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RESEARCH ARTICLE

TITLE: DIAGNOSTIC UTILITY OF CONVENTIONAL CELIAC DISEASE SPECIFIC MARKERS.

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Abstract

Background: Serology based diagnosis of celiac disease (CD) or patient selection for duodenal biopsy remains a dilemma.
Objectives:This study was performed to assess the diagnostic accuracy of conventional CD specific serological markers.
Patients and Methods: This retrospective study was performed at King Khalid University Hospital, Riyadh Saudi Arabia. Data were extracted from 237 patients investigated for CD between March 2012 and June 2014. Data for histological and serological assessment were available for 61/237 patients (36 females and 25 males; mean age28.3±14 years).CD was confirmed histologically in 37 (60.7%) patients.
Results: Frequently detected antibodies were anti-gliadinIgG in 88.5% and anti-tissue transglutaminase IgA (a-Ttg IgA) antibodies in 65.6% patients. The most reliable marker for the diagnosis of CD was a-Ttg IgA with a sensitivity of 97%, specificity of 83%, positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 95%. None of the other CD-specific marker matched the performance of a-Ttg IgA antibody. Performance of the composite marker developed using discriminant analysis surpassed all markers with a sensitivity of 97%, specificity of 92%, PPV of 95% and NPV of 96%. The area under curve for composite marker with 95% confidence interval was 96.7% (92%-100%) significantly higher (p=0.04) than that of a-Ttg 90.3% (82.3%-98.4%). The apparent prevalence of anti-gliadin IgA (62%) was almost similar to true prevalence (61%).
Conclusion: For the diagnosis of CD a-Ttg IgA displayed a high level of diagnostic accuracy as an individual marker however the performance of composite marker was significantly higher than a-Ttg IgA.

Introduction:-

Reliance on histological evidence is currently the mainstay for the diagnosis of celiac disease (CD). The presence of CD specific antibodies along with enteropathy is frequently used as surrogate markers for diagnosis of CD(Bai et al., 2005; Rostom et al., 2006).Assessment of intestinal biopsies not only require expertise but accurate diagnosis may

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be affected by variations in subjective interpretations(Ladas et al., 1994) particularly if severe villous atrophy is not present(Weile et al., 2000). Traditionally screening of candidates for duodenal biopsy is based on the presence of CD specific antibodies. Among several CD specific antibodies detection of anti-human tissue transglutanminase (a-Ttg) and anti-endomysial antibodies (EmA) are considered sufficient for identifying candidates for biopsy (Rostom et al., 2005; Dieterich et al., 1998).Detection of a-Ttg IgA as a single marker isalso considered as a strong indicator for intestinal biopsy because of its higher specificity and sensitivity(Murdock and Johnston, 2005).The recent emergence of evidence challenging the efficacy of anti-deamidatedgliadin peptide (a-DGP) test(Zucchini et al., 2016)indicates that's erological diagnosis of CD remains a dilemma.

Appropriate use of accurate and relatively simple tests may serve as a useful screening tool for diagnosis of CD to avoid the associated morbidity and mortality (Ludvigsson et al., 2009).In the presence of strong clinical suspicion reliance on CD specific serological markers is critical(Pinto Sánchez et al., 2009; Pais et al., 2008).Additional analysis in the form of new generation of CD specific serological markers has contributed significantly to the diagnostic effectiveness of the panel of conventional serological markers (Rashtak et al., 2008). Evaluation of individual tests along with combination of other relevant tests, patient diversity and the extent of intestinal mucosal damage have to be taken in consideration to develop a serology based non-invasivealgorithm for diagnosis of CD (Hill, 2005; Tursi et al., 2001).This study was performed to assess the diagnostic utility of conventional CD specific antibodies for screening and accurate diagnosis of CD in different clinical situations thus obviating the need for small bowel biopsy.

Materials and Methods:-

This retrospective study was performed at King Khalid University Hospital, Riyadh. A total of 237 patients were investigated for celiac disease between March 2012 and June 2014. Out of the total only 61 patients who underwent serological testing and duodenal biopsy were included in the study. This group of patients included 36 (59%) female and 25 (41%) male patients with the mean age of 28.3±14 years. Data forceliac-specific antibodies including anti-endomysium antibodies (EmA), anti-gliadin IgA and IgG, anti-human tissue transglutanminase (a-Ttg) IgG and IgA and anti-reticulin antibodies along with biopsy results were extracted from patient records. Histological diagnosis of CD was confirmed in 37 (60.7%) patients harboring CD-specific antibodies. EmA and anti-reticulin antibodies were detected by indirect immunofluorescence (Immco Diagnostics, Inc. Buffalo, NY, USA). Anti-gliadin IgA and IgG along with a-Ttg IgA and IgG were detected by enzyme linked immunosorbent assay (Quanta Liite™Inova Diagnostics, Inc. USA) in accordance with manufacturer instructions.

Statistical analysis:-

Data analysis was performed using SAS computer software version 9.2 (SAS Institute, Inc. Cary, NC). Categorical data were summarized as number and percentages and numeric data were summarized as mean and standard deviation, to study the diagnostic accuracy of different markers sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) were calculated. For estimation of the predictive power of study markers receiver operator curve (ROC) analysis was used with 95% confidence interval. To combine markers for improved accuracy and prediction linear discriminant function of the study markers was constructed using linear discriminant analysis. McNemar's test was used to compare the true prevalence with apparent prevalence. The true prevalence was based on biopsy confirmed diagnosis of CD whereas the apparent prevalence was based on the presence of CD specific serological markers.

Results:-

Fig. 1 shows data for distribution of celiac specific antibodies among 61 patients with histological evidence of CD. The most frequently detected antibody was anti-gliadinIgG (88.5%) followed by a-Ttg IgA (65.6%) and anti-gliadin IgA which was present in 62.3% of the individuals. The presence of other antibodies was less than 40%. Table 1 describes the performance of individual CD markers including the composite marker.Among these the most reliable marker was a-Ttg IgA with a sensitivity of 97%, specificity of 83%, positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 95%. The IgG a-Ttg lacked sensitivity (38%) whereas specificity was 98% with a PPV of 97% and NPV of 51%. Anti-gliadinIgG antibody had a sensitivity of 92% but lacked specificity (17%) with PPV of 63% and NPV of 57%. Anti-gliadin IgA antibody had a sensitivity of 81%, specificity of 67%, PPV of 79% and NPV was 70%. Both EmA and anti-reticulinantibodies had specificity of 96% with sensitivities of 62% and 54% respectively. PPV value ofEmA was 96% and NPV was 62% whereas PPV of anti-reticulin antibody was 95% and NPV was 57%. The composite marker was found to havethe best diagnostic accuracy with sensitivity of

97%, specificity of 92%, PPV of 95% and NPV of 96%. Figure 2 describes the ROC analysis comparing the diagnostic accuracy of composite marker with CD specific markers. The area under curve(AUC) with 95% CI of the composite marker was 96% (91%-100%) which was significantly (p = 0.04) higher than a-Ttg that had the highest diagnostic accuracy among the original markers with AUC (95%CI) of 90.3% (83%-97%).
McNemar's test was used for comparison of the true prevalence based on biopsy confirmed diagnosis of CD with apparent prevalence based on the presence of CD specific markers. The true prevalence was 61% whereas the prevalence based on anti-gliadin IgA was 62%, the difference was not significant with p-value of 0.796. Similarly the prevalence based on a-Ttg IgA and composite markers were 66% and 62% respectively with corresponding p values of 0.179 and 0.563. Statistically significant differences were observed between the true prevalence and apparent prevalence for the rest of the markers (Fig. 3).

Discussion:-

Among the conventional markers traditionally used for screening and diagnosis of CD a-Ttg IgA antibody exhibited highest diagnostic accuracy in this study. This finding was consistent with previous evidence supporting the importance of a-Ttg IgA in diagnosis of CD (Hill et al., 2005).The sensitivity and specificity of a-Ttg in the present study was 97% and 83%,respectively. A recently published study reported 96% sensitivity and99.5% specificity for a-Ttg IgA (Dahlbom et al., 2016).Depending upon the concentration of the antibody a sensitivity of a-Ttg IgA as low as 65% and specificity of 65.4%has also been reported (Aldaghi and Dehghani, 2016) suggesting that reliance on a-Ttg IgA as a single marker may not be a sufficient evidence to avoid intestinal biopsy. For the interpretation of results of CD markers a combination marker approach appears to be more useful not only for serological diagnosis but also for patient selection for duodenal biopsy.

Celiac specific a-Ttg IgA and EmA havebeen shown to exhibit a very high sensitivity and specificity and the combination is currently being used not only for identifying patients requiring duodenal biopsy but also as non-invasive tool for the diagnosis of CD (Rostom et al., 2006; Weile et al., 2000).Combined detection of a-Ttg IgA and IgG has displayed remarkable enhancement in the diagnostic accuracy of CD including children with IgA deficiency (Aldaghi and Dehghani, 2016).Among the CD specific markers tested in the present study both a-Ttg IgA and EmA were found to have a higher diagnostic accuracy compared to the other markers.Recent introduction of a-DGP test has proved to be a promising new generation investigation for diagnosis of CD with high diagnostic accuracy. It has been shown that the performance of a-DGP test is similar to a-Ttg however long term evaluation of the test over a period of time is mandatory to validate the diagnostic accuracy of a-DGP test (Health Quality Ontario, 2010). Moreover, a-DGP is believed to appear prior to a-Ttg IgA and combined detection of both antibodies may allow early diagnosis of CD particularly among children (Lammi et al., 2016).Despite the evidence supporting a-DGP as a useful addition to the existing celiac markers recent evidence suggests that combined detection of anti-transgluatminase and a-DGP antibodies has a lower PPV than that of anti-transgluatminase and anti-endomysium antibodies for diagnosis of CD (Zucchini et al., 2016). These observations not only highlight reliance on conventional CD markers in diagnosis of CD but also emphasize the need for further enhancement in diagnostic accuracy of CD specific markers.

Although the performance of anti-gliadin IgA antibodies in terms of sensitivity and specificity was not as high as a-Ttg IgA in the present study but it emerged as the best marker for apparent prevalence. Anti-gliadin IgA correlates well with the re-growth of jejunal villi and tends to disappear after gluten withdrawal (Volta et al., 1990).The strength of association between anti-gliadin IgA with gluten therefore appears to be auseful marker for monitoring compliance to gluten free diet. An experimental study on lymphopenic mice has clearly demonstrated that transfer of gliadin pre-sensitized CD4 lymphocyte subset induced duodenitis with histological features similar to CD that was accompanied by production of high levels of anit-gliadin IgA antibodies following an oral gluten challenge (Freitag et al., 2009).All the altered immune responses including high level of anti-gliadinIgA antibody production regressafter withdrawal of gluten exposure. These observations indicate that cell mediated immune responses against gluten may be involved in pathogenesis of CD and the associated gut inflammation is consequent to persistent gluten exposure. It is therefore highly likely that being the best apparent marker anti-gliadin IgA antibody may represent CD related ongoing inflammation in the small bowel and could serve as a sensitive marker of inflammation in CD particularly among the untreated cases.

A composite marker developed in the present study by combining the conventional markers using linear discriminant analysis was associated with significant enhancement in the diagnostic accuracy of CD. Despite exhaustive literature search no evidence of using conventional CD-specific markers as a composite marker was found. However, in the past development of composite marker has been shown to serve well in predicting liver

metastases preoperatively among patients with gastrointestinal cancer (Christensen and Jacobsen, 1987). Although the performance of the composite marker was best among the CD specific markers the diagnostic accuracy of a-Ttg IgA was comparable. The findings of the present study in this context require further validation in a large scale study as the sample size in the present study was relatively small. The serology based model developed in the present study by linear discriminant analysis thus offers a novel tool for screening and diagnosis of CD patients with a higher degree of accuracy.

In conclusion a-Ttg IgA displayed a high degree of diagnostic accuracy and none of the other CD specific markers was comparable to a-Ttg IgA. However the performance of composite marker surpassed the individual performance of all CD specific markers and was significantly higher than a-Ttg IgA antibody. Anti-gliadin IgA was the only marker with apparent prevalence almost similar to true prevalence. The performance of composite marker however requires further assessment in large scale studies to validate the findings of the present study.

Table I:-Performance of individual celiac disease specific serological markers and the composite marker.

Celiac-specific antibody	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Anti-glial IgG	92	17	63	57
Anti-glial IgA	81	67	79	70
Anti-tissue transglutanminaseIgG	38	98	97	51
Anti-tissue transglutanminase IgA	97	83	90	95
Anti-endomysium	62	96	96	62
Anti-reticulin	54	96	95	57
Composite marker	97	92	95	96

PPV = Positive predictive value
NPV = Negative predictive value

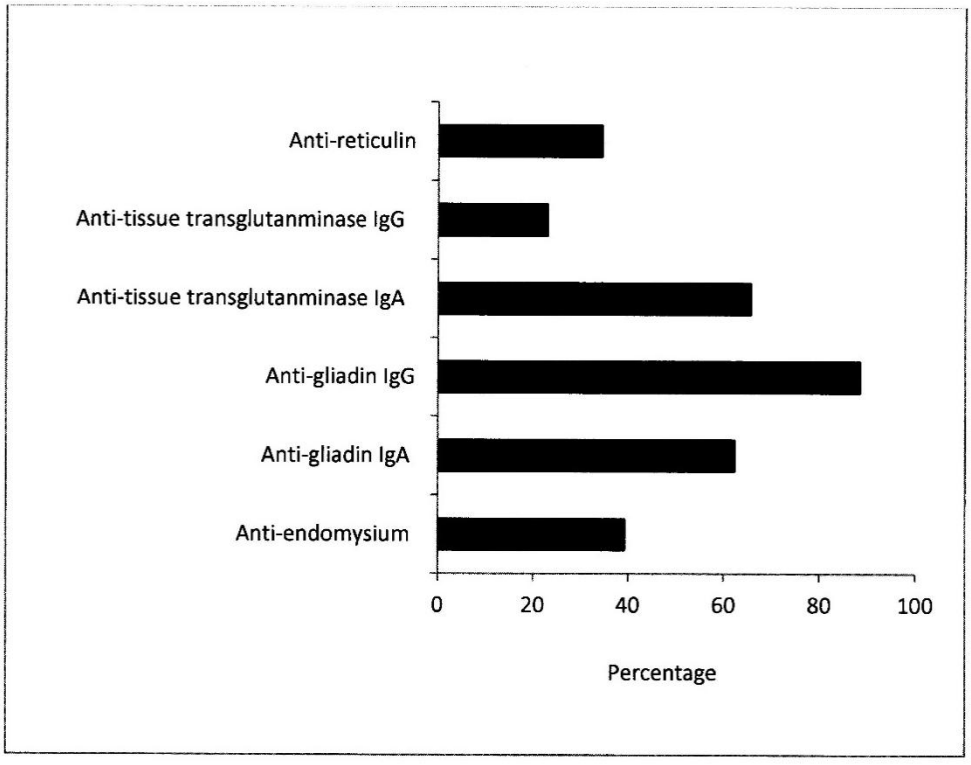

Figure 1:- Distribution of celiac specific auto-antibodies among the patients

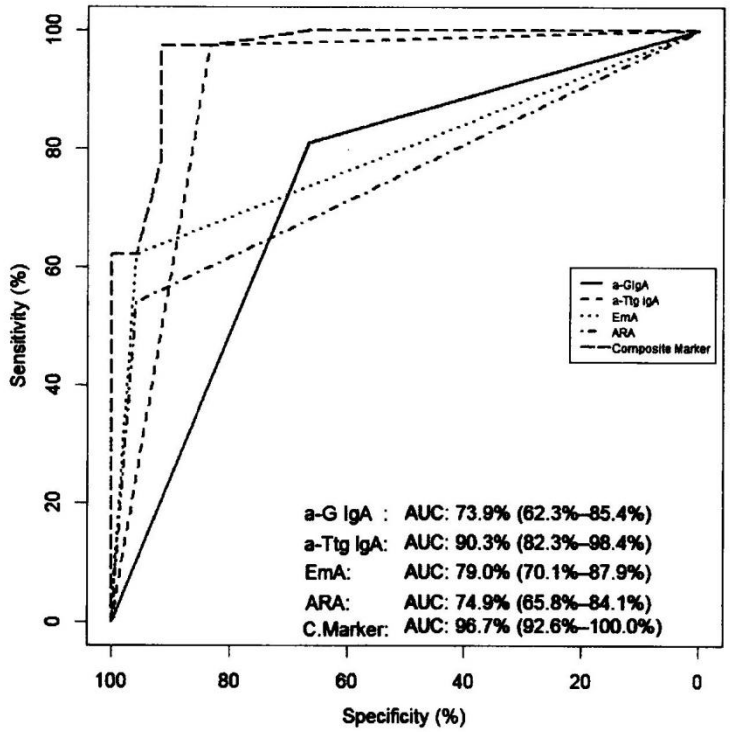

Figure 2:- Receiver operator curve analysis of individual celiac specific antibodies and the composite marker.

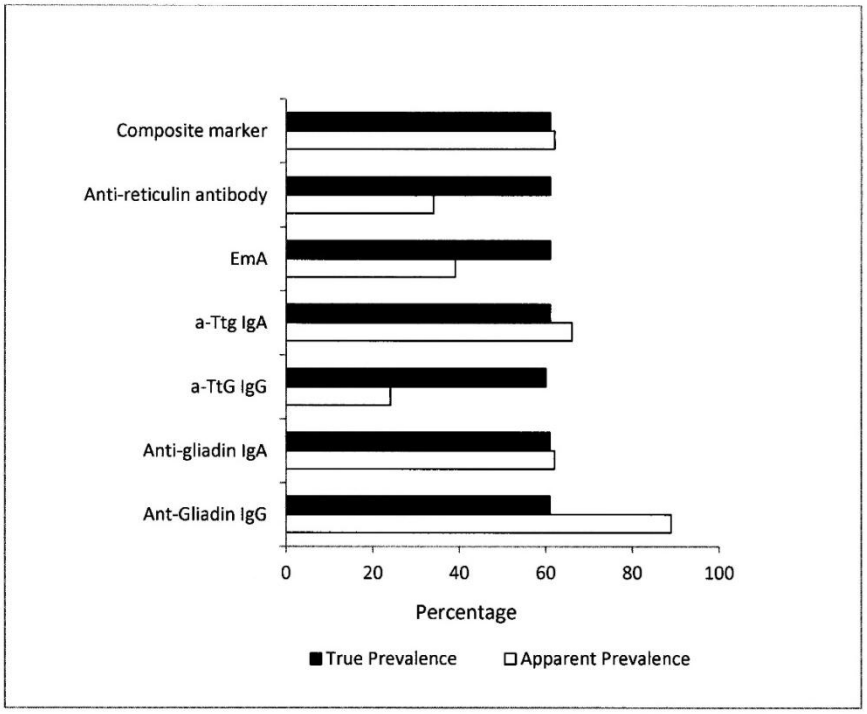

Figure 3:- Comparison of the apparent and true prevalence of celiac specific markers.

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