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**Role of orbital magnetic resonance imaging in early detection of Graves’ Orbitopathy and disease activity**

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**Abstract**--- Background and aim: we aim to evaluate feasibility of using orbital diffusion tensor imaging (DTI) magnetic resonance imaging (MRI) of extraocular muscles (EOMs) in patients with Graves’ orbitopathy (GO) versus clinical activity score (CAS) in early detection of GO activity. Methods and materials: This case control study was conducted on 20 consecutive patients (10 males, 10 females with a mean age of 35.50±13.65 years) with Graves’ orbitopathy and 10 age- and sex–matched volunteers without any thyroid abnormality as a health control (HC). The patients and HCs underwent DTI MRI of the orbit in the coronal plane. The mean diffusivity (MD) was calculated. Thyroid stimulating hormone receptor antibody (TRAb) was measured using third generation thyroid binding inhibiting immunoglobulin (TBII) with ELISA method. CAS score, according to the standardized criteria of the European Group on Graves Orbitopathy (EUGOGO),
was calculated for the patients. Results: GO patients’ EOMs showed significantly higher MD values and higher compared to HCs’ (P < 0.001). Median CAS score was 4. A statistically significant positive correlation was only detected between CAS score and inferior rectus (IR) MD (r=.360, p=.023). A statistically significant positive correlation was detected between TRAb level and MD of each of extra-ocular muscles (Superior & inferior & medial and lateral rectus) ((r=.424, p=.006) & (r=.427, p=.006) & (r=.588, p=.001) and (r=.605, p=.001) respectively). Conclusion: DTI MRI reflected microstructural changes in GO, and was found to be superior to CAS score in early detection of activity. However, use of DTI MRI in combination with CAS score could be more beneficial.

Keywords---orbital magnetic resonance, early detection of Graves’ Orbitopathy, disease activity.

Introduction

Autoimmune thyroid diseases (AITD) are the most frequent autoimmune disorders (Antonelli et al., 2015). Autoimmune thyroiditis (AT) and Graves’ disease (GD) were reported to be the main clinical presentations included (Cui et al., 2019). Presence of anti-TSH receptor antibodies (TSHRAb), resulting in an hyperthyroidism, thyroid eye disease (Graves’ orbitopathy, GO) and – very rarely – localized dermopathy (pretibial myxedema, PTM), characterizes GD (Smith and Hegedus, 2016).

TSHRAb act as agonist, stimulate thyroid gland to over secrete excessive thyroid hormone and uncouple the thyroid from pituitary control (Davies et al., 2020). They also induce thyroid gland growth via similar, but not identical, signal transduction. In the orbit, TSHRAb stimulation of fibroblasts expressing TSH receptor (TSHR) stimulates hyaluronan production, facilitated by cross-talk between TSHR and insulin-like growth factor 1 receptors. This is associated with retro-orbital inflammation, extraocular muscle fiber disruption and tissue edema (Krieger et al., 2020).

The prevalence of GD in the general population was in the order of 1% to 1.5% (Perros et al., 2017) Nearly 3% of females and 0.5% of males developed Graves’ disease throughout their lifetime (Nyström et al., 2013). Approximately 25–50 % of GD patients showed clinical manifestations of GO (Bartalena et al., 2020). Active inflammatory stage followed by a static stage are two stages in the natural course of GO (Hiromatsu et al., 2014). Therefore, duration of GO, its clinical activity and severity determine treatment decisions (Bartalena et al., 2021). The first-modality of therapy for active and moderate-severe cases recommended, is intravenous glucocorticoid (GC) (Bartalena et al., 2016). However, Its effect is significantly low after 18 months of disease duration (Terwee et al., 2005).

Clinical activity score (CAS) was established for accurate evaluation of GO activity (Menconi et al., 2017) and was correlated with the treatment response,
and if CAS > 3, immunosuppressive therapy is recommended (Mourits et al., 1989). The CAS was also based on the European Group on Graves’ Orbitopathy (EUGOGO) consensus (Negro et al., 2016). However, The significant limits of CAS include: inappropriate evaluation of the various levels of inflammation activity, so the score does not change with any improvement or exacerbation until it completely resolves, and low sensitivity and subjectivity owing to the operator dependence (Tachibana et al., 2010).

Thus, there is growing interest to find appropriate quantitative markers or more reliable imaging features to further validate CAS. Orbit Magnetic resonance imaging (MRI) was widely applied for diagnosing thyroid associated orbitopathy (TAO) and further staging TAO (Siakallis et al., 2018). Main parameters evaluated in previous imaging studies of GO, were expanded extraocular muscles and orbital fat tissues, which were thought to be significant contributors to proptosis in GO (Gontarz-Nowak et al., 2021).

Diffusion tensor imaging (DTI), which provides quantitative information about the microstructural integrities of highly oriented tissues, was applied for assessing various orbital diseases, such as glaucoma, optic neuritis, and others (Min et al., 2018). However, studies that applied DTI to assess microstructural changes in TAO patients were limited (Lee et al., 2018).

Its main parameters are fractional anisotropy (FA) and mean diffusivity. The FA determines the directionality of a diffusion signal, whereas mean diffusivity shows the magnitude of a diffusion signal (Sundgren et al., 2004). Diffusivity measures the magnitude of diffusion of water molecules within the tissue, which is dominated by interstitial space (Sotak, 2004). Diffusivity could increase in edematous muscle and decrease in fibrotic and fatty infiltrated tissue (Kilicarslan et al., 2015).

**Methods and Materials**

The Institutional Ethics Committee approved the protocol of the study and informed consent was given by all patients. This Case control study included 20 patients with moderate to severe active Graves' orbitopathy and 10 health control (HC) subjects without any thyroid abnormality. Both patients and HCs were recruited from outpatient endocrinology clinic in specialized medical hospital, Mansoura University over a period of 20 months from November 2019 to August 2021.

The inclusion criteria were patients with moderate to severe, active Graves’ orbitopathy. The control group consisted of age- and sex-matched healthy volunteers with negative results of any thyroid abnormality. The exclusion criteria were presence of other ophthalmological disease such as glaucoma & History of any chronic illness such as diabetes and hypertension & History of previous exposure to radiation and radioactive iodine & History of previous eye surgery & History of prior immune therapy such as corticosteroid in the previous 6 months.

Thyroid stimulating hormone receptor antibody (TRAb) was measured, for both patients and HCs, using third generation thyroid binding inhibiting
immunoglobulin (TBII) with ELISA method supplied by Bioassay technology china.

**Principle**

This kit is sandwich Enzyme-Linked Immunosorbent Assay. The plate has been pre-coated with Human TRAb antibody. TRAb present in the sample is added and binds to antibodies coated on the wells. And then second Human TRAb Antibody is added and binds to TRAb in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated TRAb antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TRAb. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

**Magnetic resonance imaging & clinical activity score**

The magnetic resonance imaging of the orbit was done for patients and HCs and the clinical activity score (CAS) was obtained for patients. The CAS score was calculated by one endocrinologist with 10 years' experience. A score of three or more on CAS is classified as active Graves’ orbitopathy. The time between magnetic resonance imaging and CAS score was 3–7 days.

Firstly, all subjects, patients and control group underwent conventional MR protocol of the orbit which was done using a 1.5-T machine (Ingenia, Philips, Netherland) using dStream Head and Neck 20 channel coil. Then subjects were exposed to diffusion tensor imaging (DTI).

DTI data were obtained using a single shot echo planar imaging sequence (TR/TE = 3118/93 ms). The scanning parameters were field of view of 230 × 177 mm2, data matrix of 92 × 88, and voxel dimensions of 2.43 × 2.54 × 2.5 mm3. Parallel imaging [SENSitivity Encoding (SENSE) reduction factor P 2 was used. Diffusion gradients were applied along 32 axes, using a b value of 0 and 1000 s/mm2. Thin section forty-eight slices were obtained, with a thickness of 2.5 mm, no gap, and total scan duration of about 7–8 min.

**Image analysis**

Processing of DTI data were carried out by dedicated work station (Philips Extended MR Workspace 2.6.3.2 with DTI functional tool software). Analysis was done by expert radiologist in head and neck imaging since10 years who was blinded to patients' laboratory and clinical data. Regions of interest (ROI single-pixel seed) were placed in the extra ocular muscles to calculate mean diffusivity.
Figure (1): (A) coronal STIR of both orbits (red line) refers to thickness measurement of inferior rectus (IR). (B) coronal apparent diffusion co-efficient (ADC) map, (C) coronal DTI image, (red circle) refers to region of interest (ROI) for measurement of mean diffusivity (MD).

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) and mean, standard deviation for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (0.05) level.

Results

Table (1): socio-demographic characteristics of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patients N=20</th>
<th>Health control (HCs) N=10</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/years</td>
<td>35.50±13.65</td>
<td>31.90±9.61</td>
<td>0.428</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10(50)</td>
<td>5(50)</td>
<td>1.0</td>
</tr>
<tr>
<td>Female</td>
<td>10(50)</td>
<td>5(50)</td>
<td></td>
</tr>
</tbody>
</table>
Table (1) shows that there is no statistically significant difference between studied groups regarding socio-economic data including age and sex. Among studied groups; 50% of each group were males. Smoking did not show statistically significant difference between studied groups as well.

Table (2): Clinical activity score (CAS) in patients’ group.

| CAS Median (range) | 4 (3-6) |

Table (2) Median CAS is 4 ranging from 3 to 6 in patients' group.

Table (3): Thyroid stimulating hormone receptor antibody (TRAb) of the studied groups.

<table>
<thead>
<tr>
<th>TRAb (unit/ml)</th>
<th>Patients</th>
<th>HCs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.6 (65-445)</td>
<td>34.5 (23.8-47.4)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table (4): Mean diffusivity (MD) of extraocular muscles among the studied groups.

<table>
<thead>
<tr>
<th>MD (x 10^-3 mm²/s)</th>
<th>Patients</th>
<th>HCs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR MD</td>
<td>1.48±0.10</td>
<td>1.05±0.13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IR MD</td>
<td>1.73±0.13</td>
<td>1.09±0.13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MR MD</td>
<td>1.76±0.16</td>
<td>1.14±0.11</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LR MD</td>
<td>1.48±0.147</td>
<td>1.17±0.10</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Superior rectus (SR), Inferior rectus (IR), Medial rectus (MR), Lateral rectus (LR) Table (4): MD of each of extra-ocular muscles was higher in patients' group compared to health control group, the higher MD was found in MR muscle in patients' group.
**Table (5):** Correlation between CAS & orbital muscle MD in patients’ group.

<table>
<thead>
<tr>
<th></th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR MD</td>
<td>R .289</td>
</tr>
<tr>
<td>p value</td>
<td>.071</td>
</tr>
<tr>
<td>IR MD</td>
<td>R .360*</td>
</tr>
<tr>
<td>p value</td>
<td>.023</td>
</tr>
<tr>
<td>MR MD</td>
<td>R .165</td>
</tr>
<tr>
<td>p value</td>
<td>.308</td>
</tr>
<tr>
<td>LR MD</td>
<td>R .255</td>
</tr>
<tr>
<td>p value</td>
<td>.113</td>
</tr>
</tbody>
</table>

Table (5): The only statistically significant correlation was found between MR MD and CAS score and they were positively correlated (r=.360, p=.023).

**Table (6):** Correlation between Thyroid hormone receptor antibody (TRAb) & orbital muscle MD.

<table>
<thead>
<tr>
<th></th>
<th>TRAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR MD</td>
<td>R .424</td>
</tr>
<tr>
<td>p value</td>
<td>.006</td>
</tr>
<tr>
<td>IR MD</td>
<td>R .427</td>
</tr>
<tr>
<td>p value</td>
<td>.006</td>
</tr>
<tr>
<td>MR MD</td>
<td>R .588</td>
</tr>
<tr>
<td>p value</td>
<td>.001</td>
</tr>
<tr>
<td>LR MD</td>
<td>R .605</td>
</tr>
<tr>
<td>p value</td>
<td>.001</td>
</tr>
</tbody>
</table>

Table (6): A statistically significant positive correlation was detected between TRAb level and MD of each of extra-ocular muscles.

**Discussion**

Graves’ ophthalmopathy (GO) is also known as thyroid-associated ophthalmopathy (TAO) and thyroid eye disease (TED). It is the most frequent extra thyroidal manifestation of Graves’ disease (GD) (Garrity and Bahn, 2006). Approximately 25–50 % of GD patients showed clinical manifestations of GO (Bartalena et al., 2020).

GO is characterized by an inflammatory process and subsequent tissue expansion, remodeling, and/or fibrosis of fibroadipose tissues and extraocular muscles in the orbit (Pouso-Diz et al., 2020). Although the exact pathogenesis is incompletely understood, most studies agreed with an abnormal immune response to thyroid-stimulating hormone receptor (TSHR) and insulin-like growth factor-1 receptor (IGF-1R) in the orbit, where orbital fibroblasts appear to be the major effector cells (Khong et al., 2016).

TAO patients’ orbital soft tissues were thought to experience two phases: the initial acute inflammation is characterized by hyaluronic acid accumulation and mononuclear inflammatory cell infiltration. Subsequently, chronic inactive phase
was featured with interstitial fibrosis, and fatty infiltration (Taylor et al., 2020). This pattern was identified as Rundle’s curve (Hales and Rundle, 1960). Therefore, duration of GO, its clinical activity and severity determine treatment decisions (Bartalena et al., 2021).

Clinical activity score (CAS) was established for accurate evaluation of GO activity (Menconi et al., 2017). However, it has many limitations such as its binary (yes/no) feature, (Mourits et al., 1989) low sensitivity and subjectivity owing to the operator dependence (Tachibana et al., 2010).

Thus, there was growing interest to find appropriate quantitative markers or more reliable imaging features to further validate CAS. Orbit Magnetic resonance imaging (MRI) was widely applied for diagnosing TAO and further staging TAO (Siakallis et al., 2018)

Diffusion tensor imaging (DTI) is a non-invasive MRI technology which is very sensitive to water molecules’ micromovement. Thus, it can provide quantitative information of water molecule diffusion movement (Jeon et al., 2018). Common parameters of DTI include fractional anisotropy (FA) and mean diffusivity (MD) values. The MD can reflect the magnitude of water diffusion dominated by interstitial space. Researchers found an inverse correlation between tumor cells and MD values (Razek et al., 2019). Diffusivity could increase in edematous muscle and decrease in fibrotic and fatty infiltrated tissue (Kilicarslan et al., 2015). FA can represent the directionality of water molecules, hence it can reflect tissue’s integrity of microstructural architectures (Gholizadeh et al., 2019).

Our study was a case control study case -control study that was carried out on 20 patients with active graves orbitopathy group (1) and 10 normal subjects without thyroid abnormality group (3). Socio-demographic characteristics (age & sex) as well, the effect of smoking did not show any statistically significant difference among the studied groups.

In terms of microstructural changes in extra-ocular muscles, the mean diffusivity (MD) of each of extraocular muscles (superior & inferior &medial and lateral recti) was statistically significantly higher in patients’ group compared to health control (HC) group (p<0.001). This was consistent with Kilicarslan et al., (2015) demonstrating significantly higher MD value of all the EOMs (IR, MR, LR and SR) in patients with GO versus healthy control (p=0.0001). This result was in agreement with the findings of previous studies demonstrating higher MD of medial and lateral recti in patients with TAO than health control (Chen et al., 2020, Chen et al., 2021).

This may be attributed to infiltration of the extra-ocular rectus muscles with hyaluronic acid formed by fibroblasts. Hyaluronic acid is hydrophilic glycosaminoglycan that retains water and becomes edematous (Hiromatsu et al., 2015). This changes results in increasing the diffusion space of the water protons in the extracellular and intracellular dimensions with a resultant increase in the MD value (Razek et al., 2010).
Clinical activity scales, such as the CAS (Mourits et al., 1997) and vision, inflammation, strabismus and appearance (VISA), (Dolman and Rootman, 2006) could be very helpful in assessing disease activity. Although the combination of MRI studies and clinical scores would improve diagnostic accuracy, the results were conflicting. Some researchers found no clear correlation between MRI findings and CAS indexes (Vlainich et al., 2011). In terms of microstructural changes detected by diffusion tensor imaging, Kilicarslan et al., (2015) reported that there was no correlation between the CAS and apparent diffusion co-efficient value of all EOMs. However, other researchers reported significant positive correlations (Tachibana et al., 2010). In our study, CAS score showed a statistically significant positive correlation only with IR MD (r=.360, p=.023).

There are a few limitations of this study. First, our study population was relatively small, did not include patients with mild GO. Second, we didn't conduct post-treatment assessment because this is a retrospective study and complete follow-up information was not available for every patient. However, we believe that our results could be a strong basis for further larger prospective studies.

To conclude, Mean diffusivity as a parameter in Diffusion tensor imaging (DTI) magnetic resonance imaging (MRI) reflected microstructural changes in Graves ophthalmopathy (GO), and was found to be superior to Clinical activity score (CAS) score in early detection of activity. However, use of DTI MRI in combination with CAS score could be more beneficial.

References


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