SERUM ECD LEVELS OF EGFR IN GTD

VII. SERUM ECD LEVELS OF EGFR IN GTD

INTRODUCTION

Many transmembrane growth factor and cytokine receptors have been reported to have analogous soluble, ligand-binding receptor forms detectable in the culture supernates of cell lines, and in biological fluids such as serum and urine (Heaney and Golde, 1993). The widespread distribution of soluble receptors suggests that these molecules may have important physiological roles (Reiter and Maihle, 1996).

Soluble isoforms of the *ERBB* proto-oncogene family, which encodes four homologous receptor tyrosine kinases known as EGFR/ErbB1 (HER1), ErbB2 (HER2/*neu*), ErbB3 (HER3), and ErbB4 (HER4), are being explored as serum biomarkers with potential utility in the risk assessment, screening, and diagnosis of cancer (Brandt-Rauf, 1995 and Maihle et al, 2002). Soluble ErbB (sErbB) isoforms that embody extensive portions of the receptor extra-cellular domain exist for all four members of this receptor family. sErbB isoforms are produced either by proteolytic cleavage of the full-length receptor (Zabrecky et al, 1991 and Pupa et al, 1993) or by alternate splicing of mRNA transcripts (Maihle et al, 1991 and Reiter and Maihle, 1996). sEGFR is identical to EGFR until amino acid 603 according to the nucleotide numbering system of Ullrich et al. (1984), and, hence, comprises extracellular subdomains I-IV (Baron et al, 2003)

The serum level of EGFR-ECD (sEGFR) was assessed by ELISA technique in normal and molar placenta, to evaluate its potential as a novel biomarker in GTD.

RESULTS:

Table 49 - Serum EGFR-ECD in placenta and GTD (number of cases)

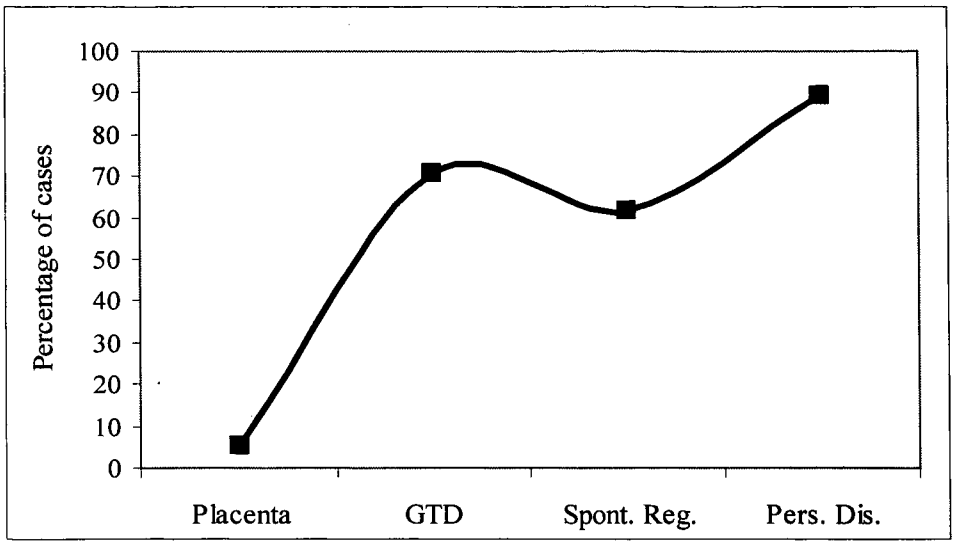
	Low	Normal	High	χ2
Placenta	0 (0%)	17 (94.5%)	1 (5.5%)	<0.001*
GTD	1 (1.7%)	16 (27.6%)	41 (70.7%)	
Spont. Reg.	1 (2.6%)	14 (35.9%)	24 (61.5%)	
Slow Reg.	0 (0%)	1 (9.1%)	10 (90.9%)	0.067**
CT	0 (0%)	1 (12.5%)	7 (87.5%)	
Pers. Dis.	0 (0%)	2 (10.5%)	17(89.5%)	0.029** 0.007*

*p-value was significant in comparison with normal placenta

**p-value was significant in comparison with spontaneously regressing group

Molar placenta and normal placenta were analyzed by EGFR ELISA, with samples tested in duplicate. The determination of a normal value was defined as the mean value for the normal patient sera samples, a case with exceptionally high level being exempted. The range for EGFR ECD in normal sera was 16-21 fmol/ml. Values obtained from the GTD sera showed an increase in 70.7 percent of the cases, statistically significant at 0.01 significance level ($p<0.001$); values ranged from 17-44 fmol/ml. Up to 61.5% of the spontaneously regressing cases exhibited high levels of EGFR-ECD in sera compared to the normal placenta, whereas 89.5% of the persisting disease cases showed high levels of sera ECD; which was statistically significant ($p=0.007$) (Table 49, Fig 49). A significant correlation was observed between presence of serum EGFR-ECD and expression of EGFR-ICD protein ($p=0.001$). The presence of higher levels of serum EGFR-ECD also correlated well with the high phosphorylation status of EGFR ($p<0.001$), with the presence of EGFR gene amplification ($p=0.004$) and with the expression of Akt protein ($p=0.038$). A significant correlation was also obtained with the diagnosis of GTD ($p<0.001$) and with disease progression ($p<0.001$).

Fig 49- Serum EGFR-ECD levels in GTD, in relation to normal placenta



The samples positive for higher levels of serum EGFR-ECD showed a relative risk of 41 fold for GTD compared to those cases that did not register positivity (95% CI=0.003-0.198, p-value<0.001). The relative risk to for serum EGFR-ECD positive patients to develop persistent disease was found to be very high (145 times) with a confidence interval of 0.001-0.084 and a significant p-value ($p<0.001$), whereas the relative risk for spontaneously regressing cases was found to be only 24 fold (95% CI=0.005-0.339, p-value<0.001)

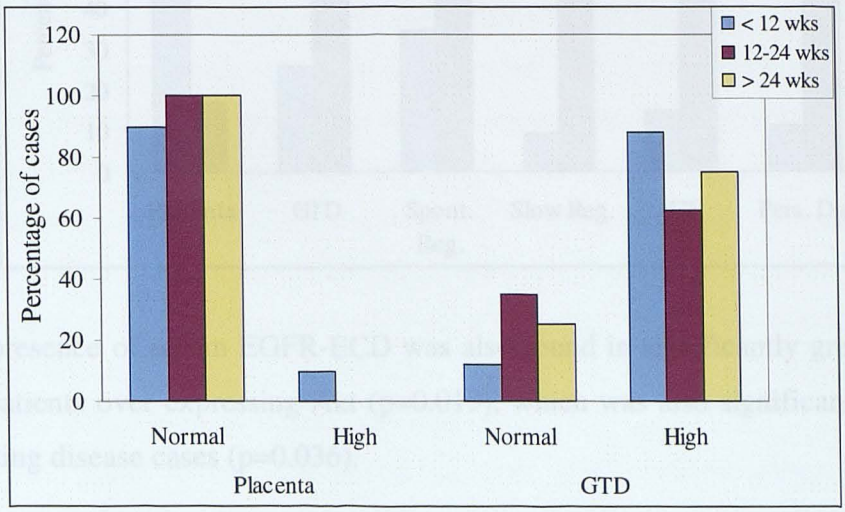
Table 50- Serum EGFR-ECD in different gestational age groups of normal placenta and GTD

	Placenta			GTD			χ^2
	Low	Normal	High	Low	Normal	High	
<12 weeks	0 (0%)	9 (90%)	1 (10%)	0 (0%)	2 (11.8%)	15 (88.2%)	0.000*
12-24 weeks	0 (0%)	3 (100%)	0 (0%)	1 (2.7%)	13 (35.1%)	23 (62.3%)	0.069*
>24 weeks	0 (0%)	5(100%)	0 (0%)	0 (0%)	1 (257%)	3 (75%)	0.048*

* p-value was significant in comparison with normal placenta

Of the GTD cases, the first trimester group showed a higher number of cases to be positive for high levels of serum-EGFR (88.2%) followed by the third trimester group (75%), and these levels revealed significant $\chi 2$ values on statistical analysis ($p<0.001$ and $p=0.048$, respectively) (Table50 & Fig 50). The mid gestational group registered high levels of serum-EGFR-ECD only in the case belonging to the persistent disease group. The early-gestation cases revealed significant correlation of serum EGFR-ECD with the expression of EGFR-ICD ($p=0.012$) and with the high levels of EGFR protein phosphorylation ($p=0.008$). The other two groups, however, did not register any such association. No significant change was evident between the different prognostic groups when analysed based on the gestational age groups.

Fig 50 - Serum EGFR-ECD levels in different gestational age groups of normal placenta and GTD



The high levels of serum secreted EGFR-ECD, was obtained in the early gestational ages, in molar placenta.

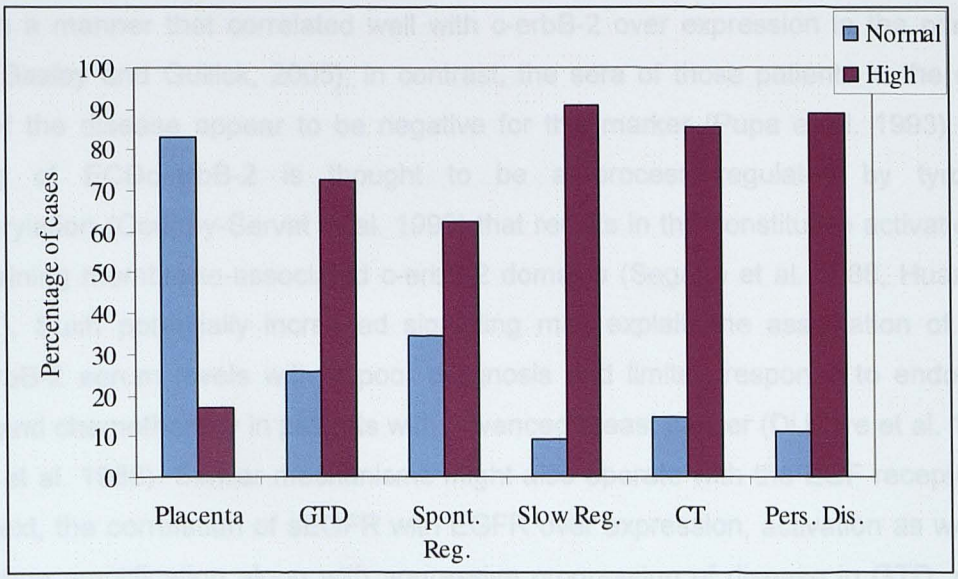
***Table 51 - Status of serum EGFR-ECD levels in EGFR-ICD immunopositive GTD and Placenta** (% cases)*

Serum EGFR-ECD	Low	Normal	High	X^2
Placenta	0	83.3	16.7	<0.017*
GTD	2.2	25.5	72.3	
Spontaneous	3.4	34.5	62.1	
Slow	0	9.1	90.9	0.076**
CT	0	14.3	85.7	NS
Persistent Disease	0	11.1	88.9	0.001* 0.046**

*p-value was significant in comparison with normal placenta
**p-value was significant in comparison with spontaneously regressing group

Serum EGFR-ECD positivity was observed to be high in majority of the EGFR-ICD immunopositive GTD cases (76.9%) analysed, compared to normal placenta ($p<0.001$). All the samples of the persistent disease group revealed presence of high levels of ECD in the serum ($p=0.001$) (Table 51, Fig 51).

Fig 51 - Serum EGFR-ECD levels in EGFR-ICD positive cases



The presence of serum EGFR-ECD was also found in significantly greater number of GTD patients over expressing Akt ($p=0.019$), which was also significantly higher in the persisting disease cases ($p=0.036$).

INFERENCE: **Serum EGFR-ECD levels are elevated in GTD**

DISCUSSION

A marked increase in levels of EGFR-ECD present in serum was noted in GTD cases. All the persistent disease group samples showed high levels of serum EGFR-ECD. The relative risk assessment conferred a very high fold chance for patients positive for this marker to develop persisting disease. The observations reported in this study indicate that serum sEGFR is a prognostic indicator of persisting disease and may have clinical utility as a new biomarker of persistent GTD. This should be validated in prospective studies. The high levels were also in correlation with the expression of EGFR-ICD and Akt, suggesting a link with the EGFR mediated Akt survival pathway.

A study by Kumar et al (2001) noted that 67.5% of breast cancer patients had elevated levels of circulating EGFR. This is in agreement with our results which revealed that 70.7% of GTD patients had higher sEGFR concentrations. Preoperative serum p105 sErbB2 concentrations have also been found to be elevated in women with ovarian cancer in comparison with healthy women (Meden et al, 1994; Meden et al, 1997; Cheung et al, 1999; Yazici et al, 2000).

Soluble ECDc-erbB-2 was found in the serum of patients with advanced breast cancer in a manner that correlated well with c-erbB-2 over expression in the primary tumour (Bazley and Gullick, 2005); in contrast, the sera of those patients in the early stages of the disease appear to be negative for this marker (Pupa et al. 1993). The shedding of ECDc-erbB-2 is thought to be a process regulated by tyrosine phosphorylation (Codony-Servat et al. 1999) that results in the constitutive activation of the remaining membrane-associated c-erbB-2 domains (Segatto et al. 1988, Huang et al. 1997). Such potentially increased signalling may explain the association of high ECDc-erbB-2 serum levels with a poor prognosis and limited response to endocrine therapy and chemotherapy in patients with advanced breast cancer (Di Fiore et al. 1987, Segatto et al. 1988). Similar mechanisms might also operate with the EGF receptor. In this pretext, the correlation of sEGFR with EGFR over expression, activation as well as EGFR gene amplification along with aggressive progression of disease in GTD, might indicate that a similar post-translational mechanism may result in the shedding of EGFR-ECD in patient sera. Thence identification of sera EGFR-ECD will also represent the constitutively active form of receptor in these cases.

It has been shown that sErbB isoforms are produced either by proteolytic cleavage of the full-length receptor (Zabrecky et al, 1991; Pupa et al, 1993) or by alternate splicing of mRNA transcripts (Ilekis et al, 1991; Ilekis et al, 1995; Maihle et al, 1991; Katoh et al, 1993; Scott et al, 1993; Das et al, 1994; Reiter and Maihle, 1996; Tong et al, 1996; Lee and Maihle, 1998; Doherty et al, 1999; Reiter et al, 2001). Perez

et al, (2002) noted that alternative splicing of transcripts arising from the human epidermal growth factor receptor (EGFR) gene generates a 3.0-kb transcript that encodes a 110 kD isoform of EGFR. The 110 kD EGFR isoform contains the four subdomains of the extracellular portion of the full-length 170 kD EGFR, and contains 78 unique carboxy-terminal amino acids. The 110 kD-EGFR is localized to the plasma membrane in transfected fibroblasts. Baron et al, (2003) demonstrated that in MDA-MB-468 and in transfected CHO cells expressing the 110 kD-EGFR cDNA, the shedding of cell surface 110 kD-EGFR is activated by phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (P1(C), and by 13-PMA, an active analogue of PMA suggesting that the shedding of 110 kD-EGFR is regulated by a PKC-dependent mechanism and by serum factors. These observations thus reveal another possible mechanism for the shedding of sEGFR.

They have also suggested that serum sEGFR may have clinical utility as a new biomarker. Baron et al, (2003) also observed that although the relationship between serum p110 sEGFR concentration and tumour EGFR expression in EOC is currently unknown, a positive immunohistochemical status of EGFR has been reported recently to be an independent prognostic factor of EOC, thus highlighting the potential importance of circulating sEGFR isoforms as prognostic factors for this disease (Skirnisdottir et al, 2001). In the light of these findings our observations on sEGFR levels and concordant results on immunohistochemical evaluation also imply the utility of sEGFR as a potential biomarker for GTD, especially for persisting disease group.

However, our findings are based on retrospective analyses on a small number of patients treated at a single institution. Hence the clinical potential of serum EGFR-ECD as a new biomarker for persisting disease must be validated in a multi-centre nation wide well designed prospective case control study.