CORRELATION OF NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE QUINONE OXIDOREDUCTASE 1 (NQO1) GENE POLYMORPHISM AND BRONCHIAL ASTHMA

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ABSTRACT

Background: Worldwide, asthma is a major public health issue due to its high prevalence and debilitating effects. Asthma prevalence in children has steadily risen over the last 20 years. Asthma affects 300 million people worldwide and is expected to rise from 45% to 59% by 2025, according to estimates. Bronchial asthma affects 5-10% of children and teenagers.

Objective: To explore the correlation of NQO1 gene polymorphism and risk factors of childhood asthma.

Patients and methods: This is a case control study. The participants were divided into two main groups, 29 non-asthmatics as a control group, and 87 asthmatic children as cases. The cases were further subdivided into 3 subgroups according to their asthma control level. All children were assessed for the pulmonary functions. Genotyping of Nicotinamide adenine dinucleotide phosphate Quinone Oxidoreductase 1 gene polymorphism was assessed by restriction fragment length polymerase chain reaction (RFLP-PCR).

Results: Patients with uncontrolled, partially controlled, and well-controlled asthma were more likely to have family members who had asthma (96.6%, 86.2%, and 72.4%, respectively). FEV1 percent and FVC differed significantly between the four groups. There was statistically significant difference between the four studied groups as regard gene polymorphism, 75.9% of control group had CC genotype and 58.6% of uncontrolled asthmatic patients had CT genotype. The asthma group in the study had less CC genotype and C allele (42.5% & 67.8%) versus the control group (75.9% & 87.9%)

Conclusion: The study has revealed a significant correlation between family history of asthma and the childhood asthma. The polymorphisms of NQO1 are correlated with the risk of childhood asthma, and it increases due to the dominant homozygous mutations of NQO1.

Keywords: Nicotinamide Adenine Dinucleotide Phosphate Quinone Oxidoreductase 1 (NQO1), Gene Polymorphism, Bronchial asthma.

I. INTRODUCTION

Childhood asthma is characterized by chronic inflammation of the airways which results in variable expiratory airflow limitation combined with a history of respiratory symptoms like wheeze, shortness of breath, tightness in the chest and cough. (1)

Throughout the world causes of morbidity and mortality, asthma is considered as a major cause. With an increasing prevalence, especially in children; as measured by absences from school/day care, visits to emergency departments (ED), and hospitalization, it is the leading cause of childhood morbidity from chronic disease (2).

Asthma incidence in children has risen steadily over the past few years, posing a serious threat to their physical and mental health (3).

Predicting the risk of asthma incidence is therefore critical, but there has been insufficient evidence to date to identify an asthma susceptibility gene both domestically and abroad (4).
Phase II reactive enzyme Nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1) reduces quinones as one of its primary functions, reduce the amount of oxygen free radicals and the toxicity of exogenous substances in cells.\(^\text{(5)}\).

Superoxide radicals are scavenged by NQO1 reactions, allowing hydrogen peroxide to contribute to inflammatory changes in the tissues of the airways. Additionally, the highly reactive hypochlorous acid is produced by hydrogen peroxide being converted by myeloperoxidase into myeloperoxidase. NQO1 has been shown to be crucial in controlling inflammatory responses induced by xenobiotics and oxidatively labile compounds. In addition, functional polymorphisms in these enzymes have been linked to an increased risk of allergic disease in people who carry them and a greater sensitivity to the xenobiotics' causative pro-allergenic effects.\(^\text{(6)}\).

However, only a correlation between NQO1 and the risk of developing respiratory cancers has been found by Liu and Zhang,\(^\text{(7)}\) and Nagata et al.,\(^\text{(8)}\). However, no definitive link has been established between it and asthma in Egyptian children. Therefore, in the hope of providing a theoretical support for the genetic polymorphism of asthma among children, Asthma children with NQO1 genes were enrolled in our department. The goal of this research is to see if the NQO1 gene polymorphism has any relationship to the risk factors and severity of childhood asthma.

Patients and methods:

Subjects
A case control study conducted on 116 children at a pulmonology & allergy unit in pediatric hospital and biochemistry department. Participants were divided into 2 main groups, 29 non asthmatics and 87 asthmatics. The asthmatic children were further subdivided into 3 subgroups according to their asthma control level, so we had 4 groups: A: 29 non asthmatic participants as a control group. B: 29 well controlled asthmatic patient. C: 29 partially controlled asthmatic patient. D: 29 uncontrolled asthmatic patients. Research ethics committee of Zagazig university's faculty of medicine approved the study after all participants provided written informed consent. (ZU-IRB#6175/14-6-2020) work was done in accordance with the Declaration of Helsinki, the ethical code of the world medical association for human studies Classified into three group regarding their level of asthma control into: controlled, partially controlled or uncontrolled according to GINA recommendation at 2008 considering the following items:

- Day time symptom
- Limitation of activation.
- Nocturnal symptom
- Need for relievers.
- Pulmonary function test.
- Exacerbation

Inclusion criteria:
Children from both sexes with bronchial asthma aged from 5-15 years with taking approval from their parents.

Exclusion criteria:
Children with chronic pulmonary disease, children suffering from liver kidney and heart disease and parents’ refusal of participation.

All patients were subjected to full history taking, general and chest local examination.

Laboratory assays
Laboratory investigations:
Routine Complete blood count.
C-reactive protein.
Liver function test.
Kidney function test.
Pulmonary function test.

Specific investigations:
Blood sample were obtained from all subject 2 ml of whole blood was collected into EDTA treated tubes for DNA extraction.

NQO1 gene polymorphism genotyping was done by Restriction fragment length polymerase chain reaction (RFLP_PCR).

PCR_RFLP for detection of 609C ~ T polymorphism of NQO1 gene was done by using the following primers.
Forward primer: 5′-TCCTCAGAGTGGCATTCTGC-3′.
Reverse primer: 5′-TCTCCTCATCCTGTACCTCT-3′.
The PCR was carried out in a final volume of 20 l containing 10 l of 2 x i Taq PCR Master mix (iNtRON Biotechnology, Seongnam-Si, Korea), 1ml of each primer (Biolegio, Nijman, Netherland), 4 ml of genomic DNA and 4 ml of deionized water.

II. STATISTICAL ANALYSIS
The acquired data were coded, entered, presented, and analyzed by computer using the Statistical Package for Social Science (SPSS) version 26 data base software application. Frequencies and percentages were used to depict qualitative data. Mean, standard deviation (SD), and (minimum-maximum) values were determined for quantitative variables. ANOVA was used to compare more than two groups of normally distributed variables, while the Kruskall Wallis test was used to compare more than two independent groups of non-normally distributed variables. The Chi square (X²) test was performed to determine the relationship between several qualitative factors. To analyse the link between various study variables, the person correlation coefficient was determined, with (+) indicating direct association and (-) indicating inverse correlation. Also, numbers close to 1 suggest significant connection, whereas values close to 0 indicate weak correlation. The results were considered statistically significant and highly statistical significant when the significant probability (P value) was <0.05* and <0.001** respectively. To assess the validity, sensitivity, specificity, predictive value for positive (PVP), and predictive value for negative (PVN) were determined.

III. RESULTS:
There were no statistically significant differences between the four studied groups as regard age (years), sex, weight (kg) and height (m). The majority of asthmatic patients had positive family history while none of controls had positive family history (Table 1).

Majority of asthmatic patients (96.6%, 86.2% and 72.4%) in uncontrolled, partially controlled and well-controlled groups respectively had positive family history to asthma (Figure 1).

There were highly significant differences between the four studied groups as regard FEV1% and FVC where higher values were among control group and well controlled group (Table 2).

There was statistically significant difference between the four studied groups as regard gene polymorphism where 75.9% of control group had CC genotype and 58.6% of uncontrolled asthmatic patients had CT genotype (Table 3).
CC genotype was highly represented in control group while CT and TT genotypes were highly represented in uncontrolled asthma patients (Figure 2).

Table (1): Basic characteristics of the four studied groups:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Uncontrolled group (n=29)</th>
<th>Partially controlled group (n=29)</th>
<th>Well controlled group (n=29)</th>
<th>Control group (n=29)</th>
<th>Tests</th>
<th>f</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.96±3.6 (5-14)</td>
<td>7.9±2.8 (5-14)</td>
<td>8.4±2.8 (5-15)</td>
<td>8.83±2.82 (5-14)</td>
<td></td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>33.8±14.6 (18-60)</td>
<td>28.7±10.4 (17-50)</td>
<td>33.2±9.2 (20-66)</td>
<td>31.2±12.86 (19-66)</td>
<td></td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.35±0.022 (1.06-1.68)</td>
<td>1.26±0.15 (1.06-1.55)</td>
<td>1.31±0.13 (1.05-1.56)</td>
<td>1.35±0.17 (1.06-1.65)</td>
<td></td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.89±3.73 (11.03-26.01)</td>
<td>17.54±3.23 (13.4-24.4)</td>
<td>18.36±2.56 (14.38-23.6)</td>
<td>16.51±2.74 (12.8-24.2)</td>
<td></td>
<td>3.2</td>
<td>0.075</td>
</tr>
</tbody>
</table>

(X2) chi-square test   (f) one way ANOVA  (NS) non significant

Figure (1): Comparison of family history between the four studied groups

Table (2): Respiratory function of the four studied groups

<table>
<thead>
<tr>
<th>Pulmonary functions</th>
<th>Uncontrolled group (n=29)</th>
<th>Partially controlled group (n=29)</th>
<th>Well controlled group (n=29)</th>
<th>Control group (n=29)</th>
<th>Tests</th>
<th>f</th>
<th>P value</th>
<th>Post hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 (%)</td>
<td>56.32±15.6 (35-81.7)</td>
<td>64.18±15.6 (35-81.7)</td>
<td>90.56±4.49 (82.3-99.5)</td>
<td>103.28±9.1 (85.8-118.6)</td>
<td></td>
<td>95.</td>
<td>&lt;0.001*</td>
<td>P1=0.071</td>
</tr>
</tbody>
</table>

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Table (3): gene polymorphism between the studied groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Uncontrolled group (n=29)</th>
<th>Partially controlled group (n=29)</th>
<th>Well controlled group (n=29)</th>
<th>Control group (n=29)</th>
<th>Tests</th>
<th>Post-Hoc Turkey's test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>x2</td>
<td>P value</td>
</tr>
<tr>
<td>CC</td>
<td>8 (27.6)</td>
<td>13 (44.8)</td>
<td>16 (55.2)</td>
<td>22 (75.9)</td>
<td>17.4</td>
<td>0.008 (S)</td>
</tr>
<tr>
<td>CT</td>
<td>17 (58.6)</td>
<td>15 (51.7)</td>
<td>12 (41.4)</td>
<td>7 (24.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4 (13.8)</td>
<td>1 (3.4)</td>
<td>1 (3.4)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(X2) chi-square test (S) Significant

**Figure (2): Comparison of gene polymorphism between studied groups**

IV. DISCUSSION

One of the most common symptoms of asthma is hyperresponsiveness of the airways, which includes chronic inflammation, increased mucus production, thickening of the airway wall, and smooth muscle dysfunction. Asthma often begins with hyperresponsiveness of the airways, but not everyone who has hyperresponsiveness goes on to develop full-blown asthma. Host atopy is also a known risk factor for asthma and airway inflammation (9).

Asthma incidence in children has risen steadily over the past few years, posing a serious threat to their physical and mental health of children (10).

People with AHR and atopy may be more susceptible to developing clinically relevant asthma phenotypes if they have certain genetic risk factors. Given the role of oxidative stress in airway inflammation and tissue damage, genes involved in oxidative stress responses may be good candidates for asthma gene discovery (5).
Toxic reactive quinines are detoxified by NQO1, which catalyses redox cycling to produce reactive oxygen species (ROS). When the amino acid proline is changed to serine (CC to TT, Pro187Ser), enzyme activity is lost (9).

This enzyme, nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1, is a phase II reactive enzyme. Its primary functions include the reduction of quinones, the elimination of oxygen free radicals, and the improvement of the toxic effects of exogenous materials on cells (5).

Regarding the basic characteristics of the studied groups, we found that there were no statistically significant differences between the four studied groups as regard age (years), sex, weight (kg) and height (m). The majority of asthmatic patients had positive family history while none of controls had positive family history, there was statistically significant difference between the studied group as regard family history.

They enrolled 102 asthmatic children as part of the Guo et al., (5) case control study, which looked at the correlations between LT and NQO1 gene polymorphisms and childhood asthma symptoms as observation group and 80 healthy children as control group, the agreed with us that there were no statistically significant age or gender differences between the studied groups, but there were significant family history differences between the studied groups.

It has been established that having a family history of asthma is a well-known risk factor for developing Asthma, and this strong and consistent correlation backs this up, shedding light on the role that genetics plays in the development of asthma. Having one affected parent increases your risk by about two times, and having two affected parents increases your risk by about four times. (11).

Asthma, on the other hand, is more than a simple genetic condition. In addition to a family history of asthma, living in a developed country is another significant risk factor for the disease. The International Study of Asthma and Allergic Diseases in Children (ISAAC) has shown over the past decade that asthma is more prevalent in more developed countries, which does not simply reflect diagnostic preferences. Asthma symptoms are on the rise, just like the number of people being diagnosed with the disease (12).

The present results showed that there were highly significant differences between the four studied groups as regard FEV1% and FVC where higher values were among control group and well controlled group.

According to our findings, the study by Ardura-Garcia et al., (13) also evaluated pulmonary function using forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), They discovered a statistically significant difference in the mean FEV1/FVC ratio between the groups they studied (p=0.001).

In contrast with our findings the results of the study by Gilliland et al., (14) revealed that in terms of FEV1 percent and FVC, there were no differences between the asthma patients and the control group (p=0.2 and 0.7, respectively).

Regarding the gene polymorphism between the studied groups, we found that there was statistically significant difference between the four studied groups as regard gene polymorphism where 75.9% of control group had CC genotype and 58.6% of uncontrolled asthmatic patients had CT genotype.

In agreement with our results the study by Guo et al., (5) found a difference in the frequency distribution of the three NQO1 rs2917666 genotypes studied between the cases and controls, and genotype CC was more common than genotypes CG and GG., and they discovered a statistically significant difference in gene polymorphism between the two groups they examined (p= 0.047).

However, Reddy et al study in (9) compared gene polymorphism among different ethnic groups. and they discovered that the CC genotype was more common in White patients (77 percent) than in African-American patients (73.7 percent) and in Indian cases, the CT+TT was more common (60 percent), Furthermore, participants with or without Airway hyperresponsiveness showed no significant differences in their results.

Comparing genotypes and alleles between the asthma group and control group, showed that there was statistically significant difference between asthma group and control group regarding genotypes and alleles with asthma group had less CC genotype and C allele (42.5% & 67.8%) versus (75.9% & 87.9%) in the control group. Those have TT genotype are at risk of asthma 7.8 times more than those have CC genotype.
We also found that there was statistically significant difference between uncontrolled group and partially controlled group regarding C &T alleles with uncontrolled group had less C allele (56.9%) versus (70.7%) in partially controlled group. Those have TT genotype are at risk of uncontrolled asthma 6.5 times more than those have CC genotype.

According to Guo et al., (5)'s findings, there was a statistically significant difference between the two groups when it came to genotypes and alleles (p= 0.047 and 0.019, respectively). They also discovered that the asthma group had a lower percentage of CC genotype and C allele (72.55% & 85.29%) than the control group (87.5% & 93.13%). They also found that the three NQO1 rs2917666 genotypes differed between the two groups. CC genotype was more common than CG and GG, and C allele was more common than G allele. CC genotype. Findings from this study suggest that the LT and NQO1 genes are linked to childhood asthma in children. There was a difference in recessive and additive modes of these two gene sites between the cases and control groups, based on their analysis of LT rs2844484 (A) and NQO1 rs2917666. Although there was no difference in dominant mode, the results suggest the LT rs2844484 and NQO1 rs2917666 genetic modes can be described by recessive and additive modes.

According to David et al., (15), people with an inactive NQO1 allele had a nonsignificantly lower risk of developing asthma. However, individuals with at least one Ser allele for NQO1 and a homozygous deletion of GSTM1 had a significant reduction in their risk of developing asthma. The discovery of a reduced risk of asthma in people with an inactive NQO1 allele and a GSTM1 deficiency, in this population that has been exposed to high levels of ozone, that supports previous research on the short-term effects of ozone exposure on the respiratory system (16).

In contrast with our results the studies by Reddy et al., (9) showed that there was no significant difference between the studied groups as regard genotypes and alleles (p=0.05).

Future studies with larger sample sizes, often in collaboration, better exposure measures, more refined asthma phenotypes, and whole genome association genotyping will enhance our knowledge of gene-environment interactions in asthma. Environmental epigenetic mechanisms should be investigated.

CONCLUSION

We found a significant correlation between family history of asthma (as genetic factor) and the childhood asthma. The polymorphisms of NQO1 are correlated with the risk of childhood asthma, and it increases due to the dominant homozygous mutations of NQO1.

REFERENCES
