CHAPTER V

ESTIMATION OF GLYCO GROUPS ATTACHED NON-ENZYMATICALLY TO PROTEINS IN HAIR AND NAIL
INTRODUCTION

The hallmark of diabetes mellitus is hyperglycemia, which leads to glycation of proteins, which in turn is thought to contribute to the long-term complications of diabetes. The discovery of increased concentration of non-enzymatic glycosylated hemoglobin in diabetes mellitus had led to intensive research into similar excess glycosylation of other tissue proteins, especially in an attempt to establish a link between this process and chronic complications of the condition. Proteins that have been shown to undergo increased glycosylation in diabetes mellitus include hemoglobin (127), lens crystallins (132), collagen (130), myelin proteins (225), membrane proteins (226), various plasma proteins (194), nail (136) and hair proteins (137). Proteins, which are suitable candidates for non-enzymatic glycosylation are those, which have longevity and which are exposed to relatively high glucose concentration.

Measurement of glyco groups in hair and nail proteins will provide a non-invasive method for detection and management of long-standing hyperglycemia. Methods have been developed earlier for the estimation of glycated protein in hair (137) and nail (136). They are based on the release and dehydration of glyco group on prolonged treatment with mild acid (oxalic acid 1 mol/l) for 2 hours at 120°C in an autoclave. The dehydrated product on condensation with thiobarbituric acid forms a yellow chromogen, which is measured at 443 nm. Cyclohexanone extraction of the chromogen improved the sensitivity of the method considerably (201). However, one major problem associated with this method is the high non-specific background absorbance which due to its variability, results in loss of sensitivity (201). Further the thiobarbituric acid method is shown to be nearly ten times less sensitive than the phenol: sulfuric acid method for glycated hemoglobin (197). The major defect of the thiobarbituric acid method is the lack of absolute proportionality between carbohydrate released (and estimated) and hemoglobin concentration (227). Pecovaro and associates (227) observed during their studies on the thiobarbituric
acid assay method, a non-linear relation between hemoglobin concentration and amount of sugar released. However, these workers suggest the use of a correction factor to avoid this interference. In the phenol: sulfuric acid method, maximal values were reached after 3-4 hours prior mild acid hydrolysis unlike in TBA method, which required much longer time (197). The differences in the values between the control and diabetic group are more significant with phenol: sulfuric acid method than with the thiobarbituric acid method (197).

Rao and Pattabiraman, who investigated the chemical basis of interaction of HMF with phenol, developed a modified phenol: sulfuric acid method, far more sensitive for estimation of glyco groups (44). They also observed that o-cresol is a better chromogen than phenol in the assay of HMF (44). Raghavendra and Pattabiraman (45) developed a sensitive method for the estimation of glycated hemoglobin based on o-cresol: sulfuric acid reaction. It was found to be useful in discriminating diabetic and normal control group. They subjected the globin solution to both one-step and two-stage o-cresol: sulfuric acid reaction for the estimation of glycated hemoglobin in normal and diabetic groups.

This chapter deals with the development of methods for the estimation of glyco groups in hair and nail proteins by o-cresol: sulfuric acid reaction. The methods have been applied to evaluate increased glycation in diabetes. Statistical analysis of the data obtained with control subjects and diabetics indicates that these estimations will be highly useful in detecting and monitoring long-standing hyperglycemic conditions.

**MATERIALS**

The present work was conducted with twenty-two confirmed diabetic patients. The patients were chosen from the outpatient clinics of the Department of Oral Medicine and General Medicine and also from patients admitted to S.D.M. College of Dental Sciences, Sattur, Dharwad-9. Twenty-five healthy,
non-diabetic subjects served as control. The criteria for diagnosis of diabetes were based on the recommendation of National diabetic data group.

The control subjects were apparently healthy dental students, para-dental staff and laboratory technicians. They were free from any sort of disease and diabetes mellitus was excluded after estimation of fasting blood sugar, examination of urine for sugar and oral glucose tolerance test, wherever indicated.

Collection of hair and nail samples

Hair taken from behind the ear was cut up finely and weighed samples were used for the study. Similarly, nail samples pared away finely were carefully weighed out into 5 mg portion and used for the study.

All other materials used are listed in Chapter II.

METHODS

Unless stated otherwise, the following routine procedures were employed.

One-step o-cresol: sulfuric acid method

Five mg of finely shaved finger nail samples was suspended in 1.0 ml of distilled water. To this, 0.1 ml of ethanolic o-cresol (20 mg) solution was added, followed by 3.0 ml of concentrated sulfuric acid. Both o-cresol solution and sulfuric acid were at room temperature before use. The contents were mixed thoroughly. Color was developed at room temperature and the absorbance values were measured at 500 nm, 30 minutes after the addition of sulfuric acid. o-Cresol and sulfuric acid reacted in the absence of nail samples gave a very small blank value. Similarly hair samples was cut up finely and 30 mg were processed as above.
Two-stage method

Five mg of finger nail samples was suspended in 1.0 ml of distilled water. To this 3.0 ml of concentrated sulfuric acid was added. The contents were mixed thoroughly. After cooling for 30 minutes at room temperature, 0.1 ml of ethanolic o-cresol (20 mg) solution was added. After 30 minutes reaction at room temperature, the chromogens formed were measured at 500 nm.

Effect of sulfuric acid concentration

To study the effect of sulfuric acid concentration on chromogen formation with o-cresol for hair and nail samples, the proportion of aqueous solutions containing hair or nail samples and sulfuric acid were altered to get a series of different sulfuric acid concentration in the range, 50-80%. In all these cases, heat of dilution generated is comparable (110-118°C). Both one-step and two-step methods were followed as above.

Reaction conditions for two-step o-cresol: 75% sulfuric acid method

a) Effect of time of interaction with o-cresol on chromogen formation

Five mg of nail samples were suspended in 1.0 ml of distilled water. To this, 3.0 ml of concentrated sulfuric acid was added and mixed thoroughly. After cooling for 30 minutes at room temperature, 0.1 ml of ethanolic solution of o-cresol (20 mg) was added and mixed. After standing for different time intervals (5-90 minutes) at room temperature, the chromogen formed was analyzed in the visible region.

b) Effect of incubation time for chromogen formation

Five mg of nail samples were suspended in 1.0 ml of distilled water. To this, 3.0 ml of concentrated sulfuric acid was added and mixed thoroughly. After cooling for different time intervals (15 -120 min) at room temperature, 0.1 ml of
ethanolic o-cresol (20 mg) was added and mixed. After 30 minutes reaction at room temperature, the chromogen formed were analyzed in the visible region.

c) Effect of o-cresol concentration

To study the effect of o-cresol concentration, different concentrations of ethanolic o-cresol (5-30 mg) in a volume of 0.1 ml were used during the assay system.

Prior hydrolysis with mild acid

To 30 mg of nail shavings or 100 mg of hair samples, 15 ml of 1.33 M HC1 was added. After treatment in a boiling water bath for varying time intervals (1-6 hours), the proteins along with the pigments (in hair) were precipitated by the addition of equal volume of 20% trichloroacetic acid (final concentration 10%). One ml of the clear supernatant was subjected to two-step o-cresol: sulfuric acid reaction.

Effect of cysteine on chromogen formation

To study the effect of cysteine on chromogen formation, 5 mg of L-cysteine hydrochloride in a volume of 0.1 ml was included at first-stage in the two-stage assay system.

RESULTS

Preliminary studies showed that hair and nail samples reacted with o-cresol in sulfuric acid medium to form chromogens, which were somewhat similar in spectral characteristics to those, formed from HMF and fructose. However, minor differences were noticed.

Data obtained with different concentrations of sulfuric acid in both one-step and two-step assay methods are shown in Table 5.1 and Figure 5.1 respectively. Nail samples showed maximum color intensity in the one-step
TABLE 5.1
EFFECT OF SULFURIC ACID CONCENTRATION ON CHROMOGEN FORMATION WITH NAIL AND FRUCTOSE IN ONE-STAGE ASSAY

<table>
<thead>
<tr>
<th>Sulfuric acid final concentration %</th>
<th>One stage assay</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nail, 5 mg</td>
<td>Fructose, 20μg</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.024</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>0.082</td>
<td>0.270</td>
<td></td>
</tr>
<tr>
<td>57.5</td>
<td>0.156</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.218</td>
<td>0.400</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>0.336</td>
<td>0.350</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.312</td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.256</td>
<td>0.270</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.1 Effect of sulfuric acid concentration in two-step assay with nail sample (5 mg) (without prior hydrolysis)
assay, when the acid concentration was 65%, whereas fructose showed maximum color at 57.5% sulfuric acid concentration. In the two-step o-cresol: sulfuric acid assay, optimum sulfuric acid concentration for nail samples was 75% as was with fructose (110). Hair samples could not be subjected to direct one-step or two-step assay, since the pigments interfered in the color development.

**Reaction conditions for two-step o-cresol: 75% sulfuric acid method**

a) **Time taken for color development**

Data obtained at different time intervals are shown in Figure 5.2. The time required for the interaction between the dehydration products of nail samples with o-cresol to form chromogens in the two-stage assay method was studied. Color intensity reached optimal value by 10 minutes for nail samples. The absorbance at 10, 20 and 30 minutes after the addition of o-cresol was the same. With fructose also color intensity reached optimal value by 10 minutes (89). A slight decrease in the values was observed at 40 minutes (5%), 60 minutes (12%) and 90 minutes (19%). (Data not shown).

b) **Effect of incubation time before the addition of o-cresol**

Nail samples were subjected to heat of dilution of sulfuric acid and cooled by allowing to stand at room temperature. o-Cresol was added at 15, 30, 45, 60, 90 and 120 minutes after the addition of sulfuric acid. Absorbance values were measured 30 minutes after the addition of o-cresol at 500 nm. Data are shown in Figure 5.3. The data show that time taken for maximal color development in two-stage assay for nail samples was much longer compared to fructose, which is 10 minutes (13). Further, after 60 minutes color intensity decreases.

c) **Effect of o-cresol concentration in two-stage assay for nail samples**

Various concentrations of o-cresol (5, 10, 15, 20 and 30 mg in a volume of 0.1 ml) were used in the assay system. It was observed that optimal
Figure 5.2 Effect of time on interaction with o-cresol in two-step assay method.

- --- Nail samples (5 mg)
Figure 5.3 Effect of incubation time before addition of o-cresol on chromogen formation with nail samples (5 mg) in two-stage assay method.
concentration of o-cresol required, was higher (20-30 mg) for the estimation of glyco groups in nails samples, whereas with fructose, a lower concentration of o-cresol (10 mg) is sufficient to obtain maximum color intensity.

**Prior hydrolysis with mild acid**

Prior hydrolysis with oxalic acid has been used to release dehydration products of glyco groups in estimation of glycated hemoglobin, albumin, and nail and hair proteins by thiobarbituric acid (194, 197, 136, 137) method. Nayak and Pattabiraman (197) used 1M oxalic acid in 2 M HCl for release of glyco groups in glycated hemoglobin. For the estimation of glyco groups in hair, prior acid hydrolysis was found to be essential. It was found that 1.33 M HCl was optimal for processing hair samples. About 30 mg of nail or 100 mg of hair samples were subjected to prior acid hydrolysis with 15 ml of 1.33M HCl in a boiling water bath at 100°C for varying time intervals (1-6 hours). The proteins along with the pigments were precipitated by the addition of equal volume of 20% trichloroacetic acid (final concentration 10%). The clear supernatant (1.0ml) was subjected to two-step o-cresol: 75% sulfuric acid reaction. The data shown in Figure 5.4 indicate that the maximal time of prior hydrolysis with 1.33 M HCl was four hours at 100°C. With nail samples also, the method was employed and the optimal time of hydrolysis was four hours. Values decreased slightly on prolonging hydrolysis time.

**Absorption spectra of the chromogens formed in 75% sulfuric acid**

The data shown in Figure 5.5 represent the comparison of absorption spectra of HMF with the absorption spectra of products formed by prior acid hydrolysis of hair/nail proteins after treatment of trichloroacetic acid filtrate with 75% sulfuric acid. Absorption spectral analysis of the dehydrated products formed by hair and nail samples after 4 hours hydrolysis with 1.33 HCl, showed that HMF is not the major product formed. While HMF, absorbed maximally at 320 nm, the products from hair samples showed two absorption maxima, one
Figure 5.4 Effect of time of prior hydrolysis with 1.33 M HCl

- Hair samples (3.33 mg equivalent)
- Nail samples (4.00 mg equivalent)
Figure 5.5 UV absorption spectra of the products formed after treatment with 75% sulfuric acid

- Hair samples (4.16 mg equivalent)
- Nail samples (4.0 mg equivalent)
- HMF (10 μg)
at 252 nm and another at 285 nm. Similar two-peak profiles were also seen with nail samples. The data suggest that partially dehydrated products still bound to amino acid residues may be present.

However, the final chromogen formed with HMF, hair and nail samples is identical with absorption maxima around 500 nm. Further there was no peak at lower wavelengths with hair and nail samples. The data obtained with hair and nail samples are shown in Figure 5.6. Based on the absorption spectra in the visible region, it can be concluded that different partially dehydrated products generated from hair and nail samples condense with o-cresol and undergo further dehydration to form the semiquinonoid of HMF in sulfuric acid medium.

Glyco groups in hair, thumb nail and toe nail samples of 10 normal individuals are compared in Table 5.2. It can be seen from the data that the extent of glycation of nail is significantly more than in hair. Values of glyco groups expressed in terms of mean optical density units ± S.D. for hair with prior hydrolysis, are 0.0358 ± 0.003 S.D. While for thumb nail and toe nail samples after prior hydrolysis, the values are 0.0999 ± 0.0151 S.D. and 0.0895 ± 0.0119 S.D. respectively. This corresponds to 2.79 and 2.50 fold increase in thumb nail and toe nail glycosylation respectively compared to hair glycosylation. Thumb nail glycosylation increase is statistically highly significant compared to the hair glycosylation (p<0.001, t=13.1). Similarly, increase in toe nail glycosylation compared to hair glycosylation is also highly significant (p<0.001, t=13.8). Thumb nail values are generally slightly higher than the toe nail values. However, the increase is not statistically significant (p>0.05, t=1.77). Further, values obtained after prior hydrolysis with acid were more than those determined directly in the individual sample of nail. Data with direct (without prior hydrolysis) method for nail samples are shown in Table 5.3.
Figure 5.6 Absorbance spectra of chromogen formed in o-cresol: sulfuric acid reaction (two-step assay).

- Hair samples (4.0 mg equivalent)
- Nail samples (4.0 mg equivalent)
## TABLE 5.2
**GLYCOSYLATION VALUES OF HAIR AND NAIL PROTEINS EXPRESSED AS ABSORBANCE UNITS/mg WEIGHT OF SAMPLES IN NORMAL SUBJECTS BY TWO-STAGE o-CRESOL: SULFURIC ACID METHOD**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Without hydrolysis mean ± S.D. (range) n=10</th>
<th>With prior hydrolysis mean ± S.D. (range) n=10</th>
<th>Correlation between hair and nail proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair</td>
<td>-</td>
<td>0.0358±0.003 (0.030-0.042)</td>
<td>-</td>
</tr>
<tr>
<td>Thumb nail</td>
<td>0.0806±0.008 (0.071-0.087)</td>
<td>0.0999±0.0151 (0.074-0.119)</td>
<td>P&lt;0.001 (t 13.14)</td>
</tr>
<tr>
<td>Toe nail</td>
<td>0.0692±0.006 (0.060-0.074)</td>
<td>0.0895±0.0119 (0.070-0.120)</td>
<td>P&lt;0.001 (t 13.8)</td>
</tr>
</tbody>
</table>

Absorbance at 500 nm
TABLE 5.3
GLYCO GROUP VALUES OF NAIL SAMPLES (μg FRUCTOSE/mg OF SAMPLE) IN NORMAL AND DIABETIC SUBJECTS BY TWO-STEP o-CRESOL: SULFURIC ACID METHOD, WITHOUT PRIOR HYDROLYSIS WITH DILUTE HCl

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diabetics mean ± S. D. (range) n=10</th>
<th>Normals mean ± S. D. (range) n=10</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thumb nail</td>
<td>0.339±0.066 (0.206-0.470)</td>
<td>0.199±0.019 (0.182-0.290)</td>
<td>P&lt;0.001 (t-6.3)</td>
</tr>
<tr>
<td>Toe nail</td>
<td>0.287±0.074 (0.193-0.445)</td>
<td>0.171±0.014 (0.153-0.225)</td>
<td>P&lt;0.001 (t-4.3)</td>
</tr>
</tbody>
</table>
Figure 5.7 and 5.8 show the scatter dot diagram of glycated protein values in hair and nail samples respectively, obtained by the two-step o-cresol: sulfuric acid method after prior hydrolysis with mild acid. The comparison of dot patterns of glycosylation of hair values shows no overlap between normal and diabetic subjects. All diabetic patients have hair glycosylation values more than 0.110 µg fructose/mg sample and normal subjects have values less than 0.100 µg fructose / mg sample. Similarly, the values for thumb nail are more than 0.30 µg fructose / mg sample and less than 0.26 µg fructose / mg sample respectively for diabetic and normal subjects. The values for toe nail glycosylation are slightly lower than the values of thumb nail glycosylation.

**Effect of cysteine on chromogen formation**

To study the effect of cysteine on chromogen formation, cysteine hydrochloride (5 mg) was included at first stage in the two-step assay system. The data obtained are shown in Table 5.4. The data show that cysteine inhibited chromogen formation marginally but it is statistically significant. This is in sharp contrast to the behavior of HMF and hexoses in presence of cysteine. Inclusion of cysteine at first-step in two-stage assay system enhanced chromogen formation from HMF and hexoses (Chapter III and IV).

The values for glycosylation of hair, thumb nail and toe nail samples of normal control group and diabetics are shown in Table 5.5 and Table 5.6 respectively. The mean value of glycosylation of hair expressed as µg of fructose/mg of sample in control and diabetic subjects are 0.089 ± 0.006 S.D. and 0.173 ± 0.056 S.D. respectively, corresponding to a 1.95 fold increase in hair glycosylation (p<0.001, t=7.28). Similarly, the mean values of glycosylation of thumb nail samples in control and diabetics are 0.237 ± 0.026 and 0.403 ± 0.088 µg fructose/mg of the sample respectively. A significant increase of 1.7 fold is observed (p<0.001, t=8.8) in thumb nail glycosylation. With toe nail samples, the mean values are 0.207 ± 0.028 S.D. and 0.356 ± 0.092 S.D. µg fructose/mg
Figure 5.7 Glycosylation of hair (μg of fructose/mg of hair) levels in normal and diabetics by two-step o-cresol: sulfuric acid method, after prior hydrolysis with 1.33 M HCl
Figure 5.8 Glycosylation of thumb and toe nail (μg of fructose/mg of nail sample) levels in normal and diabetics by two-step o-cresol: sulfuric acid method, after prior hydrolysis with 1.33 M HCl
### TABLE 5.4

EFFECT OF CYSTEINE-HCL (5 mg) ON COLOR FORMATION IN TWO-STAGE ASSAY (CYSTEINE-HCI AT FIRST STAGE) WITH HAIR, THUMB NAIL AND TOE NAIL SAMPLES. ABSORBANCE/mg WEIGHT OF SAMPLE AT 500 nm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cysteine hydrochloride</th>
<th>Absorbance/mg Sample mean ± S.D n=10</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair</td>
<td>Nil</td>
<td>0.063±0.025</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>0.058±0.024</td>
<td>(t 8.0)</td>
</tr>
<tr>
<td>Thumb nail</td>
<td>Nil</td>
<td>0.147±0.052</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>0.130±0.048</td>
<td>(t 7.9)</td>
</tr>
<tr>
<td>Toe nail</td>
<td>Nil</td>
<td>0.133±0.050</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>0.119±0.046</td>
<td>(t 4.6)</td>
</tr>
</tbody>
</table>
TABLE 5.5
GLYCOSYLATION VALUES OF HAIR (μg FRUCTOSE/mg SAMPLE) IN NORMAL AND DIABETIC SUBJECT BY TWO-STEP o-CRESOL: 75% SULFURIC ACID METHOD AFTER PRIOR HYDROLYSIS WITH 1.33M HCl.

<table>
<thead>
<tr>
<th></th>
<th>Diabetics mean ± S. D. (range) n=22</th>
<th>Normals mean ± S. D. (range) n=25</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.173±0.056 (0.093-0.270)</td>
<td>0.089±0.006 (0.070-0.110)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td>t 7.3</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 5.6
GLYCOXYLATION VALUES OF NAIL PROTEIN (µg FRUCTOSE/mg OF SAMPLE) IN NORMAL AND DIABETIC SUBJECTS BY TWO-STEP o-CRESOL: SULFURIC ACID METHOD, AFTER PRIOR HYDROLYSIS, WITH 1.33 M HCl.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diabetics mean ± S. D. (range)</th>
<th>Normal mean ± S. D. (range)</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thumb nail</td>
<td>0.403±0.088 (0.240-0.550)</td>
<td>0.237±0.026 (0.182-0.290)</td>
<td>P&lt;0.001 (t 8.8)</td>
</tr>
<tr>
<td>Toe nail</td>
<td>0.356±0.092 (0.213-0.506)</td>
<td>0.207±0.028 (0.169-0.270)</td>
<td>P&lt;0.001 (t 7.5)</td>
</tr>
</tbody>
</table>
DISCUSSION

The mean values of glycosylation of hair in control and diabetic subjects estimated by two-step o-cresol: 75% sulfuric acid assay method, after prior hydrolysis with 1.33 M HCl, are 0.089 ± 0.006 (mean ± S.D.) and 0.173 ±0.56 μg fructose/mg sample, respectively. The mean increase in hair glycosylation values in diabetics (1.94 fold) is highly significant (p<0.001). Raghavendra and Pattabiraman (45) reported a 1.45 fold increase in glycated hemoglobin values in diabetics compared to normal control subjects using o-cresol: sulfuric acid method. The reported glycated hemoglobin values (moles HMF/mole globin) were 1.24 ± 0.26 and 1.81 ±0.39 for normal and diabetics, respectively by the two-step o-cresol: sulfuric acid method. The data also agree with the values obtained by Paisey and associates by TBA method who reported a 1.79 fold increase in hair glycosylation levels in diabetics compared to normal subjects. (Normal: 0.054 ± 0.011 and diabetics 0.097 ± 0.045 μ mole fructose/100 mg hair). Our findings of hair glycosylation in these units are 0.494 and 0.961 for control and diabetics respectively, which are higher by an order of magnitude.

The present studies show that the extent of glycation is about 14 times lower in hair proteins than in hemoglobin in normal subjects. However, increase in glycation in hair is found to be more than that in hemoglobin in diabetics. The mean values of glycosylation of thumb nail in control and diabetic subjects are 0.237 ± 0.026 S.D. and 0.403 ± 0.088 μg fructose/mg of sample, respectively. The mean increase in nail glycosylation in diabetics (1.70 fold) is highly significant (p<0.001). Bakan and Bakan (136) reported 1.91 fold increase in nail glycosylation levels based on TBA method in diabetics compared to normal subjects. (Normal 8.35 ± 2.7 and diabetics 16.0 ± 7.35 nmol fructose/mg thumb nail). Our data expressed in these units are 13.16 and
22.38 nmol fructose/mg of thumb nail which are comparable to those reported by Bakan and Bakan (136). The mean values of glycosylation of toe nail in control and diabetics are 0.207 ± 0.028 S.D. and 0.356 ± 0.092 S.D. µg fructose/mg of sample respectively. The mean increase in diabetics is 1.71 fold and this is also highly significant p<0.001).

Glycosylation values obtained by the direct (without prior acid hydrolysis) method for thumb nail and toe nail samples are also found to be significantly increased in diabetes. The mean value of glycosylation of thumb nail in normal and diabetic subjects by direct two-step o-cresol: sulfuric acid method are 0.199±0.019 and 0.339±0.066 µg fructose/mg of sample, respectively. The mean increase is around 1.70 fold. The values obtained with toe nail samples are 0.171±0.014 and 0.287±0.074 µg fructose/mg of sample for normal and diabetic subjects, respectively. The mean increase in this case is also around 1.70 fold.

The values obtained by direct two-step o-cresol: sulfuric acid method is comparable with the values obtained by prior hydrolysis with mild acid in terms of fold increase in glycosylation.

To conclude, this study shows that two-step o-cresol: 75% sulfuric acid method after prior hydrolysis with mild acid is suitable for estimation of glyco groups in hairs. With nail samples the direct method is as sensitive as that of prior hydrolysis with mild acid. All the methods described are more sensitive than the thiobarbituric acid method. They clearly discriminate diabetics from normal control groups. The direct two-step o-cresol: 75% sulfuric acid method is less time consuming compared to prior hydrolysis method. Both the methods are reliable, non invasive and therefore direct two-step method can be employed for routine laboratory estimation of glycateed nail proteins and with prior hydrolysis method for hair proteins for the detection and assessment of long term glycemic control.