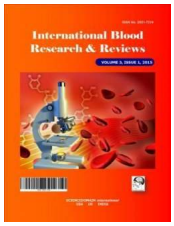

International Blood Research & Reviews
4(3): 1-8, 2015, Article no.IBRR.20213
ISSN: 2321–7219

SCIENCEDOMAIN *international*
www.sciencedomain.org

Expression of Glucocorticoid Receptor Isoforms (α, β, γ, and p) in Egyptian Primary Immune Thrombocytopenia Patients

Nahla A. M. Hamed[1], Mohamed Aldefrawy[1], Dalia Elneely[2], Omar Ghallab[1*] and Beatrice Jepngetich[3]

[1]Hematology Unit, Department of Internal Medicine, Alexandria University, Egypt.
[2]Department of Clinical and Chemical Pathology, Alexandria University, Egypt.
[3]Moi Teaching and Referral Hospital, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Author NAMH designed the study and contributed in writing protocol and manuscript. Authors MA and OG contributed in writing manuscript. Author DE did the laboratory work and contributed in writing manuscript. Author BJ collected samples, data and contributed in writing manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2015/20213
Editor(s):
(1) Felipe Samaniego, Department of Lymphoma/Myeloma, Division of Cancer Medicine, The University of Texas, MD Anderson Cancer Center, USA.
Reviewers:
(1) Ernst Glenda, British Hospital, Pulmonary Lab, Portugal.
(2) Anonymous, University of Patras, Patras, Greece.
(3) Anonymous, University of Ibadan, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/11719

Original Research Article

Received 17th July 2015
Accepted 19th September 2015
Published 7th October 2015

ABSTRACT

Glucocorticoid (GC) resistance has been demonstrated in nearly 30% of primary immune thrombocytopenia (ITP) patients even managed with high dosages GC. The biological effects of GC are mainly mediated through activation of glucocorticoid receptors (GR). An insight into the molecular mechanisms underlying GC resistance is important to avoid GC treatment in patients contraindicated from steroid use. We aimed at determining glucocorticoid receptor (GR) isoforms expression in adult ITP and its relation to glucocorticoid resistance. Thirty three ITP patients from the Hematology unit, Alexandria Main University Hospital were the subject of the study. They were subdivided into two groups (sensitive and resistant) according to their response to 4 weeks GC treatment. 15 healthy volunteers of matched age were also included. Glucocorticoid Receptor α, β,

**Corresponding author: E-mail: ghallab_2002@yahoo.com;*

γ and p gene expression were measured in cases and controls by real time Polymerase Chain Reaction. The mean age value in GC sensitive, GC resistant and control group was 33.4±11.6, 38.1±12.3 and 31.7±5.8 years respectively. Statistically significant difference between GR alpha mRNA isoform and GRα/ GRβ ratio was detected between GC resistant and GC responsive group while GRβ, GRγ and GRβ were insignificantly differed between groups. From this study, we concluded that study of GRα and GRα/ GRβ ratio is recommended early in ITP to avoid unnecessary glucocorticoid side effects.

Keywords: Glucocorticoid receptor; thrombocytopenia; glucocorticoid resistance.

1. INTRODUCTION

Immune thrombocytopenia (ITP) is an acquired immune mediated disorder characterized by isolated thrombocytopenia, less than 100 x 10^9/L in the absence of any obvious initiating and/or underlying cause of the thrombocytopenia [1]. Clinical features range from no symptoms or minimal bruising to serious bleeding which may include gastrointestinal hemorrhage, or intracranial hemorrhage [1].

Platelet counts less than 30 x 10^9/L threshold have been suggested as a cutoff for considering treatment of ITP. Glucocorticoids (GCs) are a mainstay and the most effective treatment for ITP patients. Approximately 75% of patients initially respond to corticosteroids, although tapering usually precipitates relapse. Only 10%-15% of patients are able to maintain a safe platelet count after steroid discontinuation. Use of high-dose corticosteroids early in the treatment course was suggested by some studies to induce more durable remissions [1,2].

GC resistance has been demonstrated in nearly 30% of ITP patients even managed with high dosages GC. Considering the wide range of side effects of GC, it is important to avoid GC treatment in those patients who will not benefit from its use. The biological effects of GC are mainly mediated through the activation of glucocorticoid receptors (GR). Binding of GC–GR complex to DNA [3] leads to a reduction of the cellular pool of transcription factors, such as nuclear factor κB and activator protein-1, as well as the decreased expression of genes contributing to the autoreactivity of T and B cells [3,4].

Different GR isoforms have been identified—GRα, GRβ, GRγ, GRp, GRA, and GRB—of which GRα is thought to be the primary mediator of GC action. However, GRβ behaves as an inhibitor of GRα activity. The imbalance of GRα, GRβ, or GRα/GRβ ratio was found to be related to GC resistance in several diseases. GRA and GRB have not yet been candidates for investigations. It is still unknown whether GC resistance in ITP patients is associated with GR isoforms expression [3]. So we aimed at testing the expression of GR isoforms (GRα, GRβ, GRγ and GRp) in adult ITP and its contribution to the differential GC resistance.

2. METHODOLOGY

Thirty three ITP patients and 15 age matched healthy volunteers were investigated. Enrollment took place from the Hematology unit, Alexandria Main University Hospital between April 2014 and June 2015. All the patients met the diagnostic criteria for ITP [5]. According to GC response, they were further categorized into GC-sensitive (GCS) and GC-resistant (GCR) groups. GC sensitivity was defined based on the platelet count after taking prednisone 1 mg/kg/day for 4 weeks. Patients were considered GCS if platelet more than 30×10^9/L and resistant if platelet less than 30×10^9/L. Pregnancy, chronic infections especially HCV and *H. pylori* or connective tissue diseases, such as systemic lupus erythromatosus as well as splenectomized patients were all excluded. There was no history of glucocorticoid or immunosuppressive use by any of the patients or healthy volunteers at least 2 weeks prior to the study.

Glucocorticoid Receptor α, β, γ and p gene expression were measured in cases and controls by real time Polymerase Chain Reaction (PCR) (QIAamp® RNA blood mini kit (cat no. 52304) [6]. Informed written consent was obtained from all patients and the study was approved by the Medical Ethical Committee.

2.1 Statistical Analysis

Data were analyzed using IBM SPSS 20. Qualitative data were displayed in percentages and tested by Pearson's Chi Square. Parametric data were displayed in the form of mean ± SD. t-

test was used for comparing means for 2 groups and F-test for more than 2 groups. Non-parametric data were displayed in the form of median value and range. Mann-Whitney U (Z) was used for comparing median value for two groups and kruskall-Wallis ($^{KW}X2$) for more than two groups. Spearman bivariate correlation was used for analyzing correlation between GR isoforms. P<0.05 indicated statistical significance.

3. RESULTS

Table 1 shows age and sex of the studied ITP patients and controls.

Table 2 shows GR isoforms expressions in the studied ITP patients versus the controls. Non statistically significant difference was present when GRα, β, γ, p and GRα/GR β ratio were compared between males and females of the control group (z=0.81, 1.332, 1.389, 1.504 and 0.694 respectively at p=0.463, 0.189, 0.189, 0.152 and 0.536 respectively). Statistically significant difference in GRβ, γ and GRα/GR β ratio was present between ITP cases and controls (p<0.0001*, 0.03* and 0.01* respectively).

Table 3 shows comparison between GR α, β, γ, p and GRα/GR β ratio in GC responsive (19 patients) versus GC resistant cases (14 patients). The mean age value of GCS, GCR and control group was 33.4±11.6, 38.1±12.3 and 31.7±5.8 years respectively (F=1.496, p=0.235). Half of our female patients (n=14) were GC resistant while all the males (n=5) were GC sensitive. Statistically significant difference in GRα and GRα/GR β ratio only was present between GR sensitive and GR resistant cases (p=0.038* and 0.046* respectively).

GRα showed a wide median difference between GCS and GCR patients. The GC resistant group had a lower GRα expression level even lower than that of the controls. GRα/β ratio was statistically significantly higher in GCS than GCR group. GRβ isoform expression was more than GR p and GR γ in the controls but was minimally expressed in our ITP patients. Higher GRβ levels were observed in the resistant than the sensitive group. There was a tendency for increased GRγ in the GCR group compared to the GCS group with ITP.

Table 4 shows the correlation between GR α and other GR isoforms in ITP patients. There was non-statistically significant correlation between GR α and age (r=-0.302, p= 0.087). GRα had a strong inverse correlation with GRβ and a significant direct correlation with GRγ and p.

Table 1. Personal characteristics of the studied ITP patients and controls

Personal characteristics	Cases (n=33)	Controls (n=15)	Test of significance (p value)
Age (years)			t=1.426
Mean ± SD	35.4±11.9	31.7±5.8	P=0.161
Gender (No., %)			
Male	5 (15.2%)	7 (46.7%)	X^2=5.463
Female	28 (84.8%)	8 (53.3%)	P=0.019*

*X^2: Chi-Square test, t: t-test, *significant at P≤0.05*

Table 2. Expression of GR isoforms among the studied ITP patients and controls

GR isoforms	Cases (n=33)	Controls (n=15)	Test of significance (p value)
GRα	19.6 (2.1-294.5)	7.1 (2.9-8.1)	Z=1.246
Median (Q1-Q3)			P=0.213
GR β	0.003 (0.002-0.02)	3.9(0.97-5.1)	Z=4.783
Median (Q1-Q3)			P<0.0001*
GR γ	0.3 (0.01-8.4)	0.01(0.003-0.02)	Z=2.959
Median (Q1-Q3)			P=0.003*
GR p	0.2(0.01-21.5)	0.4 (0.1-0.8)	Z=0.389
Median (Q1-Q3)			P=0.697
GR α/GR β ratio	3492.8 (7.4-109152.6)	1.4 (0.9-2.8)	Z=3.37
Median (Q1-Q3)			P=0.001*

*Z: Mann Whitney test, *significant at P≤0.05*

Fig. 1 shows ROC curve of different GR isoforms in predicting GC resistance in ITP. GRα/β ratio had the highest sensitivity (81.8%) and is the most accurate predictor of GC resistance (79.2%). GRγ had the highest specificity (86.7%). GRβ had the lowest sensitivity (9.1%) and the lowest specificity (26.7%).

Table 3. Expression of GR isoforms among the studied ITP patients

GR isoform	Cases (n=33)		Test of significance (p value)
	Sensitive (n=19)	Resistant (n=14)	
GR α Median (Q1-Q3)	191.3 (5.3-689.8)	3.9 (1.7-101.1)	Z=2.058 P=0.038*
GR β Median (Q1-Q3)	0.003 (0.001-0.006)	0.006 (0.002-0.1)	Z=1.785 P=0.077
GR γ Median (Q1-Q3)	1.4 (0.1-13.5)	0.03 (0.003-2.2)	Z=1.931 P=0.055
GR p Median (Q1-Q3)	0.5 (0.1-34.3)	0.02 (0.01-2.3)	Z=1.748 P=0.084
GR α/GR β ratio Median (Q1-Q3)	35598.2 (866.9-154775.3)	224.5 (0.9-53090)	Z=2.003 P=0.046*

*Z: Mann Whitney test, *significant at P≤0.05*

Table 4. Correlation between GR α expression and other GR isoforms among the studied ITP patients

Parameter	GR α		GR β		GR γ		GR p	
	r	P	r	r	r	P	r	P
GR α								
GR β	-0.479	0.005*						
GR γ	0.771	0.0001*	-0.585	0.0001*				
GR P	0.552	0.001*	-0.331	0.06	0.393	0.024*		
GRα/GRβ ratio	0.91	0.0001*	-0.739	0.0001*	0.847	0.0001*	0.496	0.003*

*r: Spearman Rho correlation coefficient, *significant at P≤0.05*

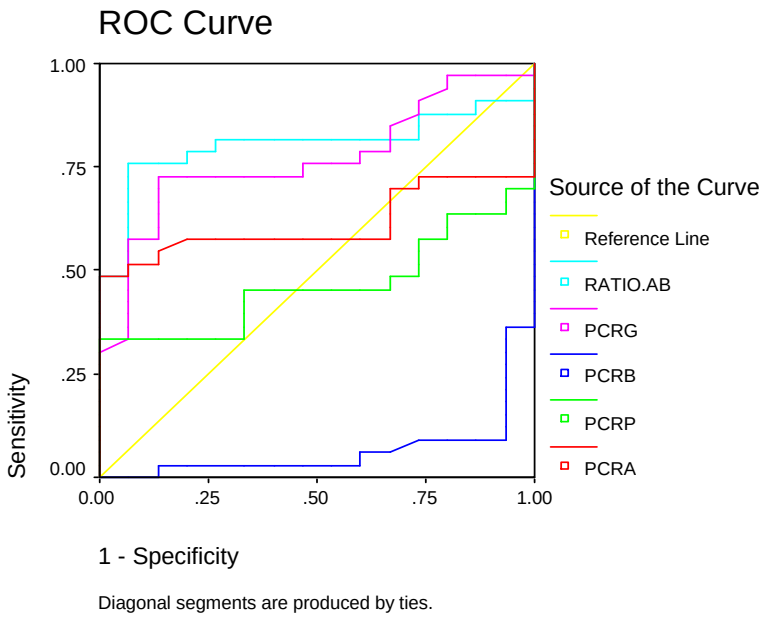

Fig. 1. ROC curve of different GR isoforms in predicting GC resistance in ITP

4. DISCUSSION

Glucocorticoid resistance has become a major barrier in effectively treating some patients with inflammatory conditions. Multiple mechanisms including GR isoforms expression contribute to glucocorticoid resistance [7]. Definitions of steroid resistance vary among papers [8,9]. Whether GC resistance in ITP patients is associated with GR isoforms expression is still unknown [7]?

As regards the influence of age on glucocorticoid resistance, we did not find statistically significant difference in age between our GCS and GCR patients. This may be explained by the prevalence of GC resistance in very elderly (>80 years) individuals while it is somewhat lower in adults [10,11].

Concerning the relation between sex and glucocorticoid resistance, half of our female patients (n=14) were GC resistant while all the males (n=5) were GC sensitive. ITP is five times more common in females than males in our study and 2 to 3 times more common in females than males in other studies [12]. Baseline serum and salivary cortisol concentrations was similar in men and women [10]. No difference in GRα / GRβ ratios was found when comparing men and women in one report [13].

We used real time PCR assay for determination of GR α, β, γ and p mRNA expression because it is a rapid and accurate method for relative quantification of gene expression level. This method is also simple and independent of standard curve construction or validation experiments [13].

GRα is expressed in most human tissues and cell line. It functions as ligand-dependent transcription factors [14]. There is a wide variability in median value of GRα between our GCS and GCR patients. GRα expression level was lower in the GCR group when compared with GCS group which was consistent with several studies [3,13]. Its lower level than controls may indicate that GRα is the primary mediator of GC action [3]. Certain proinflammatory cytokines may block the activated GRα [15] or block GRα binding affinity for glucocorticoids leading to low GRα expression [16].

An alternative isoform is GRβ. It is poorly expressed in human beings accounting for about 0.1% of GR [17]. GRβ is located in the nucleus in the absence of ligand and fails to bind hormone or activate gene transcription [17]. GRβ isoform expression was low in our ITP patients. Low GRβ expression has been also demonstrated in various diseases [18,19].

There was a non-statistically significant difference in GRβ expression between GGS and GCR group with higher levels observed in the resistant group. Some studies reported increased expression of GRβ isoform in inflammatory conditions and following exposure to interleukin (IL)-4, IL-2, IFN-γ and TNF-α [20-22]. However, other investigators have not observed any correlation between GRβ expression and glucocorticoid insensitivity [23].

The functional role of the GRβ is somewhat controversial owing to its very low expression levels compared with GRα in primary cells. GRβ may be able to modulate transcription by blocking GRα or even independent of GRα. Furthermore, the effect of GRβ on the complement of genes normally repressed by glucocorticoids is not well studied [24].

The ratio of GRα/GRβ expression is critical to the glucocorticoid responsiveness of various cells. Higher ratios correlate with glucocorticoid sensitivity, while lower ratios correlate with glucocorticoid resistance. GRα/β ratio was significantly higher in patients than in controls, being statistically significantly higher in GCS than GCR group. A study on T cell lymphoblastic leukemia reported the same [25].

This ratio can be altered by change in the expression level of GRα, GRβ, or both receptors simultaneously. The overexpression of GRβ in inflammatory diseases is suggested to be a consequence of the inflammation. There is considerable interindividual variability in GRα and GRβ mRNAs expression in the young healthy population as there is absence of acute or chronic diseases that drive inflammation and activate selective inflammatory cells [13].

GRγ and GRp isoforms expression was 4−8% and around 29% of total GR respectively in various tissues [18]. GRγ exhibits only about half of the transcriptional activity of GRα [18]. GRp was suggested by several studies to increase the activity of GRα in hematologic malignancies [3].

Expression of GRp and GR γ isoforms showed non-statistically significant difference between

our GCS and GCR group. We found a trend for increased GRγ in the GCR group compared to the GCS group. There are few data regarding GRγ and GRp isoforms expression and its correlation with GC resistance in ITP patients [18]. Similar result to ours was reported by Liangliang [3]. Beger and co-workers [26] found upregulation of GRγ mRNA in prednisone-poor responders than in prednisone-good responders after in vitro stimulation with dexamethasone.

As regard the correlation between GRα isoform and other studied isoforms in our patients. GRα had a strong inverse correlation with GRβ which may be explained by a dominant negative inhibitor effect of GRβ on GRα transcriptional regulation [17]. Other studies however do not support this finding [27,28]. There was a significant direct correlation between GRα with GR γ and p.

Among all studied parameters, GRα/β ratio had the highest sensitivity (81.8%) and is the most accurate predictor of GC resistance (79.2%). GRγ had the highest specificity (86.7%). GRβ had the lowest sensitivity (9.1%) and the lowest specificity (26.7%). There is significant direct correlation between GRα and GRα/β ratio in ITP cases.

5. CONCLUSION

The study of glucocorticoid receptor isoforms especially GRα and GRα/GRβ ratio may be of value in determining glucocorticoid resistance before starting therapy especially in those contraindicated from steroid use.

CONSENT

All authors declare that written informed consent was obtained from all the patients for publication of these results.

ETHICAL APPROVAL

All authors declare that all experiments have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The study was approved by the local Medical Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, Chong BH, Cines DB, Gernsheimer TB, Godeau B, Grainger J, Greer I, Hunt BJ, Imbach PA, Lyons G, McMillan R, Rodeghiero F, Sanz MA, Tarantino M, Watson S, Young J, Kuter DJ. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2010;115(2): 168-86.
2. Neunert C, Lin W, Crowther M, Cohen A, Solberg L Jr, Crowther MA. The American Society of Hematology 2011 evidence based practice guideline for immune thrombocytopenia. Blood. 2011;117(16): 4190-207.
3. Liangliang MA, Fang M, Liang Y, Xiang Y, Jia Z, Sun X, Wang Y, Qin J. Low expression of glucocorticoid receptor alpha isoform in adult immune thrombocytopenia correlates with glucocorticoid resistance. Ann Hematol. 2013;92:953–60.
4. Brogan IJ, Murray IA, Cerillo G, Needham M, White A, Davis JR. Interaction of glucocorticoid receptor isoforms with transcription factors AP-1 and NF-kappaB: Lack of effect of glucocorticoid receptor beta. Molecular and Cellular Endocrinology. 1999;157(1-2):95-104.
5. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113(11):2386-93.
6. Koga Y, Matsuzaki A, Suminoe A, Hattori H, Kanemitsu S, Hara T. Differential mRNA expression of glucocorticoid receptor alpha and beta is associated with glucocorticoid sensitivity of acute lymphoblastic leukemia in children. Pediatric Blood & Cancer. 2005;45(2):121-7.
7. Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends in Pharmacological Sciences. 2013;34(9):518-30.
8. Meyrier A, Simon P. Treatment of corticoresistant idiopathic nephrotic syndrome in the adult: Minimal change disease and focal segmental glomerulosclerosis. Advances in

Nephrology from the Necker Hospital. 1988;17:127-50.
9. Korbet SM, Schwartz MM, Lewis EJ. Minimal-change glomerulopathy of adulthood. American Journal of Nephrology. 1988;8(4):291-7.
10. Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE, et al. Interperson variability but intraperson stability of baseline plasma cortisol concentrations and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. The Journal of Clinical Endocrinology and Metabolism. 1998;83(1):47-54.
11. Weiner MF, Davis BM, Mohs RC, Davis KL. Influence of age and relative weight on cortisol suppression in normal subjects. The American Journal of Psychiatr. 1987;144(5):646-9.
12. Frederiksen H, Schmidt K. The incidence of idiopathic thrombocytopenic purpura in adults increases with age. Blood. 1999;94(3):909-13.
13. Colli LM, do Amaral FC, Torres N, de Castro M. Interindividual glucocorticoid sensitivity in young healthy subjects: The role of glucocorticoid receptor alpha and beta isoforms ratio. Horm Metabc Res. 2007;39(6):425-9.
14. Moraes LA, Paul-Clark MJ, Rickman A, Flower RJ, Goulding NJ, Perretti M. Ligand-specific glucocorticoid receptor activation in human platelets. Blood. 2005; 106:4167-75.
15. Adcock IM, Lane SJ, Brown CR, Lee TH, Barnes PJ. Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. The Journal of Experimental Medicine. 1995;182(6):1951-8.
16. Sher ER, Leung DY, Surs W, Kam JC, Zieg G, Kamada AK, et al. Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. The Journal of Clinical Investigation. 1994;93(1):33-9.
17. Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. J Biol Chem. 1999;274(39):27857-66.
18. Rivers C, Levy A, Hancock J, Lightman S, Norman M. Insertion of an amino acid in the DNA-binding domain of the glucocorticoid receptor as a result of alternative splicing. The Journal of Clinical Endocrinology and Metabolism 1999; 84(11):4283-6
19. Jakiela B, Bochenek G, Sanak M. Glucocorticoid receptor isoforms in steroid-dependent asthma. Polskie Archiwum Medycyny Wewnetrznej. 2010;120(6):214-22.
20. Inui S, Sumikawa Y, Asada H, Itami S. Glucocorticoid resistance in atopic dermatitis associated with decreased expression of glucocorticoid receptor-alpha in peripheral blood mononuclear cells. The Journal of Dermatology. 2010;37(5):496-9.
21. Hamid QA, Wenzel SE, Hauk PJ, Tsicopoulos A, Wallaert B, Lafitte JJ, et al. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. American Journal of Respiratory And Critical Care Medicine. 1999;159(5):1600-4.
22. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. The Journal of Allergy and Clinical Immunology 2000;105(5):943-50.
23. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: A mechanism for the generation of glucocorticoid resistance. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(12):6865-70.
24. Lavender P. Interleukin-17: A new role in steroid hypo-responsiveness? Clinical & Experimental Allergy. 2010;40:1293–4.
25. Longui CA, Vottero A, Adamson PC, Cole DE, Kino T, Monte O, et al. Low glucocorticoid receptor alpha/beta ratio in T-cell lymphoblastic leukemia. Hormone and Metabolic Research. 2000;32(10):401-6.
26. Beger C, Gerdes K, Lauten M, Tissing WJ, Fernandez-Munoz I, Schrappe M, et al. Expression and structural analysis of glucocorticoid receptor isoform gamma in human leukaemia cells using an isoform-specific real-time polymerase chain reaction approach. Br J Haematol. 2003;122(2):245-52.
27. Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor beta

isoform. Expression, biochemical properties and putative function. J Biol Chem. 1996;271(16):9550-9.

28. de Lange P, Koper JW, Brinkmann AO, de Jong FH, Lamberts SW. Natural variants of the beta isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids. Molecular and Cellular Endocrinology. 1999; 153(1-2):163-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11719