

*Full Length Research Paper*

# Clinically relevance of ectopic bone formation to the structure of TGF- $\beta$ superfamily in stress condition

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Formation of bone outside the skeletal system which can occur in all kinds of soft tissues is called Ectopic or Heterotopic Ossification (HO). It is the abnormal formation of true bone within extra skeletal soft tissues. The TGF- $\beta$  super family several physiological processes are mediate by these polypeptides such as regulates bone formation and resorption as well as osteoclasts differentiation and survival is stimulated by them. Total of 21 head injured patients admitted in the hospital between 8 to 33 years old. All individuals are treated with indomethacin to control HO formation. The red light treatment and heat lamp exposure were done for 20 min in each day of physiotherapy. Both effective members of transforming growth factor-  $\beta$ , superfamily were analyzed by molecular mechanic methods in amber force field. Montecarlo simulation was done for 200 pSec and the  $\psi$ ,  $\phi$  angles of each amino acids were calculated. Clinically, of 25 potentially relevant records identified, 21 were related to HO. Following the treatment, 11 individuals showed the response to heat exposure and physical exercises. 9 out of 21 not only never do effective response to heat treatment, but also progress the HO formation. The results reveal that 2 tgi tends to arrange the protein critical angels of  $\phi$  and  $\psi$ , into more favored and/ or allowed region and the formation and development of right handed, left handed  $\alpha$ - helices and  $\beta$ - pleated sheath will be more facilitated because of rising in temperature. It is suggested that this phenomenon cause 2tgi to act in a better thermodynamic situation.

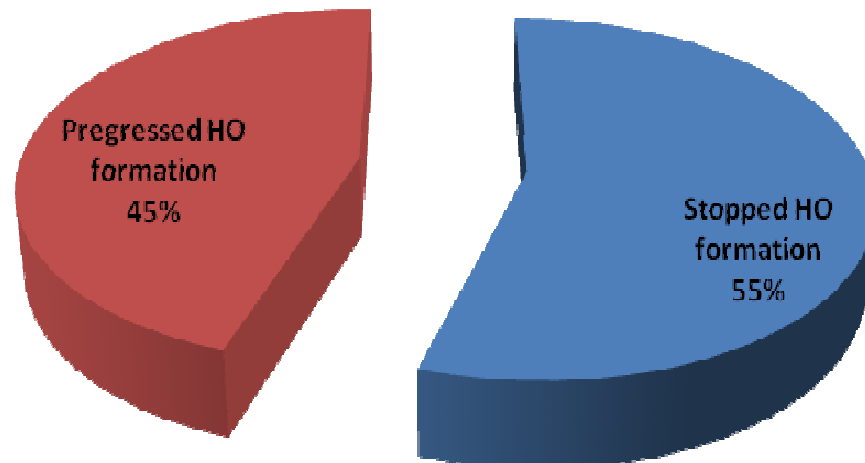
**Key words:** Ramachandran plot, Montecarlo simulation ectopic bone formation, transforming growth factor-  $\beta$ ; 1tfg; 2tgi.

## INTRODUCTION

Formation of bone outside the skeletal system which can occur in all kinds of soft tissues is called Ectopic or Heterotopic Ossification (HO). It is the abnormal formation of true bone within extra skeletal soft tissues. It is possible that the normal day life abilities lead to severe disabilities which can be caused by such a phenomenon, depending on the skeletal zones involved. It is frequently reported in head injured patients or in orthopedic surgery, after surgery, although the pathogenesis has not been fully understood (McCarthy, 2005; Kim, 2008). The TGF- $\beta$  superfamily includes a large group of polypeptides such as the bone morphogenic proteins (BMPs), differentiation factors (GDFs) and the growth factor. There are some structural variations in C-terminal amino acid sequences in this superfamily and this phenomenon makes various morphogens (Wozney, 1992; Reddi, 1997). Several physiological processes are mediate by polypeptides.

Tissue morphogenesis and regeneration, cell growth and differentiation, bone induction and modulation, the regulation of hormone secretion, immune response and neuro protection are some examples. Bone induction is mediated by the morphogens in a cascade of cellular and molecular events which occur in embryonic bone development (Reddi, 1976; Jin, 2000; Nonner, 2004). Osteoblasts express TGF- $\beta$ ; where osteoclasts differentiation and survival is stimulated by them (Chenu, 1988). Regulates bone formation and resorption (Pfeilschifter, 1987; Bonewald, 1990). The various osteogenic molecules in this family share many similar biological activities (Groeneveld, 2000), TGF- $\beta$  superfamily, initiate post-natal bone formation either singly or in combination (Langer, 1993).

Bone formation and regeneration are done by involving the recruitment of adult mesenchymal cells (Pittenger, 1999).



**Figure 1.** Heterotopic ossification progress in patients. 9 out of 21 has progression during exposure to heat.

It is not well known how the processes of proliferation and differentiation of mesenchymal cells are progressed. The signaling response by osteoprogenitor cells bearing receptors for these bone-inducing molecules is a main suggestion of TGF- $\beta$  superfamily mediated mechanism (Das, 1999; ten Dijke, 2000; Blair, 2002). Heterotopic ossification is not dependent to patient risk factors, some conditions such as ectopic bone formation after previous surgery, ankylosing spondylitis, diffuse idiopathic skeletal hyperostosis, hypertrophic osteoarthritis increase the risk of HO formation (Ritter, 1977; Vastel, 1998). Soft tissue trauma as it relates to the level of surgeon experience (Vastel, 1998) and surgical approach used to perform total hip arthroplasty (Shaffer, 1989) can contribute to HO formation. Our continuously study shows that the effect of physical treatment of patients in the early phase of HO formation is different, therefore to investigate the reason of that is necessary.

## MATERIALS AND METHODS

Total of 25 head injured patients admitted in the emergency medicine departments and general surgery between January 2003 and November 2010 were included in the study. The patients were retrospectively studied by radiologic data. The age of patients vary between 8 to 33 years old. All individuals are treated with indomethacin to control HO formation. The manifestations of HO appeared in 21 whom were followed in this study; 13 men and 8 female were included. The physically exercises in physiotherapy center were done 2 to 3 times a week, for 2 h and started after at least 6 to 8 months when patients feeling healthy situation and allowance of neurologist. The red light treatment and hot lamp exposure were done for 20 min in each day of physiotherapy. The classification of patients in present study was done as follows (Sell, 1998): In computational phase, both effective members of transforming growth factor-  $\beta$ , superfamily were analyzed by molecular mechanic methods in amber force field. Montecarlo simulation was done for 200p Sec with Hyperchem 8.0.6; the

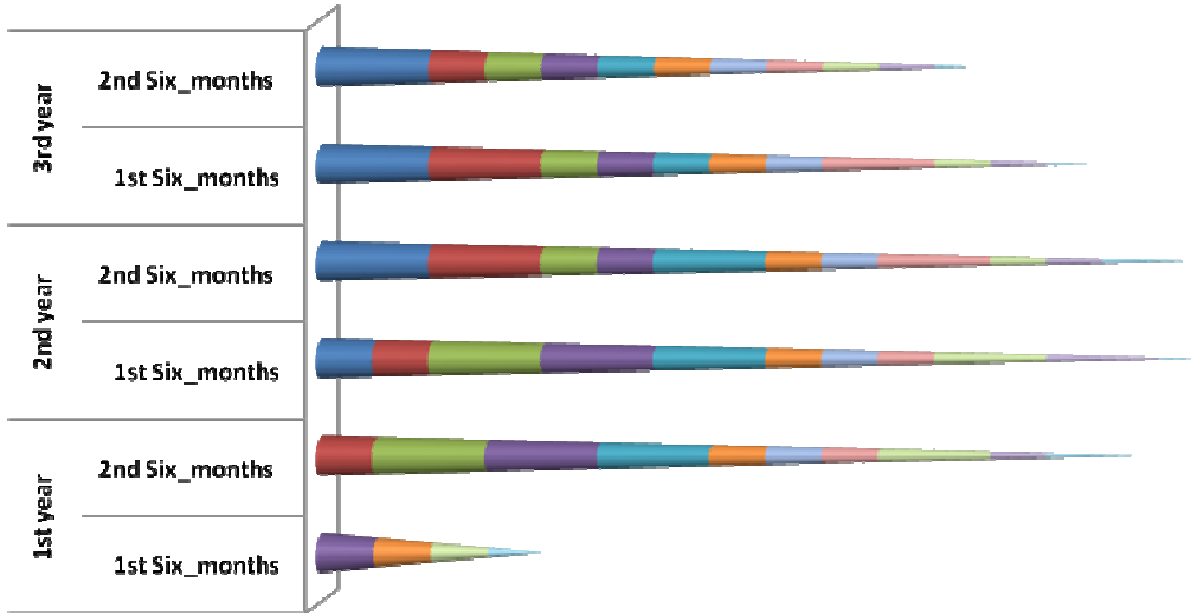
molecular mechanic method with both MM+ and Charmm force fields were done and the  $\psi$  and  $\phi$  angles of each amino acids were calculated with VMD 1.8.2.

## RESULTS

Of 25 potentially relevant records identified, 21 were related to HO. Following the treatment of 55% ( $n = 11$ ) individuals showed the response to heat exposure and physical exercises. 45% (9 out of 21) not only never do effective response to heat treatment, but also progress the HO formation (Figure 1). The results reveal that in all 11 individuals, the ectopic bone formation is decreased in response to heat exposure (Figure 2) in contrast to others who have progressed (Figure 3). The follow up were done every 6 months for a 3 years interval. It is though that 1tgi and 2tgi (Figure 4) mediated different rules in such a phenomenon. In 1st structural view, it is reveal that there are some little differences in both 2 structures in secondary structure format (Figure 5). The Montecarlo simulation in 200p Sec showed that 2tgi went toward a stable tertiary structure. The sedimentation rate of all individuals are greater than 35 mm/h and a serum alkaline phosphatase level were greater than 250 IU/L at 12 weeks postoperatively to confirm the presence of HO processes, data were not included in this paper.

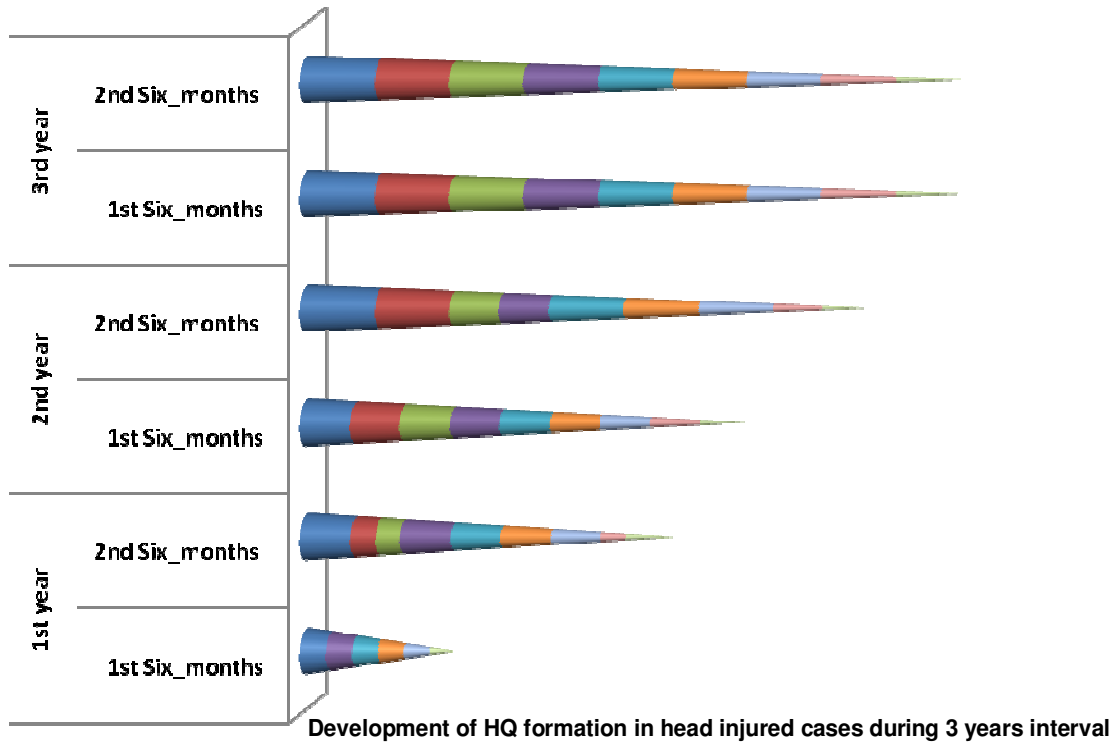
## DISCUSSION

Heterotopic ossification is a complication in some head injured patients as post traumatic effects. Pain and disability are the main effects of this ectopic bone formation. Prophylactic treatment is a preferred indication after surgery. Within 3 to 4 weeks, the findings of ossification may be visible on plain radiographs. Up to 1



Development of HQ formation in head injured cases during 3 years interval

Figure 2. The response of patients to heat exposure during a 36 month follow-up period. The progression is decreased.



Development of HQ formation in head injured cases during 3 years interval

Figure 3. The response of patients to heat exposure during a 36 month follow-up period. The progression is increased based on results.

or 2 years, the maturation of the HO would be occurred. Currently, nonsteroidal anti-inflammatory drugs (NSAIDs)

have been used as routine drugs to prevent HO. NSAID for HO prophylaxis are commonly used and Indomethacin

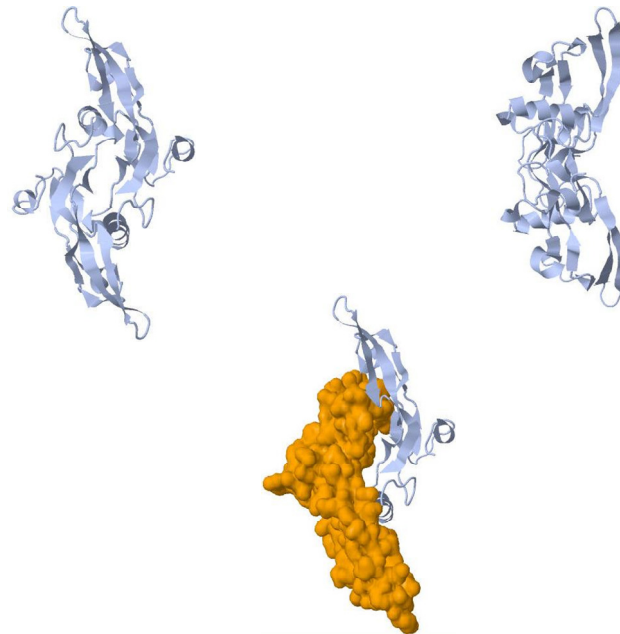


Figure 4. The tertiary structure of 1tfg and 2tgi.

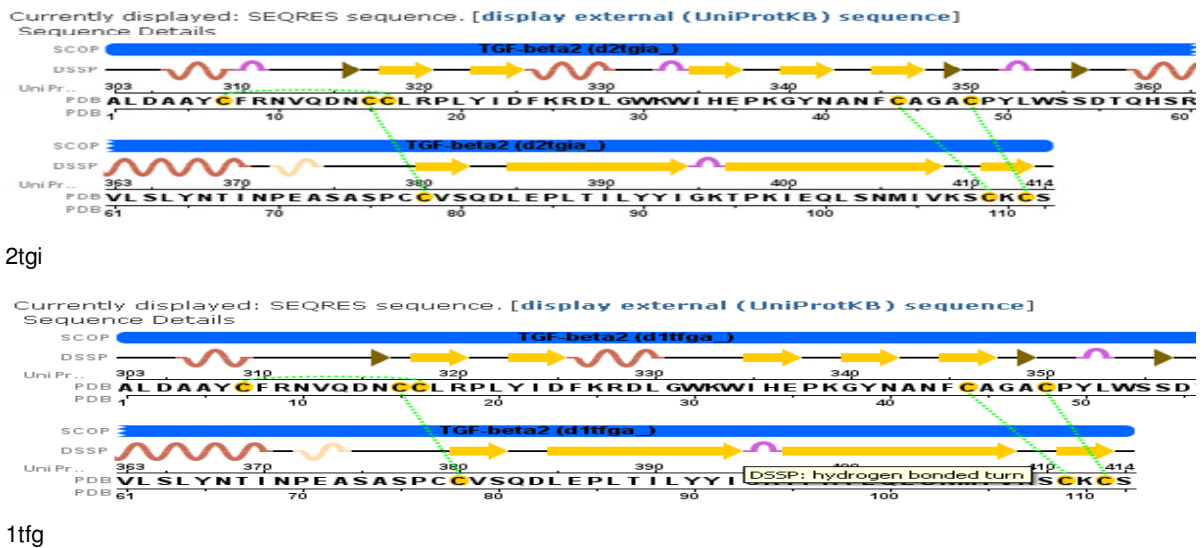
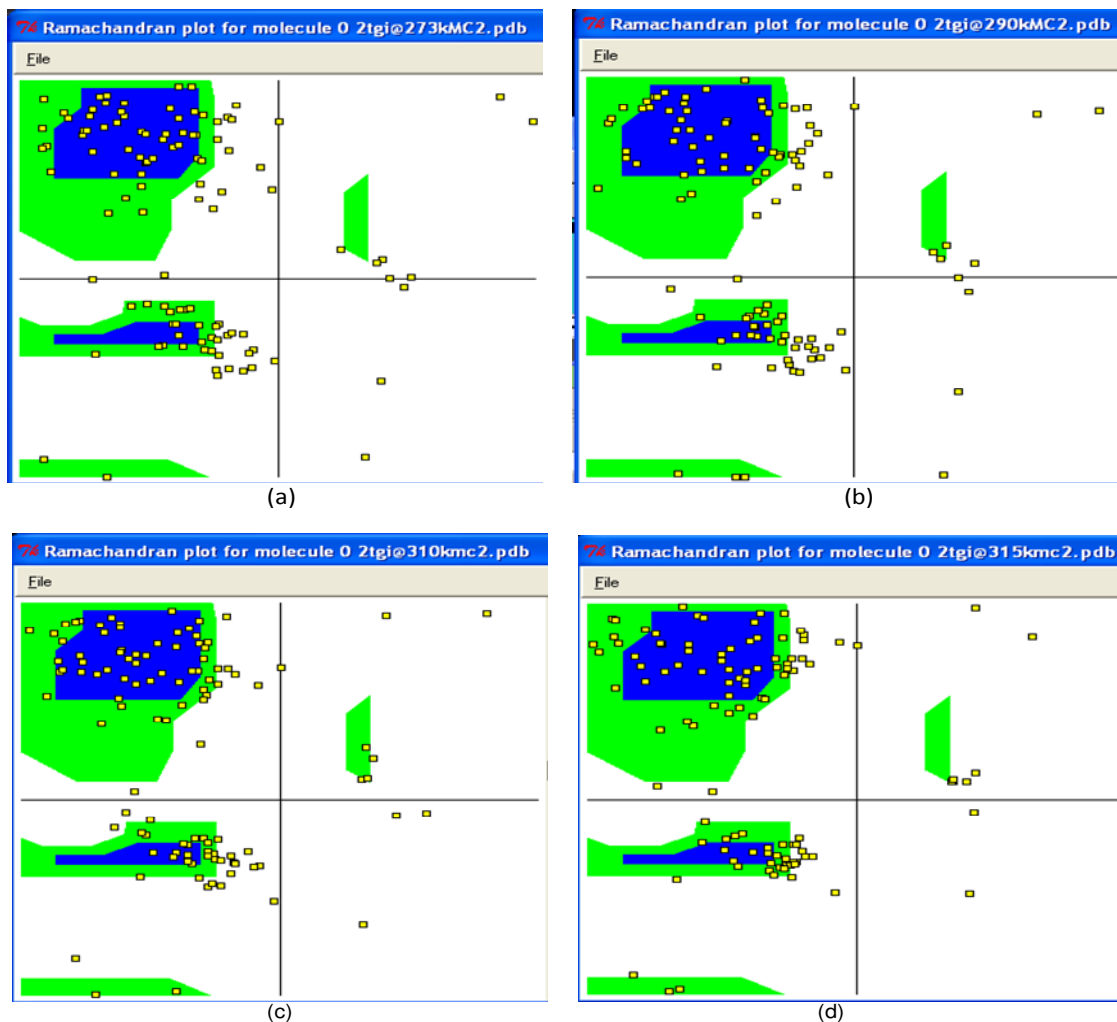


Figure 5. Comparison of secondary structure of 1tfg and 2 tgi (www.pdb.org).

is the most effective one. The recommended dose of indomethacin is 75 to 100 mg/day and should be continued for 7 to 14 days. In patients receiving indomethacin in conjunction with anticoagulation for thromboembolic prophylaxis bleeding complications are more frequent (Sell, 1998). All patients treated with 'indomethacin' with a certain dosage. The radiographic date has reveal that some patients response to the heat and physical exercises, but in some patients the sever

development of ectopic ossification is observed (Table 1) (Khalili, 2011). Data in Figure 2 shows that the development rate of HO formation is progressive but it tends to be going to a plateau phase and decrease after exposure to heat.

In contrast, Figure 3 shows increasing in the rate of HO development. Although the tertiary structure of both 1tfg and 2tgi are similar in view (Figure 4), but there are some difference secondary structure.



**Figure 6.** The ramachandran plot related to 2tgi after Montecarlo simulation for 200p Sec. The result are extracted from Montecarlo simulation in 273 K (a), 290 K (b), 310 K (c) and 315 K (d).

**Table 2.** Ramachandran plot results for 1tfg and 2tgi in various temperatures.

	2tgi		1tfg	
	Favored	Allowed	Favored	Allowed
Main data (K) (pdb.org)	106	110	104	109
273	41	61	n.i.	n.i.
290	40	60	38	78
310	44	70	31	62
315	34	74	n.i.	n.i.

n.i.= Not identified.

## Conclusion

It is tough that, increasing temperature may cause denaturation in protein and it undergoes protein dysfunction. But the results reveal that 2tgi tends to

arrange the protein critical angles of  $\phi$  and  $\psi$ , into more favored and/or allowed region and the formation and development of right handed, left handed  $\alpha$ - helices and  $\beta$ - pleated sheath will be more facilitated because of rising in temperature (Figure 6, Table 2). It is suggested

that this phenomenon cause 2tgi to works better that expected and mediated the osteoblasts mechanism in changing the connective tissue to ectopic bone. Although the rising mechanism of TGF-  $\beta$  in post trauma patients is not clearly understood, but it is suggested that the nerve protecting rule of these super family is the main cause. May be its elevating cause the connective tissue around the contusion zone, make some hard tissues to prevent local damages and prevents the response of macrophages. In other zones it is clear that some side effects would be observed, such as heterotypic ossification. 1tfg undergoes denaturation based on results explore in Table 2. Therefore it is expectable that some patients with higher 1tfg rather than 2tgi get healthier situation than those with more 2tgi.

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