . Hence the prevalence of HbE heterozygotes in Diabetes Mellitus patients is similar to that of the general population.

4. CONCLUSION

This study shows that HbE affects the determination of HbA1 and HbA1c using kits based on cation-exchange chromatography with low-pressure system. This could principally be due to the co-elution of HbE with HbA1/HbA1c thus giving falsely raised levels. This interference could be abolished by dilution.

HbE does not affect the determination of HbA1c using methods based on specific antibody to the glycated terminus of the β-chain. Thus it is recommended that for HbE positive diabetes mellitus patients, the determination of their HbA1 (or HbA1c) should be carried out using kits based on specific antibody and not on cation-exchange chromatography.

This study also show that the prevalence of HbE among diabetes mellitus patients is similar to that of the general population.

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