TUMOUR SUPPRESSOR GENE (P53) MODULATES LIF ACTIVITY - A CAUSATIVE FACTOR FOR THE DEVELOPMENT OF NEURAL TUBE DEFECTS IN INDIA

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Abstract- Neural tube defect (NTDs) is the most severe from of congenital malformation of central nervous system leading to infant mortality and morbidity. The most common form of NTDs is anencephaly (spina bifida) and myelomeningocele. The incidence in the population varies depending on racial/ethnic variation including genetic and unknown environmental factors. The etiopathology of NTDs is still unclear between two different gene, hence present study has been designed with the aim to evaluate gene mutation between tumour suppressor gene and leukaemia inhibitory factor during organogenesis. We have collected the blood samples (n= 43) of clinically diagnosed NTDs with respective controls (n= 46) for PCR based DNA analysis to evaluate the frequency of p53 and LIF gene mutation using specific primers. Present findings reveals that highly significant differences (P<0.05) were observed in LIF gene using two different exons confirming p53 gene inactivation affects neural ectoderm proliferation resulting increased “risk factor” for the development of NTDs.

Keywords- Neural tube defect, tumour suppressor gene, leukaemia inhibitory factor.

Short Title- LIF and p53 gene interaction in NTDs


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Introduction

Tumor suppressor gene (p53) is one of the most severe from of congenital malformation of central nervous system leading to infant mortality and morbidity. The most common form of NTDs is anencephaly (spina bifida) and myelomeningocele (MMC). These are accompanied by alterations of the axial skeleton, as well as the overlying meningo-vascular or dermal tissues (canalization) [7-8]. However, over 90% of the NTDs cases still have an unknown etiopathology. During normal differentiation, cell develops the ability to maintain genomic integrity by providing regulatory mechanism based on cell- cycle kinetics.

The p53, a nuclear phosphoprotein is expressed in normal as well as malignant cells condition [11], highly conserved during evolution and its mutations causes amino acid substitutions increased instability for the development of abnormal neural tube closure including exencephaly [12-13]. Since p53 has become the molecule of central focus of intensive basic and clinical research and molecular mechanisms are still not well implicit but its activation increase risk factor either alone or associated with cytokines in cancer patients [14].

Leukaemia inhibitory factor (LIF) is a pleiotropic cytokine belong to IL-6 family acts as a paracrine or autocrine fashion to regulate metabolic homeostasis through binding to a heterodimeric specific glycoprotein (gp190 & gp130), shared by several receptors of cytokines related to IL6 to maintain totipotent embryonic stem cells [15-17]. The regeneration of diverse types of neural cells during development occurs through the fate of progenitor cells regulated by intrinsic or extrinsic factors. The significant of LIF gene mutation, their functional consequences and clinical impacts are still not fully understood. To understand the role of this ubiquitous cytokine, it
becomes necessary to understand the function of LIF during organogenesis in NTDs [18]. Hence, there is considerable curiosity has been developed with the aim to determine, whether p53 gene mutation act as LIF suppressor or inducer to neural ectoderm during folding of neural tube defects. Therefore, present study becomes imperative to explore the mechanism of NTDs to understand the action of causative factors including gene-gene interactions by helping non invasive technique such as prenatal diagnose to prevent incidence of NTDs in the society.

Materials and Methods
Blood samples of NTDs (n = 43) were collected from the Obstetrics & Gynaecology & Paediatric Surgery, I.M.S., B.H.U.,Varanasi with their respective controls (n = 46), after written consent of patients/attendant. The criteria for inclusion of an individual was based on clinically diagnosed NTDs. The study was approved by ethical committee of the Institute. Genomic DNA was isolated from the whole blood using Bioner Kit (Korea) and samples were kept at 20°C till further analysis. In the present study specific primers for p53 and LIF were selected forward 5'-TGA AGT CTC ATG GAA GCC AGC. and reverse 5'-GCC AAG GTA CAC GAC TAT GC and reverse 5'-GCC AAG GTA CAC GAC TAT GC and reverse 5'-CCG TAG GTC ACG TCC ACA TG while for LIF exon 3a and 3b, forwards 5'-ACA ATT CCA GAT GCT TAC AGG G 3' and reverse 5'-GCC AGC. and reverse 5'-GCC AGC. and reverse 5'-CCG TAG GTC ACG TCC ACA TG-3' respectively. We have used two different PCR specific strategies for p53 and LIF exon 3a and 3b gene using specific forward and reverse primers in volume of 25 μl contain 50-100 ng of DNA, 20 pmole of each primer, 200μM of each dNTPs mix with Taq buffer (10mM Tris HCl pH 8.3, 50mM KCl), 3.0mM MgCl2 and 3 unit of Taq polymerase (New England Biolab). For p53, cycling conditions were 3 min at 94°C for initial denaturation, 60°C/30sec for annealing followed by 35 cycles and 72°C/5min for final extension while LIF exon 3a & 3b having same cycling conditions were 1 min at 94°C for initial denaturation, 56°C/1min for annealing followed by 35 cycles and 72°C/7min for final extension. The PCR product was separated on 1.5% agrose gel, stained with Et.Br and bands were visualized and characterize on Gel Doc system (SR Biosystem).

Results
Table-1 showing the details finding of the p53 and LIF gene activity using polymerase chain reaction (PCR) based DNA analysis with specific amplicons to characterize mutational spectra in NTDs cases and compare the same with controls. We are able to characterize the gene mutation in terms of either complete disappearance (null) or up regulation (over expression)/down regulation (regression). To confirm the above findings the study was repeated using polymerase chain reaction (PCR) based DNA analysis with specific amplicons to characterize mutational spectra in NTDs cases and compare the same with controls. We are able to characterize the gene mutation in terms of either complete disappearance (null) or up regulation (over expression)/down regulation (regression). To confirm the above findings the study was repeated

Table 1- p53 and LIF gene mutation showing variable (%) frequency and O.R. at 95% C.I. between NTDs cases and controls

<table>
<thead>
<tr>
<th>Markers</th>
<th>Case</th>
<th>Control</th>
<th>O.R at 95% C.I.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up-regulation</td>
<td>30</td>
<td>8.3</td>
<td>4.7 (0.9-25.15)</td>
<td>0.023</td>
</tr>
<tr>
<td>Down-regulation</td>
<td>20</td>
<td>5.5</td>
<td>4.2 (0.6-33.60)</td>
<td>0.073</td>
</tr>
<tr>
<td>Null/Absent</td>
<td>11.36</td>
<td>22.2</td>
<td>2.0 (0.6-6.8)</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIF exon3a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up regulation</td>
<td>32.3</td>
<td>13</td>
<td>3.2 (0.9-10.7)</td>
<td>0.028</td>
</tr>
<tr>
<td>Down regulation</td>
<td>41.8</td>
<td>10.8</td>
<td>5.2 (1.5-18.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Null/Absent</td>
<td>6.9</td>
<td>2.1</td>
<td>3.3 (0.0-2.24)</td>
<td>0.274</td>
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<tr>
<td>LIF exon3b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up regulation</td>
<td>36</td>
<td>11.4</td>
<td>4.3 (1.0-20.3)</td>
<td>0.023</td>
</tr>
<tr>
<td>Down regulation</td>
<td>24</td>
<td>5.7</td>
<td>5.2 (0.8-41.9)</td>
<td>0.040</td>
</tr>
<tr>
<td>Null/Absent</td>
<td>16</td>
<td>2.8</td>
<td>6.4 (0.6-163.2)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Statistical analysis showing significant differences p < 0.05 using x² square test.

Discussion
During differentiation of central nervous system, neural crest cells proliferation is the consequences of a combination of both extrinsic and intrinsic factors arise from the embryonic niche. The protective nature of p53 during cell damage provides genomic stability to cells. Several published studies have examined the role of p53 associated with congenital malformations including exencephaly, pre axial polydactyly and ocular abnormalities in mice [19-20]. Present study showing the variable frequency of p53 and LIF distribution in clinically classified NTDs and similar findings has also been

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Hence, plausibly hypothesize that significant association of two disorders: between p53 gene and LIF gene in NTDs with four possible reasons associated with such disorders: 
1. p53 gene inactivation increased “risk factor” for development of neural tube defects. 
2. Severity of the disease such as anencephaly is equally important for developing complications during organogenesis due to p53 & LIF gene mutation. 
3. LIF gene activity confirm their association either as an independent or association with p53 gene mutation. 
4. Genetic heterogeneity in population varying age, sex and belong to different ethnic groups modulate LIF activity either due to severity of the disease or tissue specific genetic susceptibility. 

Hence, plausibly hypothesize that significant association of two different genes are associated for the development of clinically diagnosed NTDs. Although, our study is small but promising to further evaluation to confirm gene-gene interaction to induce congenital malformations of central nervous system leading “Birth Defects”.

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References