High Serum Endostatin Level in Egyptian Children with Down Syndrome: Gene Dosage Effect

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Abstract: The present study was carried out on 51 individuals with Down syndrome (DS), 39 patients with trisomy 21 and 12 patients with mosaicism as well as their 22 matched controls. Their ages ranged from 2 months to 18 years. The purpose of this work was to study the level and the gene dosage effect of serum endostatin in DS children and control subjects. Present results showed significant high levels of endostatin in the population with complete trisomy 21 compared to mosaicism and control subjects, whereas, in cases with mosaicism, endostatin levels showed no statistical difference compared to control subjects. Congenital heart disease was present in 58.8%. No significant difference in endostatin levels between cases with congenital heart and cases without. Reviewing the literature showed that DS patients are resistant to solid tumours and rarely have haemangiomas. This study concluded that the increased levels of endostatin is a gene dosage effect (three copies of the protein) and it could be used as a preventive protein for high risk population up to the level seen in DS without side effects. The present work is important in the field of angiogenesis, not only from research area, but also from product development safety.

Key words: Endostatin, Egyptian, Down syndrome

INTRODUCTION

In recent years it has become clear that angiogenesis not only is important in physiological processes such as embryonic development, wound healing, organ and tissue regeneration, but also plays a pivotal role in tumor progression and metastasis[1].

The malignant transformation of a normal cell into a cancer cell requires no vasculature. Growth of solid tumors, however, requires angiogenesis to provide oxygen and nutrients to support cell proliferation. The switch from an avascular to a vascular phenotype is typically associated with acceleration of tumor growth. Antiangiogenic therapy, starving a tumor of its blood supply, is an attractive addition to the anticancer armamentarium. Animal tests of antiangiogenic therapy have shown remarkable potential. Initial human trials have proven antiangiogenic therapy to be remarkably non-toxic. Numerous antiangiogenic agents have been isolated as proteolytic fragments of endogenous polypeptides of the extracellular matrix[2]. Endostatin is expressed at high levels in human liver and kidneys and freely circulating in serum. Efficient endostatin generation requires a moderately acidic pH similar to the pericellular milieu of tumors[3]. Endostatin is produced by hemangioendothelioma cells and it is a 20 kDa (184 aa)

C-terminal fragment of collagen XVIII. It is encoded by the COL18A1 gene which has been mapped to 21q22.3[6].

Down syndrome (DS) is the most common multiple malformation syndrome occurring in humans and is associated with an increased risk of childhood malignancies, in particular leukemias. However, solid tumors seem to be under represented in DS patients and brain tumors occurring in association with DS have so far been limited to sporadic case reports, predominantly in children and young adults[8]. In Down syndrome, the incidence of solid tumors including lung cancer is considerably lower than that of the general population. The low risk of lung cancer in individuals with Down syndrome may be related to the gene-dosage effect of the extra chromosome 21. It was suggested that tumor suppressor genes playing a role in the pathogenesis of lung cancer may be present on chromosome 21[9]. The aim of the present study is to assess the level and the gene dosage effect of serum endostatin in DS children and control subjects.

MATERIALS AND METHODS

The present study was carried out on 51 Egyptian children with Down syndrome referred to the clinic of children with special needs, National Research Centre
(39 patients with trisomy 21 and 12 patients with mosaicism). They were 23 males and 28 females. Their ages ranged from 2 months to 18 years. Twenty two cases were included as matched controls (12 males and 10 females).

All cases were subjected to:
1. Detailed history taking, including pedigree analysis, developmental history and similarly affected family members.
2. Thorough clinical examination to detect any associated anomalies.
3. Echocardiography to detect any cardiac anomalies.
4. Chromosomal analysis.
5. Endostatin immunoassay: Endostatin concentrations in serum were detected using competitive enzyme immunoassays as a commercially available assay (endostatin enzyme immunoassay Kits, Accucyte, Cyrimmune Science, Inc., College Park, MD, USA[3]). According to the manufacturer’s instructions for usage ACCUCYTM Human EndostatinTM EIA Kit, (Article No. CY-AC215-K). The ACCUCYTE Human Endostatin kit has been developed using recombinant human Endostatin as the antigen for polyclonal antibody production and is used in the kit as the assay standard.

Reading the plate: The developed red colour was read at 492 nm.

Evaluation of results
1. The average of the duplicate absorbance values for each standard, including the Zero and all the sample values was calculated.
2. On semi-log graph paper, plot the mean absorbance values for each of the standards on the (linear scale) Y-axis versus the concentration of each standard (ng ml⁻¹) on the (log scale) X-axis was plotted. The standard curve should have a sigmoid shape that shows an inverse relationship between Endostatin concentrations and the corresponding ODs (absorbances). In other words, the greater the concentration of endostatin in the sample, the lower the OD, or less red color.

Statistical analysis: Data are expressed as mean±standard deviation. Statistical analysis was performed using SPSS statistical software for window, release 9.0 (Chicago, IL). Homogeneity of variance was tested using the Levene test. If the variances were homogenous, data were analyzed by using 1 way ANOVA test with Bonfereoni’s correction for multiple comparisons when more than two groups were analyzed. For data sets with non-homogeneous variances, ANOVA test with Tamhane’s post hoc analysis was applied. Individual comparisons were made using student’s 2-tailed t-test, unpaired t test which performed using the SPSS software package for window. The criterion for significance was P value of <0.05 for all comparisons.

RESULTS

Fifty one patients aged between 2 months and 18 years diagnosed as Down syndrome were included in this study. Thirty-nine cases were diagnosed as trisomy 21 and twelve patients were diagnosed as mosaic Down syndrome. Male to female ratio was 23 to 28, respectively. Only two sits with Down syndrome were included in the study. Cardiac anomalies were diagnosed in 58.8% of the studied cases (26 cases with trisomy 21 and 4 cases with mosaicism).

Present results showed that serum endostatin levels ranged from 13.36 to 58.28 ng ml⁻¹ in the control group. As regards the group of Down syndrome, its levels ranged from 15.20 to 126.55 ng ml⁻¹ in cases with mosaicism and from 15.21 to 199.80 ng ml⁻¹ in cases with trisomy 21.

Result revealed that serum endostatin levels were significantly higher in the group of Down syndrome compared to normal control group (99.69±46.36 and 34.36±13.04, respectively) (Table 1) (Fig. 1). There was no statistical difference in endostatin levels by age or sex of DS group when compared to the control group (Table 2 and 3).

Results revealed significantly higher levels of serum endostatin in the population with complete trisomy 21 than in mosaicism and control subjects (117.11±35.63; 43.07±28.44 and 34.36±13.04, respectively). Whereas in cases with mosaicism, endostatin levels showed no statistical differences compared to the control subjects (Table 4) (Fig. 2). On the other hand, no significant difference in endostatin levels between cases with congenital heart and cases without.

DISCUSSION

Present study revealed wide variation in the level of endostatin in both control group and DS patients, which is consistent with the previously reported data by Hebaru et al[3]. This study found that serum endostatin levels were significantly higher in the group of Down syndrome compared to the normal control group (99.69±46.36 and 34.36±13.04, respectively). There was no statistical difference in endostatin levels by age or sex for
Table 1: Basic statistical analysis of serum endostatin levels (ng ml⁻¹) in control (C) and Down syndrome (DS) groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of cases</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>Mean</th>
<th>± SD</th>
<th>± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>22</td>
<td>13.36</td>
<td>58.28</td>
<td>33.33</td>
<td>34.36</td>
<td>13.04</td>
<td>2.78</td>
</tr>
<tr>
<td>DS</td>
<td>51</td>
<td>15.20</td>
<td>199.80</td>
<td>126.55</td>
<td>99.68*</td>
<td>46.35</td>
<td>6.49</td>
</tr>
</tbody>
</table>

* Highly significant increase when compared down syndrome (DS) to control (C) group (P<0.0001)

Table 2: Basic statistical analysis of serum endostatin levels (ng ml⁻¹) in control (C) and Down syndrome (DS) groups (According to gender (male and female))

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gender</th>
<th>No. of cases</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>Mean</th>
<th>± SD</th>
<th>± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Male</td>
<td>12</td>
<td>13.36</td>
<td>58.28</td>
<td>37.14</td>
<td>35.90</td>
<td>13.43</td>
<td>3.87</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>15.72</td>
<td>58.20</td>
<td>20.42</td>
<td>32.50</td>
<td>13.00</td>
<td>4.11</td>
</tr>
<tr>
<td>DS</td>
<td>Male</td>
<td>23</td>
<td>27.64</td>
<td>157.39</td>
<td>126.55</td>
<td>93.63</td>
<td>48.39</td>
<td>9.04</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>28</td>
<td>15.20</td>
<td>199.80</td>
<td>126.26</td>
<td>104.66</td>
<td>48.87</td>
<td>9.23</td>
</tr>
</tbody>
</table>

- Non Significant difference between means values of male and female in control (C) group
- Non Significant difference between means values of male and female in Down syndrome (DS) group
- Significant increased when compared male or female in Down syndrome (DS) group to the same gender or others in Control (C) group, (P<0.05)

Table 3: Basic statistical analysis of serum endostatin levels (ng ml⁻¹) in control (C) and Down syndrome (DS) groups (According to age <24 month>)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age</th>
<th>No. of cases</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>Mean</th>
<th>± SD</th>
<th>± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>&lt;24 month</td>
<td>3</td>
<td>24.84</td>
<td>34.58</td>
<td>32.08</td>
<td>30.50</td>
<td>5.05</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>&gt;24 month</td>
<td>19</td>
<td>13.36</td>
<td>58.28</td>
<td>34.97</td>
<td>34.97</td>
<td>13.83</td>
<td>3.18</td>
</tr>
<tr>
<td>DS</td>
<td>&lt;24 month</td>
<td>19</td>
<td>15.21</td>
<td>199.80</td>
<td>125.97</td>
<td>97.47</td>
<td>51.22</td>
<td>11.75</td>
</tr>
<tr>
<td></td>
<td>&gt;24 month</td>
<td>32</td>
<td>15.20</td>
<td>182.20</td>
<td>126.55</td>
<td>101.00</td>
<td>44.02</td>
<td>7.78</td>
</tr>
</tbody>
</table>

Table 4: Basic statistical analysis of serum endostatin levels (ng ml⁻¹) in control (C) and Down syndrome (DS) groups (trisomy 21 (T) and mosaic (M))

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type</th>
<th>No. of cases</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>Mean</th>
<th>± SD</th>
<th>± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>C</td>
<td>22</td>
<td>13.36</td>
<td>58.28</td>
<td>33.33</td>
<td>34.36</td>
<td>13.04</td>
<td>2.78</td>
</tr>
<tr>
<td>DS</td>
<td>M</td>
<td>12</td>
<td>15.20</td>
<td>126.55</td>
<td>37.28</td>
<td>43.07</td>
<td>28.44</td>
<td>8.21</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>30</td>
<td>15.21</td>
<td>199.80</td>
<td>126.55</td>
<td>117.10*</td>
<td>35.63</td>
<td>5.70</td>
</tr>
</tbody>
</table>

* Significant increased when compared mean of trisomy 21 group (T) to mosaic (M) and control (C) group (P>0.005)

Fig. 1: Distribution patterns of Endostatin levels (ng ml⁻¹) in healthy control (C) and Down syndrome (DS) groups

Fig. 2: Distribution values of serum endostatin levels (ng ml⁻¹) in control (C), mosaic (M) and trisomy (T)
DS group or the control group. These results agreed with those reported by Zorick et al.\textsuperscript{[9]} However, present work compare the level of endostatin of those DS with trisomy 21 and mosaic group. We emphasize the gene dosage effect where in cases with mosaicism there was no statistical difference of the level of endostatin compared to control group.

Evidence emerged that angiogenesis is tightly regulated by a balance of activating and inhibiting factors\textsuperscript{[10]}. Therefore, continuous over expression of angiogenic factors by gene therapy, for instance, should counteract the tumor-induced angiogenesis. Many tumor and non-tumor-associated angiogenic factors have been described. The proteolytic cleavage of larger precursor molecules associated with the vascular system (proteins of the coagulation cascade and basement membrane proteins) is thought to play an important role in the generation of several of these angiogenic proteins and thus, in the control of angiogenesis.

The significantly higher levels of serum endostatin in trisomy 21 cases compared to mosaic and control cases in present study is the result of gene dosage effect of endostatin precursor which has been mapped by Suzuki et al.\textsuperscript{[4]} at 21q22.3.

Barlow et al.\textsuperscript{[14]} reported a region on chromosome 21 which is responsible for the production of subsets with DS congenital heart disease. This region does not include genes encoding collagen XVIII. It was reported by Lorenza et al.\textsuperscript{[22]} that during the development of the cardiac valves, transformation, migration and proliferation of endothelial cell derivatives occur. This process is indispensable for the proper formation of the valves. The gene which encodes collagen XVIII is localized on chromosome 21. They hypothesize that an increase in the expression of collagen XVIII is directly related to cardiac malformations of DS individuals. Present results showed no significant difference in endostatin levels between cases with congenital heart and cases without congenital heart.

The population with Down's syndrome has a different cancer profile compared to the general population, even after taking into account issues of survival and ageing. Several solid tumours are unusually rare, whereas in contrast leukaemias are increased. Risk of female breast cancer was found to be very low in patients with DS compared to general population which could be partly explained by over expression of genes linked to gene dosage effects on chromosome 21, playing a role in cell growth, differentiation, survival and death: An additional protective effect could come from the marked and continued decreased exposure to oestrogens, starting in utero for women with trisomy 21 and lasting all over life\textsuperscript{[13]}.

High endostatin levels have been reported in patients with systemic sclerosis\textsuperscript{[9]}. However, Distler et al.\textsuperscript{[14]} reported normal levels of endostatin or even low levels in patients with systemic sclerosis. Whether enhanced levels of endostatin might be specific for certain subgroups of systemic sclerosis, it remains to be examined in further studies.

It was proposed that in Down syndrome, the overdose of otherwise perfectly normal genes causes disorders of human health, indistinguishable from major public health problems of the general population, such as mandatory early onset Alzheimers degeneration, increased risk of leukemia and protection from cancer of solid tissues. The DNA sequence of human chromosome 21 is, at the moment, the most complete piece of DNA sequence known in the whole of human genome\textsuperscript{[26]}.

Haemangiomas occurred in 1/100 of all new borns worldwide and up to 30% in prematures. However, it is very rare to be associated in individuals with DS. There are few reports about the co-incidence of haemangioma and Down syndrome. Musarella and Vermes\textsuperscript{[40]} reported a case of double aneuaploidy involving Down and Turner cell lines in a female child with a massive capillary hemangiomma of the left orbit and mild clinical features of Down syndrome. Needless to say that the reported observation could be due to the double aneuaploidy.

Present study concluded that elevated serum endostatin is a gene dosage effect up to the level seen in DS patients (3.5 folds of the control levels) without side effects. So, it can be used as a preventative substance for high risk population and its deficiency can be used as a marker for solid tumour susceptibility which may help in further studies in the field of cancer treatment.

REFERENCES