Gene Expression in Proliferative Diabetic Retinopathy Using RNA-Seq Data: A Computational Study on Saudi Patients

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Abstract
Proliferative diabetic retinopathy is the widespread type of DM which causes chronic as well as progressive alterations at microvascular level, which particularly affects the retina of eye as well as at the posterior region of eye segments. For our computational analysis 155 differentially expressed genes calculated through paired t test statistics analysis using the GenePattern platform, of proliferative diabetic retinopathy in Saudi patients were downloaded. Among the 155 genes, 95 were upregulated, and 60 were downregulated. The Annotation Cluster (FAC) tool in the (DAVID) (http://david.abcc.ncifcrf.gov/home.jsp) was used to identify biological processes that are abundant in proliferative diabetic retinopathy (PDR). The functions required for response to mRNA splicing, intracellular protein transport, mRNA processing, microtubule cytoskeleton structure, and atrioventricular canal formation are represented by the GO keywords that are abundant in genes. We used the KAAS web server to identify the biological pathways of these DEGs in addition to DAVID functional analysis and found that the majority of the DEGs were associated with important biological processes, with many being classified in metabolic pathways, Spliceosome, Cell cycle, or being involved in the mRNA surveillance pathway. findings are consistent with those of earlier research. To corroborate the predictions stated in this work, which will demonstrate the role enhanced functional processes, experimental validation will be necessary.

Key Words: Computational Analysis, Diabetes Mellitus, Gene Expression, Proliferative Diabetic Retinopathy.

Introduction
Diabetes mellitus is one of the multifactorial diseases and a leading cause of death in world and especially in Saudi Arabia. Proliferative diabetic retinopathy is the widespread type of DM which causes chronic as well as progressive alterations at microvascular level, which particularly affects the eye, along with other body parts. If the disease is left untreated it will grow gradually and ultimately leading to the blindness. Progression of disease is not rapid, but gradual starting from mild alterations, moving towards moderate and ultimately severe proliferative diabetic retinopathy. The main characteristic of this disease is the development of few new blood vessels around the retina of eye as well as at the posterior region of eye segments i.e., vitreous (El-Bab et al. 2012; Lee et al. 2015; Alharbi and Alhazmi 2020).

The mechanism by which the DM progresses to diabetic retinopathy is not clearly understood and that’s why the disease pathology is thought to be complex and unclear. However, a lot of studies has been carried out to examine the disease progression by considering the disease history along with other aspects. It has been suggested that multiple interactive mechanisms are playing an important role, causing the damage at cellular level and adaptive changes, which cause the devastation in this disease(El-Asrar et al. 1998; Sinclair and Schwartz 2019; Alharbi and Alhazmi 2020).

Earlier it was considered that DM and especially PDR is not a prevalent disease at Saudi Arabia, due to healthy diet and routine. However, recent studies have reported that prevalence of disease is increasing in Saudi Arabia as bell and the possible risk factors for this progression are supposed.
to be consumption of more westernized diet leading to increased chances of obesity and ultimately complications of diabetes. Earlier the disease was 23.7% prevalent in Saudi Arabia while by the year (2011), it has reached to increase 30% and increasing day by day with men more affected that females (Ali et al. 2008; Al Dawish et al. 2016; Alharbi and Alhazmi 2020).

Different treatment strategies can be used to treat diabetic retinopathy. Photocoagulation is one of them. Studies has shown that photocoagulation approach causes a decrease in chances of loss of vision by up to 50% (Cantrill 1984). It causes the decrease in visual acuity as well as constricts the posterior visual regions. Intravitreal administration of about 1.25 mg bevacizumab at the time of cataract surgery could be safe as well as protective in preventing the progression of DR and diabetic maculopathy in patients with cataract and diabetic retinopathy (Cheema et al. 2009; Alghamdi et al. 2021).

MATERIAL AND METHODS

155 differentially expressed genes calculated through paired t test statistics analysis using the GenePattern platform, and identified based on the statistical cutoff of proliferative diabetic retinopathy in Saudi patients with type 2 diabetes were downloaded (Pan et al. 2016). Among the 155 genes, 95 were upregulated, and 60 were downregulated, and has been taken for computational analysis shown in Table 1. For the functional analysis, on the list of differentially expressed genes with a fold change of >1, DAVID (http://david.abcc.ncifcrf.gov/home.jsp) functional annotation cluster analysis was done. For analysis, only terms with a value of 0.05 and a count number of 5 genes were chosen. DAVID was used to classify enriched biological themes in the collection of DEGGs using the gene ontology (GO) term biological process (BP). The KEGG Automatic Annotation Server (KAAS) (http://www.genome.jp/kegg/kaas/) was used to map pathways (Moriya et al. 2007). The amino acid sequences of these DEGGs were submitted to the KAAS online site as input, and orthologs were assigned using the single-directional best hit (SBH) technique. KAAS uses BLAST similarity searches against a carefully selected set of ortholog groups in the KEGG GENES database to provide functional annotation of genes in a genome. Genes in the data sets that were mapped to one of KEGG’s reference pathways were given a KEGG orthology (KO) number by KAAS (Amoaku et al. 2020).

RESULTS AND DISCUSSION

We downloaded the precomputed list of 155 differentially expressed genes for our computational analysis shown in Table 1. Among the 155 genes, 95 were upregulated, and 60 were downregulated (Pan et al. 2016; Amoaku et al. 2020).
For the functional annotation analysis, the Annotation Cluster (FAC) tool in the Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used to identify biological processes that are enriched in proliferative diabetic retinopathy (PDR) ([http://david.abcc.ncifcrf.gov/home.jsp](http://david.abcc.ncifcrf.gov/home.jsp)). For annotations and GO terms with statistically significant values from the resultant functional analysis, the name "Biological Process" was utilized. The functions required for response to mRNA splicing, intracellular protein transport, mRNA processing, microtubule cytoskeleton structure, and atrioventricular canal formation are represented by the GO keywords that are abundant in genes in this table (Table 2).

Recent studies have reported prevalence of Proliferative diabetic retinopathy (PDR) disease is increasing. Proliferative diabetic retinopathy is the widespread type of DM which causes chronic as well as progressive alterations at microvascular level, which particularly effects the eye. The main characteristic of this disease is the abnormal growth of new vessels occurs (Tarr et al. 2013; Safi et al. 2014). Study shows Involvement of angiogenesis, inflammation, and fibrosis in proliferative diabetic retinopathy and Enrichment of genes and pathways related to lymphatic development indicates that targeting lymphatic involvement in PDR progression. Several pro-angiogenic cytokines have been described as being involved in the pathogenesis of PDR, although VEGF is accepted as the most significant cytokine in PDR (Amoaku et al. 2020). The present finding shows significance of mRNA splicing, intracellular protein transport, mRNA processing, microtubule cytoskeleton organization and atrioventricular canal development, and associated with important biological processes, many being classified in metabolic pathways, Spliceosome, Cell cycle or being involved in mRNA surveillance pathway These are consistent with those of other studies (Korhonen et al. 2021).

### Table 2. Significantly enriched gene ontology (GO) terms detected by FAC in differentially expressed genes. Only those terms which reported a value of ≤0.05 and count number ≥2 genes were selected for the analysis.

<table>
<thead>
<tr>
<th>Term</th>
<th>Count</th>
<th>P-Value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0002564</td>
<td>mRNA splicing, via spliceosome</td>
<td>7</td>
<td>0.00381823</td>
</tr>
<tr>
<td>GO:0006688</td>
<td>mRNA splicing</td>
<td>5</td>
<td>0.00451622</td>
</tr>
<tr>
<td>GO:0042182</td>
<td>Regulation of mitotic cell cycle</td>
<td>4</td>
<td>0.0159074</td>
</tr>
<tr>
<td>GO:0006514</td>
<td>Splicing</td>
<td>5</td>
<td>0.01633239</td>
</tr>
<tr>
<td>GO:0043735</td>
<td>Positive regulation of interleukin-3 production</td>
<td>2</td>
<td>0.04708976</td>
</tr>
<tr>
<td>GO:0010467</td>
<td>Gene expression</td>
<td>3</td>
<td>0.04140273</td>
</tr>
<tr>
<td>GO:0043128</td>
<td>Activating intracellular signaling by G-protein coupled receptor</td>
<td>2</td>
<td>0.04718423</td>
</tr>
<tr>
<td>GO:0070078</td>
<td>Intracellular transport, pathway of</td>
<td>2</td>
<td>0.05431318</td>
</tr>
<tr>
<td>GO:0009843</td>
<td>Negative regulation of IL-1beta-induced inflammation</td>
<td>3</td>
<td>0.05494312</td>
</tr>
<tr>
<td>GO:0044453</td>
<td>Regulation of protein localization to pre-autophagosomal structure</td>
<td>2</td>
<td>0.07405295</td>
</tr>
<tr>
<td>GO:0002564</td>
<td>Intracellular protein transport</td>
<td>5</td>
<td>0.07117601</td>
</tr>
<tr>
<td>GO:0002267</td>
<td>Regulation of microtubule cytoskeleton organization</td>
<td>3</td>
<td>0.08226181</td>
</tr>
<tr>
<td>GO:0043254</td>
<td>Regulation of protein complex assembly</td>
<td>2</td>
<td>0.09582973</td>
</tr>
<tr>
<td>GO:0000656</td>
<td>Alternative mRNA splicing, via spliceosome</td>
<td>2</td>
<td>0.09582973</td>
</tr>
<tr>
<td>GO:0006514</td>
<td>Splicing</td>
<td>5</td>
<td>0.09498518</td>
</tr>
<tr>
<td>GO:0006888</td>
<td>Cell cycle</td>
<td>2</td>
<td>0.09636227</td>
</tr>
</tbody>
</table>

CONCLUSION

The findings of the present study have used a Bioinformatics approach to identify the DEGs enrichment indicate the...
significance of mRNA splicing, intracellular protein transport, mRNA processing, microtubule cytoskeleton organization and atrioventricular canal development, and associated with important biological processes, many being classified in metabolic pathways, Spliceosome, Cell cycle or being involved in mRNA surveillance pathway. The present study’s findings are consistent with those of earlier research. To corroborate the predictions stated in this work, which will demonstrate the role enhanced functional processes, experimental validation will be necessary.

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