Retinal Vascular Geometry: Examination of the Changes between the Early Stages of Diabetes and First Year of Diabetic Retinopathy

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***Abstract*—Controversy exists on how the functional impairment triggered by diabetes affects the retinal vascular structure, leading to diabetic retinopathy (DR). DR remains a main cause of blindness in United Kingdom with thousands of new cases per year. Diagnosing DR early is crucial for planning the treatment more effectively, before the retina of the eye is severely affected. In this study we investigated the hypothesis that significant vascular changes occur during the progression of diabetes until the first lesions appear in the retina (background retinopathy). The analysis is approached from a novel perspective in which the selected images for pre-/post-DR come from the same patients who have progressed from diabetes to DR. The measurements were analyzed using a statistical model. Veins' and arteries' widths and also arteries' angles were found to differ significantly between the two cases, but no statistical significance was observed for the veins' angles.**

Keywords—Retina; Diabetic retinopathy; Diabetes; Progress; Vasculature

I. Introduction

Retina is a reliable and direct way of non-invasively monitoring the blood vessels in the human body. It has been vastly studied for association with a variety of diseases, like hypertension [1],[2], cardiovascular diseases [3],[4], and especially diabetes [5],[6],[7],[8]. This phenomenon has led many researchers to work on both how to improve the tools for taking retinal measurements and how to use these tools for studying retinal and/or other diseases.

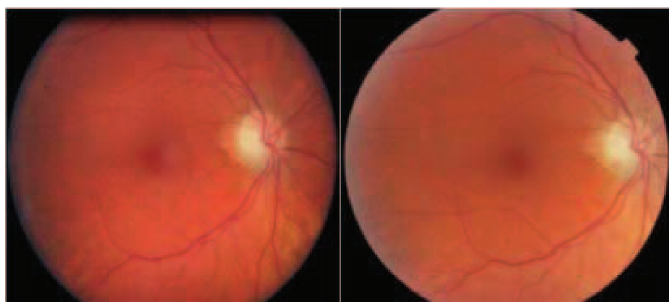

Fig. 1. Two images of the same patient. First year of diabetic retinopathy (left) and early stages of diabetes (right). Micro-aneurysms have already appeared, defining the beginning of diabetic retinopathy

Research supported by a Marie Skłodowska-Curie grant from the EC in the framework of the REVAMMAD ITN. Project number 316990

Diabetic retinopathy (DR) is a major disease affecting the retina of the eye. It constitutes a complication of the systemic disease of diabetes and affects the lives and well-being of thousands of people in United Kingdom (UK), with numerous new cases per year. Every patient in UK diagnosed with diabetes, enters automatically to a public screening program in order to monitor his/her retina at least once per year.

The underlying mechanisms that provoke diabetes are more or less known, but the exact way that diabetes affects the haemodynamic functionality and the retina vessel wall structure and how this leads to DR is still under investigation [9],[10]. The impaired physiological functionality includes changes in the retinal blood flow rate, blood flow velocity, oxygen diffusion, intraocular pressure and so on.

All these parameters are believed to start changing much before the first stage of DR, affecting the vascular geometry before the first lesions appear. To investigate how these hemodynamic parameters affect the vasculature, forty high resolution images (3216-by-2316 pixels) from twenty patients were selected, who have progressed from diabetes to DR. For each patient one image from the early stages of diabetes (6-8 years before DR) and one image from the year that DR appeared in the retina (background retinopathy) were included. From every image eight junctions were selected, making sure that can reliably be measured at the same level in both images. Moreover, these junctions, four for veins and four for arteries, were exactly at the same position in the two images of the same patient. In total 960 widths were measured, 480 for veins and 480 for arteries, for early diabetes and first year of DR. Similarly, 320 angles were measured from the same junctions. The labelling and marking of each junction were fulfilled by using a semi-automatic tool, which is described in details from Al-Diri et al. [11].

II. Methods

A. Background

In literature, many methods have been proposed for extracting measurements from the retinal vasculature. Xu et al. [12] implemented a technique based on a graph-theoretic algorithm, which includes initial vessel segmentation for building the graph. Lupascu et al. [13] presented a bagged decision trees approach using an extended hermite model

where they estimate the local width from the parameters of the best-fit surface. Gang et al. [14] used an amplitude modified second-order Gaussian filter and they proved that the vessel width can be measured in linear relationship with the spreading factor of the matched filter after adjusting the magnitude coefficient of the filter accordingly.

The main problem with the automatic algorithms still remains the fact that they are implemented based on specific databases and/or specific features. Their accuracy is measured in accordance with these datasets and they need to be parametrized constantly for new images that have different inherent characteristics of resolution, reflections, distortions, blurriness etc. For this reason, for these types of sensitive studies, where we need to minimize the errors that we insert in our measurements, the semi-automatic tools are still a more robust option, although being time-consuming.

B. *Marking tool*

For all the measurements, both for widths and angles, a semi-automatic tool was used, creating a Graphical User Interface (GUI) that gives the user the ability to label specific junctions inside the retina and then mark them manually. In this way we actually measure the blood column width by defining its boundaries within an adjustable rectangular. Regarding the angles, we define them by drawing the vectors, creating the correct angle that corresponds to the direction of the vessels when they start branching out of the junction (Fig. 2). This method is preferred over the fully automatic algorithms for two reasons: a) the fully automatic tools are less accurate, adjusted to specific datasets and more prone to errors, since the images are almost never of the same quality and they include different levels of noise, center-line reflections and so on. That means that the accuracy is not consistent either within the same patient or within the whole dataset, leading us to inserting many unpredictable errors. B) With the semi-automatic tools someone can repeat the measurements as many times as he/she wants, improving the accuracy, and therefore ensure that the final measurements are as consistent as possible. By taking multiple measurements either within the same rater (intra-rater) or using multiple raters (inter-rater), someone can statistically measure the intra- and inter- rater variability, benchmark the measurements and thus help improve the absolute agreement.

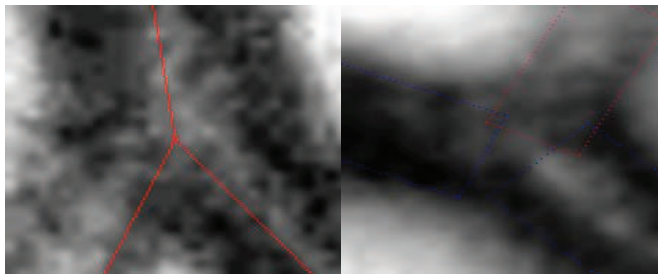

Fig. 2. Example of the measurement technique both for angles (left) and widths (right), using the semi-automatic tool

III. DESIGN

A. *Data structure*

To the best of our knowledge, most of the similar studies in literature, follow the approach of using different datasets, with different subjects per group, e.g. pre-DR, post-DR, Proliferative DR, in order to compare and evaluate the significance of the differences between normal subjects (no diabetes/no other disease that is known to influence the vessel structure), diabetic subjects (diagnosed with diabetes but still not progressed to DR) and DR subjects.

Since the retinal vasculature includes different sizes of vessels, which are smaller the further they are from the optic nerve head, it has to be predefined which vessels (near optic disk, periphery) or which combination of them to include and be consistent within-groups and between-groups. Moreover, since duration of diabetes has been found to have an influence on the progress to DR, then the within-diabetic group subjects have to be matched according to the duration of diabetes, otherwise the measurements will be inaccurate with multiple levels of errors [15]. The same problem is enhanced if we take into account that differences exist between different ages (younger vessels are more elastic than older ones), between men and women as well as from other possibly unknown diseases.

All of the aforementioned enhance the statistical error since the observed values are more likely to differ from the expected ones, increasing the standard error of the means. Having all these parameters influencing the reliability of the observed values, someone would ideally need thousands of measurements to compensate for these errors and finally converge to the expected (actual) values. By not being consistent with the chosen sample, the residual error is enhanced because the sample mean will likely include inconsistent measurements. However, we can usually standardize the statistical error in a z-score and the residual in t-statistic. The stochastic error plays its role as well since it denotes the error that is random from one measurement to the next one. However, going into this topic in depth is out of the scope of this manuscript.

On the contrary, our approach aims to overcome these limitations by analyzing images from the same progressed patients. The novel proposed approach of analyzing the data, tries to minimize the aforementioned errors by including the same conditions within the groups of comparison (pre-/post-DR). The independent group includes the two possible cases of Retinopathy/Non-retinopathy, which is the case under investigation. For each case, twenty subjects are included as a subgroup, which are the same for both cases. Under each subject we find the individual measurements (Fig. 3).

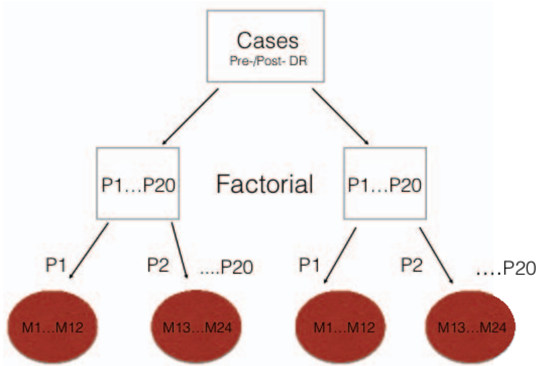

Fig. 3. Diagram showing the design and the structure of the data. The cases refer to the two possible conditions of early stage of diabetes and first year of DR, the P1-P20 are the patients and M1, M2…., the corresponding measurements of widths. For the Angles the measurements are from M1-M4 for every patient instead

B. Analysis

The design in Fig. 3 was used for the statistical analysis using an Analysis of Variance (ANOVA) method [16]. The ANOVA test is known to be robust and assumes that all sample populations are normally distributed with equal variance and all observations (samples) are mutually independent. To verify that our data conform to these requirements two tests of normality were ran, one based on the Anderson-Darling goodness-of-fit and one on the Shapiro-Wilk test. Both failed to reject the null hypothesis that the data are normally distributed (p-values=0.34 and 0.41 respectively) [17].

For building the ANOVA model, the Mathworks Matlab 2014b suite was employed. All the variables were defined according to the described structure and the statistical analysis was performed for the arteries and the veins both for widths and angles. IBM SPSS 21 suite was used in order to define the intra-rater variability and the effect size was also calculated using the eta square-η^2 according to the formula:

$$\eta^2 = \frac{Sum\ of\ Squares_{effect}}{Sum\ of\ Squares_{total\ (within-subjects)}} \quad (1)$$

Where, Sum of $Squares_{effect}$ refers to the sum of squares for whatever effect is of interest, here the pre-/post- DR, whereas the Sum of $Squares_{total}$ is the total sum of squares for all effects, interactions and errors in the ANOVA.

IV. RESULTS

The purpose of our study was to evaluate whether there are any differences in the vessels geometry between the early stages of diabetes and the first year of DR. As aforesaid, both width and angles measurements were included in preselected matched junctions. According to previous studies, junctions are affected during the progression of diabetes, probably due to impaired blood flow and also the changes in the vascular wall structure, which make the wall less capable of autoregulating [9],[10],[18]. Intra-rater variability was calculated in order to-

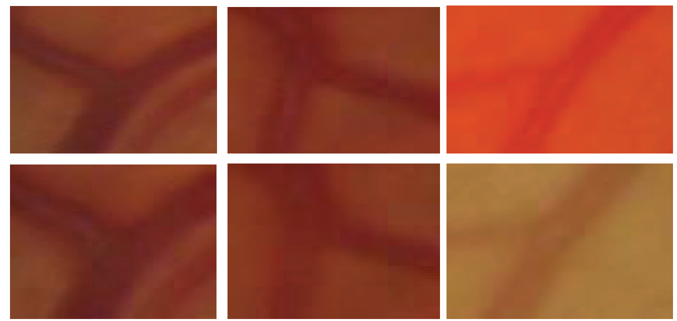

Fig. 4. Vessel junctions before (down) and after retinopathy (up), which show the effect of diabetes in the angles and widths during the progression of the disease

evaluate the performance of the rater by repeating the measurements twice. In literature an agreement of more than 80% is considered adequate.

As can be seen in table 1, the variability between the repeated measurements was over 80% in all the cases, thus we kept both measurements for the analysis, by taking their average. In Fig.4 we can see three examples of segments from the analysis in which the differences in widths and angles are visually apparent.

A. Veins

1) Widths

For each of the two groups, 240 measurements from 80 matched junctions of twenty patients were taken. From every junction three widths were measured, one of the parent and two of the children vessels. The groups' mean values were 11.97(±4.03) and 13.01(±4.21) pixels, for first year of DR and early stages of diabetes respectively. ANOVA analysis was conducted based on the proposed model structure, in order to calculate the F-statistics and test the null hypothesis that there-

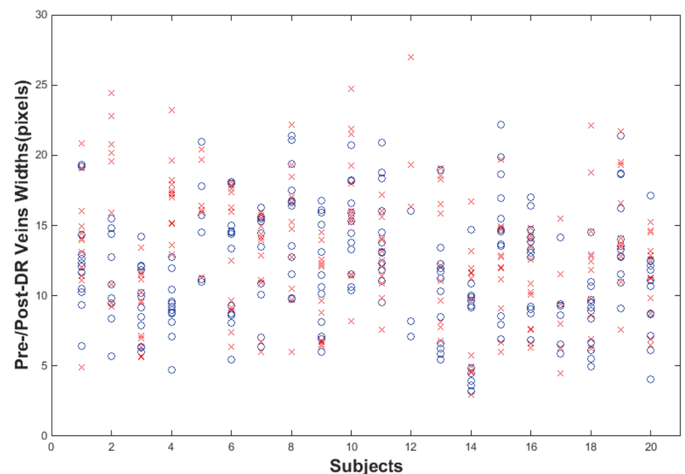

Fig. 5. Measurements for the veins' widths (y axis in pixels) per subject (x axis). (Blue color for the first year of DR and red for the early stages of diabetes)

is no relationship between the two measured cases (early diabetes/first year of DR) and any difference can be attributed to random observations.

Based on our analysis, we reject the null hypothesis since the veins in pre-/post- DR appear to be affected significantly (F(1,448)=6.23, p-value=0.01). This result can be explained as a consequence of the progression of diabetes, which makes the vessels decrease their size based on the local metabolic needs and their autoregulation. In Fig. 5 we can view directly the differences of the measurements between the pre-/post- DR for every patient.

2) Angles

For the angles the same procedure as for the widths was followed using the same 80 junctions. For each junction we measured the angle that is created from the two children vessels (Fig. 2). For the two groups the mean values were 81.23^o (±10.52) and 81.40^o (±11.42) respectively. In contrast with the widths, no statistical difference was observed between the pre-/post- DR, so we adopted the null hypothesis that any observations result entirely by chance (F(1,136)<0.000, p-value=0.96). In Fig. 6 we can view the changes between the two groups for each of the twenty patients.

B. Arteries

1) Widths

Regarding the arteries, the exact same procedure as for the veins was followed. The groups' mean values for first year of DR and early stages of diabetes were 10.01(±3.24) and 10.74(±3.74) pixels respectively. Similarly with veins, the null hypothesis was rejected, since the impaired functionality due to diabetes, affects the arterial network as well (F(1,448)=7.71, p-value=0.006). A relevant illustration of the changes can be seen in Fig. 7.

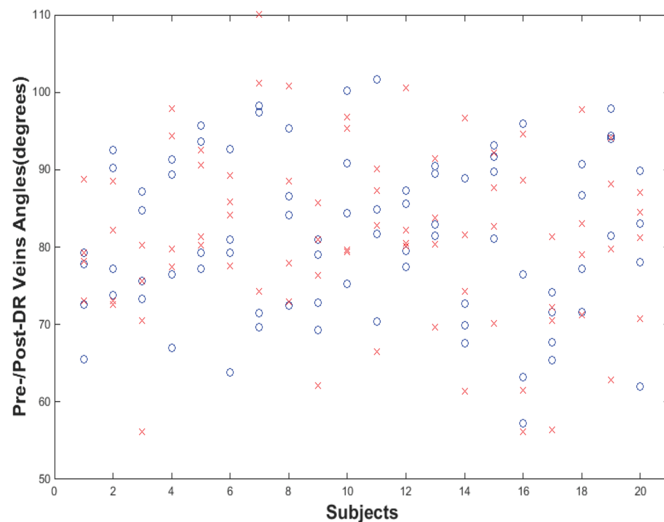

Fig. 6. Distribution of the measurements for the veins' angles (y axis in degrees) per subject (x axis). (Blue color for the first year of DR and red for the early stages of diabetes)

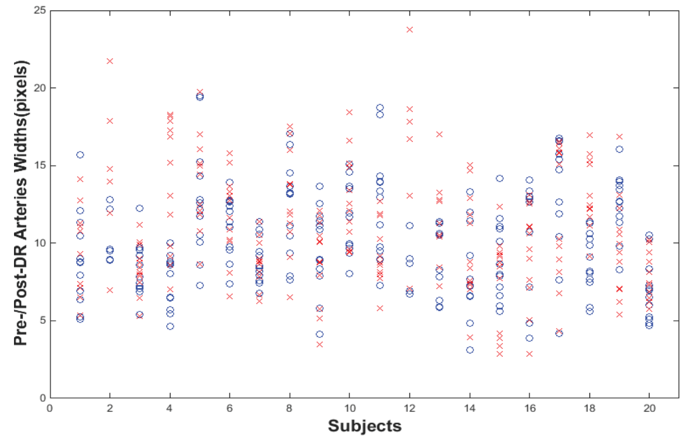

Fig. 7. Measurements for the arteries' widths (y axis in pixels) per subject (x axis). (Blue color for the first year of DR and red for the early stages of diabetes)

2) Angles

In contrast to the veins' angles, the arteries' angles are affected from the disease, making them adjust to the new underlying conditions (F(1,136)=4.21, p-value=0.04). The groups' mean values for first year of DR and early stage of diabetes were 86.11^o (±12.11) and 89.31^o (±13.31) respectively. During the progression of diabetes, changes in the blood flow velocity and/or blood pressure make the vessels adjust dynamically. This can include constricting or dilating and/or changing their angles in order to regulate the conditions locally, based on the local oxygen or nutrient needs. These two parameters seem to be affected during the progression of diabetes [19], [20]. Since arteries carry the oxygenated blood with increased pressure, any structural changes can alter the way they autoregulate, making them more vulnerable.

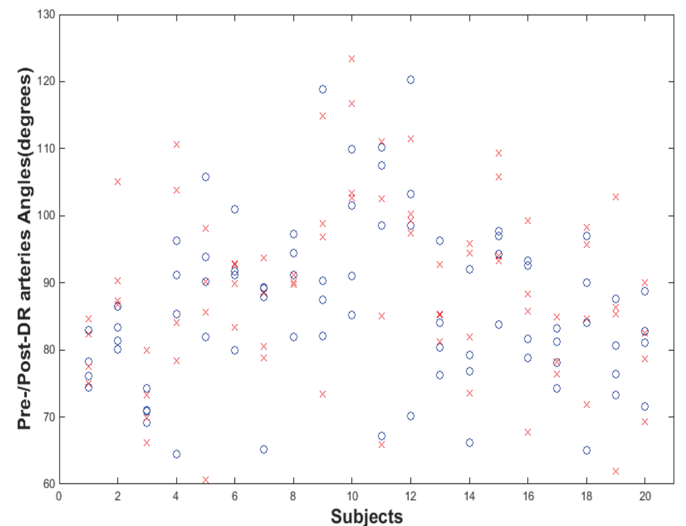

Fig. 8. Corresponding measurements for the arteries' angles (y axis in degrees) per subject (x axis). (Blue color for the first year of DR and red for the early stages of diabetes)

TABLE I. SUMMARY OF ALL THE MEASUREMENTS

Early diabetes/1st year of DR	P-value (a=0.05)	F-value	Sum of squares	Intra-rater variabilty (ICC)	Eta-square η^2
Veins' angles (81.40°/81.23°)	0.96	<0.00	1.2	89%	<0.00
Arteries' angles (89.31°/86.11°)	0.04	4.21	259.3	91.3%	0.012
Veins' widths (13.01/11.97) (pixels)	0.01	6.23	127.7	88.2%	0.018
Arteries' widths (10.74/10.01) (pixels)	0.006	7.71	57.7	93.6%	0.01

V. CONCLUSION

Diabetes is a major disease, with millions of people facing its consequences, one of which remains diabetic retinopathy. Studying the changes in the vasculature during the progression of diabetes and measuring them is a difficult task. Robust tools are needed for long-term studies and also a properly designed model that will take into account the normal changes of the vasculature and discriminate over the pathologies. The alterations are so subtle that sometimes is very hard to measure and identify them. Hence novel methods of obtaining and analyzing data are crucial for evaluating the progression and create reliable prediction models with robust biomarkers.

In this paper a study of the changes in the retinal vasculature between the early stages of diabetes and the first year of DR was presented. The importance of organizing and analyzing these types of data efficiently based on statistical models was also exploited. Finally it can be concluded that the vascular geometry of the retina could be used as an indicator of the progression to diabetes, subject to the proper analysis of the data. Logical next step to our research study will be to improve the statistical model, based on the nature of the data and our novel approach, and also use other type of features, e.g. the length from junction to junction, ratios of the geometric features or even global features, like fractal dimension.

ACKNOWLEDGMENT

Special thanks to Greg Russell and Evi kotsiliti from the Health Intelligence company in UK for their support in providing images for analysis as well as Phil Assheton for his advice on improving the analysis.

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