P10

Investigations Into The Creation Of A Strip Test Capable Of Monitoring Glucose Concentration Using Tears

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ABSTRACT

Background: To create a strip test to monitor blood sugar levels in diabetics using tears. For most diabetics, the current available methods for testing blood glucose are either invasive, inconvenient or both. A test such as a glucose tear strip would improve patient compliance and disease control.

Methods: 50 diabetic and 50 non-diabetic subjects, participated in this study. A sample of tears was collected from each subject's right eye using a glass microcapillary tube. Following tear collection, the blood glucose level of each subject was tested and recorded. The tear samples were then frozen and stored for lab analysis later on. At the end of sample collection, 100 samples of tears were tested for glucose concentration. The results were examined to determine the correlation of glucose in tears to that in blood.

Results: Based on this study conducted, the average TG concentration in non-diabetics was found to be 1.89 ± 0.54 mg/dL and in diabetics, 3.10 ± 0.55 mg/dL, whilst the PPBG concentration in non-diabetics and diabetics was found to be 118.60 ± 4.67mg/dL and 233.98 ± 16.21 mg/dL respectively.

Conclusion: There was a significant difference between the glucose concentration in tears and the glucose concentration in blood. There was a weak correlation between the two variables. The development of a non-invasive, convenient and patient-friendly method of monitoring glucose concentrations would give rise to improved patient compliance and disease control. Based on our current findings, the creation of a strip test to detect glucose concentration may not be possible but with advancements in technology, a strip sensitive to low concentrations of glucose present in tears can be developed.

INTRODUCTION

Diabetes is one of the most prevalent diseases existing, affecting as much as 1 in every 4 adults in some Caribbean countries. It affects the ability of the body's pancreas to produce insulin, a hormone vital for blood glucose regulation, or to effectively use the insulin that is produced. This results in elevated blood glucose levels. There are 3 main types of diabetes, however, in this study, only Type 2 diabetics were used, which is the most common type. Diabetes can be treated with regular insulin injections, glucose regulation tablets, exercise and proper dieting. Disease and complications of diabetes related to the eye include diabetic retinopathy, macular edema, cataract, exudates and hemorrhages. It is also associated with cardiovascular disease, stroke, neuropathy and nephropathy.

The two main cell populations responsible for tear production are plasma cells and acinar and duct cells of the lacrimal gland. Plasma cells migrate from lymphoid organs to the interstitial spaces within the lacrimal gland. The acinar and duct cells manufacture water and proteins specific to tears. The tear film produced over the ocular surface consists of 3 layers: inner mucous layer, middle aqueous layer and outer lipid layer. Glucose is a component of the aqueous layer. Overall, the rate of tear secretion can be decreased by factors such as: age, medication, ocular disease and systemic disease like diabetes in this case.

There are 3 types of tears produced: basal tears, reflex tears, and psycho-emotional tears. Their components vary according to type. However, for collection, basal tears were the target in this study since it is expected to give the truest glucose concentration and can be compared to that of blood plasma. Reflex tearing can be induced by exposure to any foreign bodies in contact with the ocular surface, such as the glass microcapillary tube used in this study.

At present, the methods available for blood-glucose testing are either invasive, inconvenient or both as it uses blood and urine. However, most recent findings have shown other body fluids such as tears to be a feasible alternative means of determining glucose levels. The advantages of developing a method of using tears will be non-invasive, convenient and patient-friendly especially for those who may be reluctant to blood or urine testing. In addition, this alternative method of glucose testing could improve patient compliance and disease control.

Based on other studies, there are contradicting opinions on whether or not the glucose concentration in tears can be an effective means on monitoring glucose concentrations.

OBJECTIVES

General:

To create a strip test which can be used to monitor blood sugar levels in diabetics using tears.

Specific:

- To investigate the efficiency of using human tears to indicate sugar levels in diabetics.
- To develop an alternative means to test sugar levels in diabetics by using strips sensitive to tears.
- To establish the correlation between tear glucose concentration and post prandial blood glucose concentration for both diabetics and non-diabetics.

MATERIALS AND METHODOLOGY

This study was divided into a four-step process: Phase 1 to Phase 4. Phase 1 was to develop a glucose detection strip, Phase 3 was to test the response of the strip to glucose concentration in tears and Phase 4 was to use the strip as a clinical test. However only Phase 2 was carried out in this study which was to determine the glucose content in tears for diabetics and non-diabetics. Tear samples were collected from 50 diabetics and 50 non-diabetics using a glass microcapillary tube. The tear samples were temporarily stored in an iced cooler then at the end of each day of sample collection, the samples were stored in a freezer at -20 ˚C. Immediately after tear collection, the finger prick method was used to obtain 10 µl of blood from each subject. The fixed volume of blood was measured using a micropipette and transferred onto the glucose test strip to obtain a blood glucose concentration reading from the Perfect 3 glucometer. After 100 tear samples were obtained, the correlation between blood [PPBG] and tear [TG] glucose concentration for diabetics and non-diabetics was obtained by use of an assay and spectrophotometer in the lab analysis conducted at the biochemistry research lab in Mt. Hope.

The data was analyzed using SPSS software. The means between PPBG and TG for diabetics and non-diabetics were compared using the Mann-Whitney U test for non-parametric data.

RESULTS

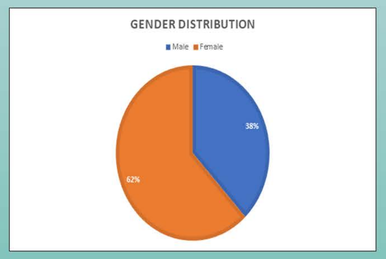

Figure 1.0 Pie Chart showing the Gender Distribution among participants, Males= 38, Females= 62

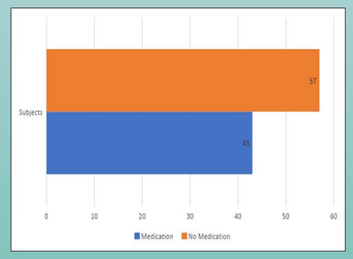

Figure 2.0 Bar Graph showing the number of participants who were on medication for Diabetes and who were not on medication for Diabetes.

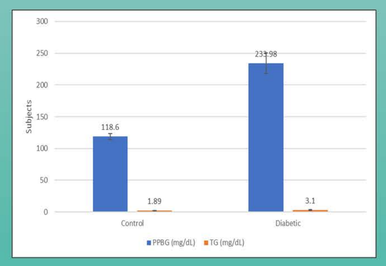

Figure 3.0 Column Graph comparing the mean concentration of TG to PPBG in diabetic subjects and the mean concentration of TG to PPBG in control subjects.

- Standard Error (SE):

Control: PPBG: ± 4.67 mg/dL
TG: ± 0.54 mg/dL
Diabetic: PPBG: ± 16.21mg/dL
TG: ± 0.55 mg/dL

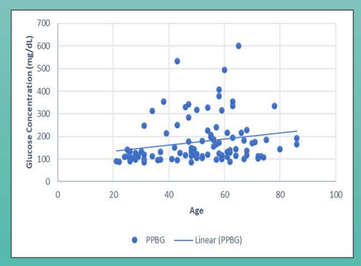

Figure 4.0 Scatter plot showing the correlation between Age and PPBG Concentration.
Kendall's tau-b correlation coefficient for PPBG = 0.194
Significant (p) value = 0.01

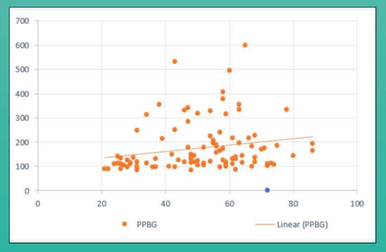

Figure 5.0 Scatter plot showing the correlation between Age and TG Concentration.
Kendall's tau-b correlation coefficient for TG = 0.182,
Significant (p) value = 0.01

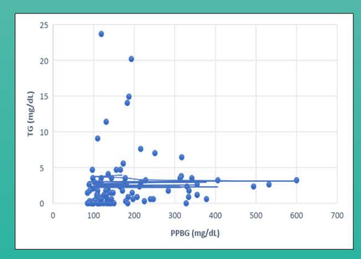

Figure 5.0 Scatter plot showing the correlation between PPBG Concentration and TG Concentration.
Spearman's rho correlation coefficient = 0.357
Significant (p) value = 0.01

DISCUSSION

The purpose of this study was to explore more convenient, non-invasive alternatives to glucose concentration monitoring. This four-phase study was developed to possibly create a strip test which can be used to monitor blood sugar levels in diabetics using tears; however only phase 2 was completed. All correlations which will be discussed are significant at $p<0.01$.

Figure 1.0 shows the gender distribution of the study which comprised of 38 males and 62 females. Figure 2.0 shows the number of subjects who were on medication for diabetes and those who were not. Figure 3.0 shows the mean glucose concentrations in tears and in blood compared between diabetics and non-diabetics. The average TG concentration in non-diabetics was found to be 1.89 ± 0.54 mg/dL and in diabetics, 3.10 ± 0.55 mg/dL, whilst the PPBG concentration in non-diabetics and diabetics was found to be 118.60 ± 4.67mg/dL and 233.98 ± 16.21 mg/dL respectively. The data had to be analyzed using the non-parametric, Mann-Whitney U test, since the data was not normally distributed.

Using Kendall's tau-b Bivariate Correlation test, the correlation coefficient between age and TG and age and PPBG were calculated to be +0.182 and +0.194 respectively. The correlations between age and TG, and age and PPBG were therefore found to be weak and is shown as a linear relationship in Figure 4.0.

Using the Spearman's rho Bivariate Correlation test, the correlation between PPBG and TG was calculated to be +0.357. which is of most value to this study and proves the existence of a moderate linear relationship between the variables. This linear relationship is shown in Figure 5.0.

Like other findings exploring the use of tears as a reliable alternative to monitoring glucose levels; the main challenge of this study is establishing a strong enough correlation between blood and tear glucose concentration and then creating a safe and easy test, sensitive enough to detect and give accurate glucose concentrations in tears.

Limitations: Some of the main limitations in the study include: Inability to eliminate reflex tearing, failure to locate a blank detection strip, the use of Random glucose concentration readings instead of fasting glucose concentrations and the presence of anti-diabetic medication in a diabetic's system would have reduced the actual glucose concentration in blood and hence tears.*Recommendations:* Ensure that the internal diameter of the collection tube is as small as possible to allow for greater capillary action. Multiple trials, at least 3, should be conducted for each sample so that an average tear glucose concentration can be obtained. Sampling should be done in fasting state before any medication is taken, for all subjects, including non-diabetics.

CONCLUSION

In conclusion, although there is a significant difference at $p<0.01$, between PPBG and TG concentration in diabetics compared to non-diabetics, the correlation between the two variables was found to be of moderate strength with a correlation coefficient of +0.357. The average TG concentration in non-diabetics was found to be 1.89 ± 0.54 mg/dL and in diabetics, 3.10 ± 0.55 mg/dL, whilst the PPBG concentration in non-diabetics and diabetics was found to be 118.60 ± 4.67mg/dL and 233.98 ± 16.21 mg/dL respectively. Based on our findings at this point in time, the creation of a strip test to detect glucose concentration may not be possible but with hope of future advancements in technology, a glucose tear strip sensitive to low concentrations of glucose present in tears can be developed.

REFERENCES

1. Sen, D. and Sarin, G. (2017). Tear glucose levels in normal people and in diabetic patients. [online]

2. Zhang, J., Hodge, W., Hutnick, C. and Wang, X. (2017). Non-invasive Diagnostic Devices for Diabetes through Measuring Tear Glucose. [online]

3. Ascaso F, Huerva V. Noninvasive Continuous Monitoring of Tear Glucose Using Glucose-Sensing Contact Lenses [Internet]. 2017 [cited 5 October 2017].