Interpretation of Biochemical Tests Using the Reference Change Value in Monitoring Adverse Effects of Oral Isotretinoin in 102 Ethnic Turkish Patients

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ABSTRACT

Objectives: The aim of this study was to model the use of reference change values (RCVs) for the follow-up of 4 parameters of patients using oral isotretinoin which is gaining widespread popularity for monitoring the side effects of the treatment.

Method: 102 patients received 30 mg/day oral isotretinoin for 24 weeks for the diagnosis of acne vulgaris.

Results: Repetitive measurements of the patients were interpreted with RCVs, after comparing the first and second doses based on RCVs: TC, TG, AST and ALT results increased in 12%, 20%, 14% and 12% of the patients respectively. When the first dose was compared with the last dose, the increases were 20%, 29%, 22% and 18% respectively interpreted as significant changes based on laboratory medicine.

Conclusions: A more sensitive follow-up is possible in the monitorization of adverse effects by using RCVs method.

Keywords: isotretinoin, acne vulgaris, references values, side effects, data interpretation, clinical chemistry test

In the clinical laboratory, patient results are interpreted for diagnosis, follow-up, and differential diagnosis. If the medical record of the patient does not include any previous results, the use of reference-based intervals is helpful in establishing a diagnosis. However, in instances of medication use that require consecutive measurements derived from testing, the adequacy of using population-based reference values is controversial.[1] In the calculation of the reference change value (RCV), analytical and biological variations are taken into account for test interpretation. Thus, in addition to the population-based reference range, laboratory scientists must also include RCV in the patient reports. In serial measurements, biological and analytical variation in the laboratory should also be taken into consideration.[2] Thus, in recent years, especially for patients undergoing follow-up, unidirectional calculated RCV and reference change factor ($RCF_{UP}/_{DOWN}$) are used to define upper and lower limits. The current approach is that, in repetitive measurements, these limits are used to include laboratory variations in the evaluation and interpretation of the results.[3]

Acne vulgaris is a commonly observed dermatologic condition that affects millions of individuals and can be psychosocially disabling. Severe and recalcitrant acne is typically treated with oral isotretinoin. Isotretinoin is a synthetic vitamin A derivative that has been available since 1982.[4] In the treatment of acne vulgaris, benzoyl peroxide gel, topical retinoids, topical antibiotics, oral antibiotics, hormonal therapy, and light/laser treatments have also been used.[5] The use of oral isotretinoin is

Abbreviations

RCV, reference change value; $RCF_{UP/DOWN}$, reference change factor; TC, total cholesterol; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CBC, complete blood count; PT, prothrombin time; NCI, National Cancer Institute; CTCAE, Common Terminology Criteria for Adverse Events; CV_A, analytic coefficient of variation; QC, quality-control; NADH, nicotinamide adenine dinucleotide hydrogen; EQAS, external quality-assurance; CV_W, intraindividual coefficient of variance; CV_G, interindividual coefficient of variance; CLSI, Clinical Laboratory Standards Institute

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recommended for a period of 16 to 24 weeks at different doses. Teratogenicity is the most well-known adverse effect; dryness of the eyes and the skin, cracked lips, and eye irritation are the most frequently observed mucocutaneous effects.[6] Because certain patients who have taken oral isotretinoin have developed systemic complications such as pancreatitis, drug-induced hepatotoxicity, leukopenia, and thrombocytopenia, it is recommended that patients have specimens drawn for laboratory testing during follow-up. To this end, before starting treatment with the drug, glucose, total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, complete blood count (CBC), prothrombin time (PT), and pregnancy testing are recommended. In the follow-up after treatment, TG, AST, and ALT levels should be tested within the first 30 days and in the following months.[7]

Recently, we reviewed the literature to find monitoring studies performed using RCV. For instance, we perused clinic investigations related to routine troponin levels in healthy groups and patients undergoing hemodialysis, troponin and natriuretic substance levels in acute coronary syndrome and coronary failure, tumor marker levels in patients harboring malignant neoplasms, and thrombocyte levels in patients who have undergone stem cell transplantation.[8-11]

Regarding the use of RCV in treatment follow-up to track the adverse effects of medications, we found only 2 clinic studies of antivirals. One of those studies contains a comparison of routine parameters that are used in follow-up of treatments with ribavirin along with other methods.[12] The other study reported the RCV of the creatinine test, which is used in follow-up measures designed to track the adverse effects of ritonavir and tenofovir antivirals.[13]

In the literature, we found no study that follows up on the adverse effects of oral isotretinoin by using the RCVs of respective tests. The aim of our study is to focus on patients using oral isotretinoin for the treatment of acne vulgaris and to observe the changes in laboratory follow-up parameters by using population-based reference values and to contribute to the interpretation of risky follow-up results by using RCV and RCF_{UP} .

Materials and Methods

We retrospectively analyzed laboratory data from 102 patients (51 female and 51 male) being followed up at the Dermatology Outpatient Center, Izzet Baysal Training and Research Hospital, Abant Izzet Baysal University, Bolu, Turkey, with the diagnosis of acne vulgaris and taking oral isotretinoin. We also examined laboratory data from a control group of 30 healthy volunteers who work in our hospital (15 female and 15 male). This analysis was performed after obtaining the approval of the local ethics committee on April 28, 2014.

The patients included in this study were taking 30 mg per day of oral isotretinoin.[14] Patients were undergoing monthly systematic and laboratory adverse effects follow-up. After taking predose measurements, we performed TC, TG, AST, and ALT tests at 1 and 6 months after the start of treatment; we included these increases in our reporting of study results.

Our analyses of normal and abnormal TC, TG, AST, and ALT levels were based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.[15] CTCAE consists of a 5-level system for grading severity that is used to classify adverse reactions. TC levels were classified as follows: normal, 200 mg per dL or less; grade I, 201 to 300 mg per dL; grade II, 301 per 400 mg per dL; grade III, 401 to 500 mg per dL; grade IV, greater than 500 mg per dL. TG levels were classified as follows: normal, 150 mg/dL or greater; grade I, 151 to 375 mg per dL or greater; grade II, 376 to 750 mg per dL; grade III, 751 to 1500 mg per dL; and grade IV, greater than 1500 mg per dL. AST and ALT levels were classified as follows: normal, 40 IU per L or less; grade I, 41 to 100 IU per L; grade II, 101 to 200 IU per L; grade III, 201 to 800 IU per L; and grade IV, greater than 800 IU per L.

In our study, we calculated the RCV of 4 clinical chemistry analytes. We used analytic coefficient of variation (CV_A) from our internal quality-control (QC) program. Intraindividual biological variations were obtained from current updated data by Ricos et al, as published on the Westgard website (the Westgard database was updated in 2016).[16-19]

We analyzed 2 control specimens at 2 different concentrations at 8:00 AM every day for 20 consecutive days. The within-run (intra-assay) analytical variation was minimized by assaying all the control specimens with the same lots of reagents on the same day. All analytes were included in each assay run, and we used Westgard Multirules to accept or reject these runs.[20] One analyst (H.O.) performed all the analyses, using the same batches of reagents, QC materials, and calibrators. We included control material in each analytical series to guarantee that the

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analytical imprecision was within acceptable limits, according to our internal QC protocol.

We analyzed 4 serum analytes using kits from Abbott Laboratories, Inc. that were manufactured for use with the Abbott ARCHITECT c8000 Clinical Chemistry Analyzer. On this analyzer, we used Level 1 Reference No. 05P78-10; Level 2, Reference No. 05P7811; and level 3 Reference No. 05P78-12 as internal QC material (Multichem S Plus, Lot No: 13111120, Techno-Path Manufacturing Ltd.). The analytical method used for each analyte included the following: TC (enzymatic method with cholesterol esterase, cholesterol oxidase, and peroxidase), triglyceride (enzymatic method with glycerol kinase, glycerol phosphate oxidase, and peroxidase), AST (nicotinamide adenine dinucleotide hydrogen [NADH] method), and ALT (NADH method). We practiced an external quality-assurance (EQAS) program (EQAS Program, Cycle 12; Jul 2013-2014, Bio-Rad Program) for all analytes during the study period.

Statistical Analyses

We evaluated the distribution of 3 measurements performed for 4 analytes using the Kolmogorov-Smirnov test. The Student's *t*-test was used in normal distribution groups, and nonparametric Wilcoxon testing was used in the normal distribution group. We compared the results with predose measurements. The CV_A was calculated retrospectively through control materials. We calculated mean (SD) values of the results of control materials for each analyte and used the formula $CV_A = SD/mean$ to calculate CV_A. The index of individuality was calculated by the ratio CV_W/CV_G. (CV_W stands for the intraindividual coefficient of variation, and CV_G stands for the interindividual coefficient of variation.) We calculated the RCVs for analytes at various probabilities that were applied to a single quantity or the 2 quantities combined according to the formula $RCV = Zx\,2^{1/2} \times (CV_A^2 + CV_w^2)^{1/2}$.[2] The formulas to be used for the RCVs of all the tests in the study are as follows:

RCV formula for patient data $= Zx2^{1/2} \times (_{CVA}{}^2 + CV_w{}^2)^{1/2}$

Intraindividual variation coefficient $CV_W = (CV_{A+W}{}^2 - CV_A{}^2)^{1/2}$

Interindividual variation coefficient $CV_G = (CV_T{}^2 - CV_W{}^2 - CV_A{}^2)^{1/2}$

We calculated the RCV as unidirectional for $P < .05$ (Z-scores = 1.65). RCF_{UP} was calculated as exp (Z_n. $2^{1/2}$. $(CV_A{}^2 + CV_W{}^2)^{1/2}$/100), and RCF_{DOWN} was calculated as ($1/RCF_{UP}$). Our laboratory total precision was calculated according to the formula ($CV_{Total\ precision}{}^2 = CV_{Within\ run}{}^2 + CV_{Between\ run}{}^2 + CV_{Beetween\ day}{}^2$), which is suggested in the Clinical Laboratory Standards Institute (CLSI) guideline EP5-A2.[18]

Table 1. Analyte Levels at Predose, End of 1 Month, and End of 6 Months of Patients Undergoing Treatment for Acne Vulgaris With Oral Isotretinoin[a]

Analyte	Mean (SD)		
	Predose[b]	End of 1 Month of Treatment[b]	End of 6 Months[b]
TC, mg/dL	155.50 (27.70)	166.22 (31.88)	167.85 (33.42)
TG, mg/dL	79.37 (37.91)	94.97 (42.1)	100.06 (45.14)
AST, IU/L	17.20 (5.15)	18.28 (4.23)	19.24 (5.38)
ALT, IU/L	15.79 (7.87)	15.84 (7.48)	16.75 (10.18)

[a] *n = 102 Mean dose: 20 mg/day for a 24-week period.*
[b] *P < .05.*

Results

We studied data from 102 patients (51 male and 51 female; age range, 15 to 37 years, mean [SD], 21.22 [2.49] years) who were using oral isotretinoin for acne vulgaris; the mean daily dose for those patients was 30 mg per day for a period of 24 weeks. The results of measurement of laboratory values at predose, 1 month, and 6 months of treatment in the patient group are demonstrated in **Table 1**. After predose measurements, TC, TG, AST, and ALT assays were used as measurements at 1 month and 6 months of treatment, for follow-up purposes, and the increases in the values yielded by these tests were also included in the study. In all measurements, in the patient group in which follow-up was performed, TC, TG, AST, and ALT parameters showed statistical significance ($P < .05$) values (**Table 1**).

We also studied the change in RCV in the control group (15 male and 15 female; age range, 18 to 49 years; mean [SD], 31.40 [7.21]) within an interval of 6 months (starting at the predose point). All levels of the analytes in this paragraph were measured at 1 month and 6 months after initial treatment; all values are shown as mean (SD). TC, TG, AST, and ALT activity level follow-up results in the control group ($n = 30$) were as follows: TC levels were measured as 180.70 [36.80] mg per dL and 182.83 [33.37] mg per dL, respectively. TG levels were measured as 83.79 [37.51] mg per dL and 82.17 [38.00] mg

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Table 2. Example That Provides Explanation of How to Use RCV, RCF_{UP}, and RCF_{DOWN} Data for the Interpretation of Patient Results

Variable	TC	TG	AST	ALT
RCV[a]	14.09	46.92	28.80	45.48
RCF_{UP}[b]	1.15	1.60	1.33	1.58
RCF_{DOWN}[c]	0.87	0.63	0.75	0.63
Predose levels	116 mg/dL	65 mg/dL	13 IU/L	12 IU/L
After 1 month	139 mg/dL	141 mg/dL	20 IU/L	25 IU/L
After 6 months	166 mg/dL	213 mg/dL	21 IU/L	35 IU/L
RCV	19.83 >14.09	116.92>46.92	58.84>28.80	108.33>45.48
	43.10 >14.09	227.69>46.92	61.53>28.80	191.67>45.48
RCF_{UP}	116×1.15=133.40	65×1.60=104.00	13×1.33=17.29	12×1.58=18.96
RCF_{DOWN}	116×0.87=100.92	65×0.63=40.95	13×0.75=9.75	12×0.63=7.56

[a]Using an unidirectional probability of 95%, Z = 1.65, P <.05, $RCV = 1.65x2^{1/2} \times CV_T$, $CV_T = (CV_A^2 + CV_w^{/2})^{1/2}$.
[b]RCF_{UP} calculated as exp $(Z \times 2^{1/2} \times CV_T/100)$ or $RCF_{UP} = exp\ (Z \times 2^{1/2} \times (CV_A^2 + CV_w^{/2})^{1/2}/100$.
[c]RCF_{DOWN} calculated as $(1/RCF_{UP})$.

Table 3. Analytical (CV_A), Intraindividual (CV_W), and Interindividual (CV_G) Coefficients of Biological Variation and Index of Individuality of 4 Serum Analytes, Compared With Their Clinical-Chemistry Parameter Values

Variable	TC	TG	AST	ALT
CV_A Level 1, %	0.86	3.84	1.66	2.94
CV_A Level 2, %	1.14	1.63	0.70	1.24
CV_A Level 3, %	1.06	3.15	0.64	1.43
CV_A mean, %	1.02	2.87	1.00	1.87
CVw, %	5.95	19.9	12.30	19.40
CV_G, %	15.3	32.7	23.10	41.60
Index of individuality (CVw/CV_G)	0.39	0.61	0.53	0.47

TC, total cholesterol, TG, total triglycerides, AST, aspartate aminotransferase; ALT, alanine aminotransferase.

per dL, respectively. AST activity was measured as 18.23 [3.78] IU per L and 16.97 [3.19] IU per L, respectively. ALT activity was measured as 19.27 [11.19] IU per L and 17.07 [9.82] IU per L, respectively.

For TC, the RCV was 14.09%; for TG, 46.92%; for AST, 28.80%; and for ALT, 45.48%. The RCF_{UP} was 1.15 for TC, 1.60 for TG, 1.33 for AST, and 1.58 for ALT (**Table 2**). In all measurements, in the control group in which the follow-up was performed, TC, TG, AST, and ALT parameters were statistically insignificant ($P > .05$). When we interpreted repetitive measurements of the control groups using the RCV, after comparing measurements after the first and sixth months of treatment based on RCV values, we discovered that TC, TG, AST, and ALT values increased by 10%, 7%, 0%, and 3%, respectively.

The analytical variations of the measured tests, along with the interindividual and intraindividual variations index of individuality results are shown in **Table 3**. The between-run, within-run, and day-to-day CV% and SD data are demonstrated in **Table 4**.

To show how we used these formulas, we provide examples from 4 different parameters in **Table 2**. When a patient has a predose result of 116 mg per dL and when that patient arrives for follow-up care 1 month later and has a result of 139 mg per dL, in interpretation of the results, we calculated to show a change of 19.83% (139 to 116). This result is higher than the RCV value of 14.09 for TC calculated for our laboratory and should be interpreted as a clinically significant increase. In the same patient specimen, the RCF_{UP} and RCF_{DOWN} range is identified as 101 to 133 mg per dL. Because the second measurement for this patient is outside this range, it was interpreted as a clinically significant increase. Likewise, in **Table 2**, patient specimens showing clinically significant increases in TG, AST, and ALT are provided as examples for the interpretation of these results.

When we interpreted repetitive measurements from patients using RCV, after comparing measurement at predose and after 1 month of treatment based on RCV values, TC, TG, AST, and ALT results had increased in 12%, 20%, 14%, and 12% of patients, respectively. When we compared predose measurements with those after 6 months of treatment, the increases were 20%, 29%, 22%, and 18%, respectively; we interpreted these values as representing significant changes. When repetitive analyte measurements from our patients

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Table 4. Calculation of the Total Precision of Our Laboratory Values

Analyte	Units of Measurement	L1 Mean	L2 Mean		Within-Run		Between-Run		Between-Day		Total Precision	
					L1	L2	L1	L2	L1	L2	L1	L2
TC	mg/dL	105.7	162.5	SD	1.98	0.78	1.01	1.03	0.91	1.85	2.40	2.89
				CV%	0.80	0.60	0.40	0.80	0.83	1.14	1.22	1.45
TG	mg/dL	55.6	132.4	SD	1.42	0.80	0.75	0.64	2.13	2.16	2.67	2.69
				CV%	7.00	0.80	0.40	0.60	3.84	1.63	7.99	7.20
AST	IU/L	26.9	105.1	SD	0.36	0.61	0.24	0.32	0.69	0.88	0.81	0.98
				CV%	1.10	0.40	0.80	0.20	2.94	1.24	3.24	1.84
ALT	IU/L	41.5	124.1	SD	0.44	0.39	0.38	0.54	0.79	1.31	0.98	1.43
				CV%	1.50	0.50	1.30	0.70	1.66	0.70	2.59	2.10

TC, total cholesterol; TG, total triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

were interpreted with CTCAE, after comparing measurement at predose and after 1 month of treatment based on CTCAE values (Grade 1; for TC, 201 to 300 mg/dL; for TG, 151 to 375 mg/dL; and for AST/ALT, 41 to 100 IU/L), we discovered that TC, TG, AST, and ALT results had increased in 10%, 12%, 1%, and 1% of the patients, respectively. When we compared predose values with those after 6 months of treatment, the increases were 18%, 14%, 1%, and 4%, respectively, which we interpreted as significant changes based on CTCAE values.[15]

Discussion

In our study, 102 patients were taking oral isotretinoin for the treatment of acne vulgaris. For our interpretation of laboratory test follow-up results, we used RCV and RCF_{UP} for retrospective evaluation. By using the RCV method that we recommend, when we compared measurements at predose and after 1 month of treatment, based on RCV values, 12%, 20%, 14%, and 12% of the patients had increases in TC, TG, AST, and ALT results, respectively. When we compared predose values with those measured at the end of treatment, 20%, 29%, 22%, and 18% of the patients had significant increases in TC, TG, AST, and ALT results, respectively (**Table 3**). The presentation of these data from follow-up of patients who have taken oral isotretinoin is novel in the literature, to our knowledge. The method we describe herein is more sensitive than previous methods, in interpretation of patient results and in follow-up for adverse effects, so we recommend its use because doing so is likely to decrease the occurrence and severity of potential long-term adverse effects.

In patients using isotretinoin for the treatment of acne vulgaris, once monthly measurement-of-hepatic-function tests and once-monthly lipid analyses have been recommended as follow-up laboratory tests. Study results reported in the literature[19,20] show us that despite the increases that might be observed in these parameters, increases that necessitate discontinuation of treatment are reported in less than 1% of such patients.

Zane et al[7] performed a retrospective cohort study of 13,722 patients with acne vulgaris (age range, 13 to 50 years) who were being treated with oral isotretinoin. These researchers identified changes in the baseline levels of serum lipids and transaminases; however, they did not identify such a change in hematological parameters. Zane et al do not recommend follow-up of CBC if there is no clinical suspicion of adverse effects. In our study, we performed CBC measurements at the beginning, but we did not perform this measurement after that time. Compared with baseline levels, TG levels changed by 44%, TC levels by 31%, and serum aminotransferase levels by 11%.

In studies performed on patients using oral isotretinoin for the treatment of acne vulgaris, certain studies report increases of 25% of patients in serum TG and in 15% of patients in serum TC, whereas other studies report increases in serum TG in 5% to 18% of patients and in serum TC in 6% to 32% of them.[21-23] In follow-up of individuals using oral isotretinoin, 5% to 44% has increases in TG, 6% to 31% has increases in TC, and 3% to 11% has increases in transaminase levels.[7] Vieira et al[24] performed a follow-up study on 70 patients (55.7% of whom were female) who were receiving a daily dose of 20 mg to 80 mg per day of oral isotretinoin. These investigators reported a statistically significant increase in the mean (SD) levels of triglycerides

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(87.01 [48.25] versus 105.32 [48.76] mg/dL), AST (20.44 [6.26] versus 24.38 [11.92] IU/L) and ALT (18.24 [8.31] versus 23.34 [20.03] IU/L) performed before and 3 months or more after the start of oral isotretinoin treatment. After treatment with oral isotretinoin, TG levels had increased beyond the normal range in 11% of the patients, whereas 8.6% had elevated AST levels and 7.3% had increased ALT levels.

In our study, when we compared analyte levels at predose and 1 month after treatment initiation, we observed increases of 7% in TC, 15% in TG, 12% in AST, and 14% in ALT. In comparison of predose levels after 6 months of treatment, we noted increases of 8% in TC, 32% in TG, 18% in AST, and 21% in ALT. In our study, the patients were taking a relatively low dose of oral isotretinoin, namely, 30 mg per day, which is why the number of patients with analyte levels higher than the reference level was relatively low. Based on the classical follow-up we used, no dose changes were made. In recent years, the classical dose recommended for acne vulgaris treatment is 0.5 to 1.0 mg per kg (of body weight) per day. In a study by Brito et al,[25] in 150 patients (of whom 48% were female and 52% male), aged 15 to 32 years, who were taking a daily dose of 0.5 to 1.0 mg per kg per day of oral isotretinoin, systemic adverse effects were followed up. In this study group, which was similar to ours, increases in TC, TG, AST, and ALT levels were reported during laboratory follow-up after treatment. However, these researches did not report any systemic adverse effects that would necessitate a change in the treatment dose, as happened in our study.

Carobene et al[26] reviewed 10 publications in their study and reported the ranges of within-subject variation as follows: ALT, 11.1% to 58.1% and AST, 3.0% to 32.2%. The median values (ALT, 18% and AST, 11.9%) were similar to those listed in the biological-variations database used as a common reference source. The CV_G value used in our study was 12.35% for AST and 19.4% for ALT, as obtained from the updated database reported by Westgard and mentioned in Qi et al.[10] This study showed, once again, that for test results with index of individuality of less than 0.6, the use of reference values in the evaluation of repetitive patient results is controversial, as is the case in our study. In patients undergoing follow-up evaluation, the changes between the initial result and later results should be evaluated by calculating RCV or RCF.

Our study had certain limitations. Due to difficulty in finding a retrospective sample that would yield data from the tests for the 4 target analytes within an interval of 6 months, we used data from 30 healthy, young volunteers as a control group.

The patient group that was followed up with CTCAE and completed its treatment without changing medicine dosage makes up our working group. Our thesis was that it will be possible to protect future patients from the adverse effects of oral isotretinoin by following more current patients closely, to determine the amount of adverse effects that occur during their follow-up period.

When interpreting patient results, using RCV and/or $RCF_{UP\text{-}DOWN}$ in addition to reference values will include analytical variations of any laboratory and biological variations from the patient into the evaluation process, making more-precise interpretation possible. After this preliminary study, we are considering calculating RCV and RCF_{UP} in a larger patient group to evaluate the interpretation of patient results. **LM**

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