HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2013-004441</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>13-Nov-2013</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Fernández-Crehuet, Pablo; Alto Guadalquivir Hospital, Dermatology Unit Serrano, José Luis; Alto Guadalquivir Hospital, Dermatology Unit Allam, Moahmed; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health Navajas, Rafael; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Gastroenterology and hepatology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Dermatology, Gastroenterology and hepatology, Pharmacology and therapeutics</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Acne &lt; DERMATOLOGY, Lipid disorders &lt; DIABETES &amp; ENDOCRINOLOGY, Adult gastroenterology &lt; GASTROENTEROLOGY, Hepatobiliary disease &lt; GASTROENTEROLOGY, Hepatology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME

Pablo Fernández-Crehuet Serrano. MD, PhD

José Luis Fernández-Crehuet Serrano. MD, PhD

Mohamed Farouk Allam. MPH, PhD

Rafael Fernández-Crehuet Navajas. MD, PhD

1. Dermatology Unit. Alto Guadalquivir Hospital. Andújar, Jaén, Spain.

Correspondence: Mohamed Farouk Allam, Department of Preventive Medicine and Public Health, Faculty of Medicine, University of Cordoba. Avda. Menéndez Pidal, s/n Cordoba 14004, Spain.

Phone: +34 957 218 278; Fax. +34 957 218 573

E-mail: fm2faahm@uco.es

Word count: 1944

Short Title: Hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome.

Keywords: Acne, bilirubin, Gilbert’s syndrome, triglycerides, isotretinoin.
ABSTRACT

Objectives. The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.

Setting. Dermatology Outpatient Clinics of 3 regional hospitals of Jaen (Spain).

Participants. All patients with severe acne who attended the Dermatology Outpatient Clinics over 4 years were eligible to be included in our study. We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome.

Interventions. All patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks. Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles.

Primary outcome. Blood analyses were repeated in the follow-up visits.

Results. In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks. None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow up. Liver enzymes were maintained within normal levels in both groups. Both studied groups did not show significant pathological increase in lipid profile levels. LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients.
Conclusion. Oral isotretinoin is not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment.

Ethical Committee: Study approved by the Ethical Committee of Hospital de Andjuar.

Article summary

'Strengths and limitations of this study'

- All patients with severe acne who attended the Dermatology Outpatient Clinics over 4 years were eligible to be included in our study.

- We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome. Patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks.

- Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Blood analyses were repeated in the follow-up visits.

- Oral isotretinoin was not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment.

- Lipid profile levels in Gilbert’s syndrome patients were maintained within the normal recommended levels.
INTRODUCTION

The use of isotretinoin for the treatment of severe acne has been widely used over the last 30 years. Many adverse reactions to isotretinoin have been reported. Several studies
have shown that hepatotoxicity could occur in about 10% and hyperlipidemia in 20-45%. [1-3]

Gilbert’s syndrome is a benign and inherited state characterized by intermittent unconjugated hyperbilirubinaemia with accompanying jaundice in the absence of haemolysis or underlying liver disease. [4] Previous studies have shown that Gilbert's syndrome affects 5% to 7% of the population, mainly postpubertal male patients. [4-5]

In patients with hepatic dysfunction, like Gilbert’s syndrome, we would expect an increased sensitivity to isotretinoin that is metabolized by hepatic oxidation and biliary excretion. [6-7]

The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.

**METHODOLOGY**

All patients with severe acne who attended the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala la Real and Alcaudete (Jaen, Spain) over 4 years were eligible to be included in our study. Owning to the location of these 3 regional hospitals in the centre of Andalusia, they serve over one million persons.

Only patients with severe acne and treated with isotretinoin from September 1, 2008 to August 31, 2012 were included consecutively in the study.

Inclusion criteria were age between 14 and 20 years old, severe acne, treatment with oral isotretinoin (0.5-0.8 mg/kg) and normal LFTs and lipid profiles.
Meanwhile, exclusion criteria were contradictions for isotretinoin, concomitant use before or during the study of other treatment inducer of Cytochrome P450, alcohol consumption, significant change in weight (>10%) and suspension of the treatment.

We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome.

All patients were informed about the aim of the study and signed an informed consent.

Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, liver function tests and lipid profiles.

Basal levels of bilirubin in patients with Gilbert’s syndrome were measured in 3 consecutive mornings. Of the 3 measures, the mean basal level was calculated. Fasting levels of bilirubin in patients with Gilbert’s syndrome were >1.1 mg/dl.

All patients were followed up in our outpatient clinics after 10 and 20 weeks. Blood analyses were repeated in the follow-up visits.

No adverse effects of isotretinoin treatment were noted and in all patients the acne was in complete remission at the last follow-up visit.

Statistical methods:

First, the following descriptive analysis was done: frequency, percent, mean, standard deviation. Comparisons between patients with Gilbert’s syndrome and control patients were done using Mann-Whitney test for continuous variables and Pearson’s Chi square test for categorical variables. Comparisons of LFTs and lipid profile levels over the follow-up period were done using Friedman Test. Level of significance was set at p
<0.05. All data variables were encoded and computerized. Data entry and statistical analysis were performed using the Statistical Package for Social Science (SPSS) version 15.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Our follow-up study included 37 patients with severe acne; 11 patients with Gilbert’s syndrome and 26 control patients. No statistical significant differences were found between the 2 studied groups regarding age and gender.

In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks compared with the basal level (P value <0.001). Meanwhile, AST, ALT and GGT levels over the 20 weeks follow-up did not vary significantly; P values 0.37, 0.77 and 0.46 respectively (Table 1).

In control patients, bilirubin and ALT levels did not vary significantly over the 20 weeks follow-up; P value 0.23 and 0.28 respectively. Meanwhile, AST and GGT varied significantly over the 20 weeks follow-up; P value 0.018 and 0.002 respectively (Table 1).

In Gilbert’s syndrome patients, LDL levels showed substantial increase over the 20 weeks follow-up, with more increase after 10 weeks compared with the basal level (P value <0.001). Meanwhile, cholesterol, triglycerides and HDL levels over the 20 weeks follow-up did not vary significantly; P values 0.18, 0.65 and 0.32 respectively (Table 2).

In control patients, cholesterol, triglycerides and LDL levels showed substantial increase over the 20 weeks follow-up; P values <0.001. Meanwhile, HDL levels over the 20 weeks follow up did not vary significantly; P values 0.34 (Table 2).
DISCUSSION

The main objective of the current follow-up study was to evaluate the hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome. In theory, hepatotoxic drugs like isotretinoin would increase bilirubin and liver enzymes levels in patients with hereditary hepatic disorders like Gilbert’s syndrome.[4]

Before reaching conclusions based on the present results, it is necessary to consider a number of potential objections to the methodology. Limitations such as the small sample size could affect the validity of the results. Our study included only 37 patients with severe acne and treated with oral isotretinoin. It was difficult to identify more patients according to our strict inclusion and exclusion criteria, and that is why we included all patients treated over 4 years in 3 regional hospitals. Other possible limitations were the placebo effect and the natural evolution. Placebo effect was avoided by repeated measures; before and 10 and 20 weeks after treatment. Natural evolution was controlled by the follow-up comparative control group without Gilbert’s syndrome.

By 10 weeks all patients with Gilbert’s syndrome had a significant decrease in total bilirubin levels and they maintained this decrease after 20 weeks.

The cause for decline in bilirubin levels in patients with Gilbert’s syndrome treated with isotretinoin is still unknown. Only few studies were published about this unexpected decline in bilirubin levels by a hepatotoxic drug like isotretinoin.[8-10] Several drugs like phenobarbitals and corticosteroids induce microsomal enzymes as UDP-GT and stimulate the conjugation of bilirubin producing its decrease. In contrary,
isotretinoin inhibits the inducer effect of other drugs and thus this does not seem to be its mechanism of action in patients with Gilbert’s syndrome.[11-12]

Currently, there two hypothesis to explain this strange effect of isotretinoin in patients with Gilbert’s syndrome; reversible decrease in the serum levels of testosterone increasing the activity of UDP-GT or stimulating hepatocytes to generate transporting proteins that eliminate bilirubin.[13-14]

None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow-up. Of note, liver enzymes (AST, ALT and GGT) were maintained within normal levels in both studied groups.

Our study also explored the adverse effect of isotretinoin on lipid profile levels. Both studied groups did not show significant pathological increase in lipid profile levels (cholesterol, triglycerides, HDL and LDL). All lipid profile levels in Gilbert’s syndrome patients and control patients were maintained within the normal recommended levels.

Recent follow-up study with 248 patients treated with isotretinoin because of acne vulgaris, showed that only 1.2% suffered impaired LFTs and 1.61% had high lipid profile levels after 16 weeks of treatment.[15] These findings agree with our results after 10 and 20 weeks of follow-up in both Gilbert’s syndrome and control patients.

LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Again, these findings are contradictory to what would be expected from hepatotoxic drug administrated in patients with hepatic dysfunction.

Previous studies showed that patients with Gilbert’s syndrome have lower prevalence of atherosclerosis and ischemic heart disease.[16-17] A systematic review confirmed that
there is a reliable inverse and dose–response relationship between serum bilirubin and
the atherosclerotic process.[18] Recent case control study showed that LDL, well
known mediator of initial stages of atherosclerosis, was lower in patients with Gilbert’s
syndrome when compared to healthy controls.[19] This agrees with the results of our
study where basal levels of LDL and after isotretinoin treatment were lower in patients
with Gilbert’s syndrome compared with control patients.

In conclusion, oral isotretinoin is not only an effective safe treatment for patients with
Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment.
Identification of the mechanism of action of oral isotretinoin in patients with Gilbert’s
syndrome could help in elaborating new protective drugs against hepatotoxicity.
CONTRIBUTORSHIP STATEMENT:

Each author declares having participated in the activities described below and that they have seen and approved the final version. They also declare not having conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

Pablo Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

José Luis Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

Mohamed Farouk Allam: Data analysis, interpretation of results, decision making on content and paper write-up and revision of final draft.

Rafael Fernández-Crehuet Navajas: Author of original idea, study design, field work supervision, data analysis, decision making on content and paper write-up and revision of final draft.

Funding: none.

Conflicts of interest: none.
REFERENCES


Table 1. Follow-up and comparative study of liver function tests in controls and patients with Gilbert's syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal bilirubin levels</td>
<td>1.78 ± 0.65</td>
<td>0.50 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 10 weeks</td>
<td>1.03 ± 0.18</td>
<td>0.52 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 20 weeks</td>
<td>1.38 ± 0.54</td>
<td>0.48 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal AST levels</td>
<td>22.1 ± 5.1</td>
<td>21.6 ± 14.7</td>
<td>0.14</td>
</tr>
<tr>
<td>AST levels after 10 weeks</td>
<td>23.8 ± 5.4</td>
<td>21.4 ± 7.8</td>
<td>0.11</td>
</tr>
<tr>
<td>AST levels after 20 weeks</td>
<td>21.5 ± 7.1</td>
<td>21.4 ± 7.6</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Table 2. Follow-up and comparative study of lipid profile in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cholesterol levels</td>
<td>143.6 ± 19.5</td>
<td>155.6 ± 24.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cholesterol levels after 10 weeks</td>
<td>164.4 ± 23.4</td>
<td>176.3 ± 30</td>
<td>0.32</td>
</tr>
<tr>
<td>Cholesterol levels after 20 weeks</td>
<td>162.7 ± 12.4</td>
<td>181.6 ± 30.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal triglycerides levels</td>
<td>71.2 ± 27.2</td>
<td>72.1 ± 43</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides levels after 10 weeks</td>
<td>84.3 ± 50.7</td>
<td>93.5 ± 46.4</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides levels after 20 weeks</td>
<td>67.2 ± 31</td>
<td>103.8 ± 56.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Basal HDL levels</td>
<td>HDL levels after 10 weeks</td>
<td>HDL levels after 20 weeks</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Basal HDL levels</td>
<td>53.5 ± 15.2</td>
<td>56.7 ± 11.4</td>
<td>0.33</td>
</tr>
<tr>
<td>HDL levels after 10 weeks</td>
<td>53.5 ± 14.8</td>
<td>56.7 ± 12.8</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL levels after 20 weeks</td>
<td>62.4 ± 17.5</td>
<td>54.8 ± 13.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Basal LDL levels</td>
<td>78.1 ± 13.9</td>
<td>82.3 ± 20.5</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL levels after 10 weeks</td>
<td>95.3 ± 19</td>
<td>99.4 ± 25.6</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL levels after 20 weeks</td>
<td>88.3 ± 14.1</td>
<td>107.3 ± 24.5</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

1 Mann-Whitney Test
### STROBE Statement—checklist of items that should be included in reports of observational studies

<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| **Title and abstract** | 1  
(a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | 2 Explain the scientific background and rationale for the investigation being reported |
| **Objectives** | 3 State specific objectives, including any prespecified hypotheses |
| **Methods** | 4 Present key elements of study design early in the paper  
5 Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  
6  
(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants  
(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed  
Case-control study—For matched studies, give matching criteria and the number of controls per case  
7 Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  
8* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group  
9 Describe any efforts to address potential sources of bias  
10 Explain how the study size was arrived at  
11 Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why  
12  
(a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) Cohort study—If applicable, explain how loss to follow-up was addressed  
Case-control study—If applicable, explain how matching of cases and controls was addressed  
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy  
(e) Describe any sensitivity analyses |

Continued on next page
Results

Participants 13* (a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
(b) Give reasons for non-participation at each stage
(c) Consider use of a flow diagram

Descriptive data 14* (a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders
(b) Indicate number of participants with missing data for each variable of interest
(c) Cohort study—Summarise follow-up time (e.g. average and total amount)

Outcome data 15* Cohort study—Report numbers of outcome events or summary measures over time
Case-control study—Report numbers in each exposure category, or summary measures of exposure
Cross-sectional study—Report numbers of outcome events or summary measures

Main results 16 (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included
(b) Report category boundaries when continuous variables were categorized
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses 17 Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results 18 Summarise key results with reference to study objectives

Limitations 19 Discuss limitations of the study, taking into account sources of potential bias or imprecision.
Discuss both direction and magnitude of any potential bias

Interpretation 20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

Generalisability 21 Discuss the generalisability (external validity) of the study results

Other information

Funding 22 Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME: CASE SERIES

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2013-004441.R1</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>26-Feb-2014</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Fernández-Crehuet, Pablo; Alto Guadalquivir Hospital, Dermatology Unit Serrano, José Luis; Alto Guadalquivir Hospital, Dermatology Unit Allam, Moahmed; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health Navajas, Rafael; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Gastroenterology and hepatology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Dermatology, Gastroenterology and hepatology, Pharmacology and therapeutics</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Acne &lt; DERMATOLOGY, Hepatobiliary disease &lt; GASTROENTEROLOGY, Physiology &lt; BASIC SCIENCES, CLINICAL PHARMACOLOGY, Hepatology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME: CASE SERIES

José Luis Fernández-Crehuet Serrano. MD, PhD

Pablo Fernández-Crehuet Serrano. MD, PhD

Mohamed Farouk Allam. MPH, PhD

Rafael Fernández-Crehuet Navajas. MD, PhD

1. Dermatology Unit. Alto Guadalquivir Hospital. Andújar, Jaén, Spain.


Correspondence: Mohamed Farouk Allam, Department of Preventive Medicine and Public Health, Faculty of Medicine, University of Cordoba.

Avda. Menéndez Pidal, s/n Cordoba 14004, Spain.

Phone: +34 957 218 278; Fax. +34 957 218 573

E-mail: fm2faahm@uco.es

Word count: 1944

Short Title: Hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome.

ABSTRACT
Objectives. The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.

Setting. Dermatology Outpatient Clinics of 3 regional hospitals of Jaen (Spain).

Participants. Over 4 years, we included all patients diagnosed with severe acne. Only 37 patients were identified, of which 11 had Gilbert’s syndrome.

Interventions. All patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks. Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Data and results of patients with severe acne and Gilbert’s syndrome were compared with these of the 26 patients with only severe acne (control group).

Primary outcome. Blood analyses were repeated in the follow-up visits.

Results. In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks. None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow up. Liver enzymes were maintained within normal levels in both groups. Both studied groups did not show significant pathological increase in lipid profile levels. LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Conclusion. Oral isotretinoin is not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment. Limitations of the study include the small numbers of participants and the fact that it is restricted to one region of Spain.

Keywords: Acne, bilirubin, Gilbert's syndrome, triglycerides, isotretinoin.
Ethical Committee: Study approved by the Ethical Committee of Hospital de Andjuar.

Article summary

'Strengths and limitations of this study'

- All patients with severe acne who attended the Dermatology Outpatient Clinics over 4 years were eligible to be included in our study.

- We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome. Patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks.

- Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Blood analyses were repeated in the follow-up visits.

- Oral isotretinoin was not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment.

- Lipid profile levels in Gilbert’s syndrome patients were maintained within the normal recommended levels.

- Limitations of the study include the small numbers of participants and the fact that it is restricted to one region of Spain.
INTRODUCTION

The use of isotretinoin for the treatment of severe acne has been widely used over the last 30 years. Many adverse reactions to isotretinoin have been reported. Several studies have shown that hepatotoxicity could occur in about 10% and hyperlipidemia in 20-45%.[1-3]

Gilbert’s syndrome is a benign and inherited state characterized by intermittent unconjugated hyperbilirubinaemia with accompanying jaundice in the absence of haemolysis or underlying liver disease.[4] Previous studies have shown that Gilbert's syndrome affects 5% to 7% of the population, mainly postpubertal male patients.[4-5]

In patients with hepatic dysfunction, like Gilbert’s syndrome, we would expect an increased sensitivity to isotretinoin that is metabolized by hepatic oxidation and biliary excretion.[6-7]

The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.
METHODOLOGY

All patients with severe acne who attended the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala la Real and Alcaudete (Jaen, Spain) over 4 years were eligible to be included in our study. Owning to the location of these 3 regional hospitals in the centre of Andalusia, they serve over one million persons.

Only patients with severe acne and treated with isotretinoin from September 1, 2008 to August 31, 2012 were included consecutively in the study.

Severe acne was diagnosed according to the European evidence-based (S3) guidelines for the treatment of acne.[8]

Inclusion criteria were age between 14 and 20 years old, severe acne, treatment with oral isotretinoin (0.5-0.8 mg/kg) and normal LFTs and lipid profiles.

Meanwhile, exclusion criteria were contradictions for isotretinoin, concomitant use before or during the study of other treatment inducer of Cytochrome P450, alcohol consumption, significant change in weight (>10%) and suspension of the treatment.

We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome.

All patients were informed about the aim of the study and signed an informed consent.

Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, liver function tests and lipid profiles. All questionnaire data were collected by the first author (P F-C S) in the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala
For peer review only

la Real and Alcaudete, and the blood tests were done the laboratories of the same hospitals.

Basal levels of bilirubin in patients with Gilbert’s syndrome were measured in 3 consecutive mornings. Of the 3 measures, the mean basal level was calculated. Fasting levels of bilirubin in patients with Gilbert’s syndrome were >1.1 mg/dl.

All patients were followed up in our outpatient clinics after 10 and 20 weeks. Blood analyses were repeated in the follow-up visits. All participants were followed up over 20 weeks with no lost follow-up.

Data and results of patients with severe acne and Gilbert’s syndrome were compared with these of the 26 patients with only severe acne (control group).

No adverse effects of isotretinoin treatment were noted and in all patients the acne was in complete remission at the last follow-up visit.

Statistical methods:

First, the following descriptive analysis was done: frequency, percent, mean, standard deviation. Comparisons between patients with Gilbert’s syndrome and control patients were done using Mann-Whitney test for continuous variables and Pearson’s Chi square test for categorical variables. Comparisons of LFTs and lipid profile levels over the follow-up period were done using Friedman Test. Level of significance was set at p <0.05. All data variables were encoded and computerized. Data entry and statistical analysis were performed using the Statistical Package for Social Science (SPSS) version 15.0 (SPSS Inc., Chicago, Illinois).
RESULTS

Our follow-up study included 37 patients with severe acne; 11 patients with Gilbert’s syndrome and 26 control patients. No statistical significant differences were found between the 2 studied groups regarding age and gender.

In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks compared with the basal level (P value <0.001). Meanwhile, AST, ALT and GGT levels over the 20 weeks follow-up did not vary significantly; P values 0.37, 0.77 and 0.46 respectively (Table 1).

In control patients, bilirubin and ALT levels did not vary significantly over the 20 weeks follow-up; P value 0.23 and 0.28 respectively. Meanwhile, AST and GGT varied significantly over the 20 weeks follow-up; P value 0.018 and 0.002 respectively (Table 1).

In Gilbert’s syndrome patients, LDL levels showed substantial increase over the 20 weeks follow-up, with more increase after 10 weeks compared with the basal level (P value <0.001). Meanwhile, cholesterol, triglycerides and HDL levels over the 20 weeks follow-up did not vary significantly; P values 0.18, 0.65 and 0.32 respectively (Table 2).

In control patients, cholesterol, triglycerides and LDL levels showed substantial increase over the 20 weeks follow-up; P values <0.001. Meanwhile, HDL levels over the 20 weeks follow up did not vary significantly; P values 0.34 (Table 2).
DISCUSSION

The main objective of the current follow-up study was to evaluate the hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome. In theory, hepatotoxic drugs like isotretinoin would increase bilirubin and liver enzymes levels in patients with hereditary hepatic disorders like Gilbert’s syndrome.[4]

Before reaching conclusions based on the present results, it is necessary to consider a number of potential objections to the methodology. Limitations such as the small sample size could affect the validity of the results. Our study included only 37 patients with severe acne and treated with oral isotretinoin. It was difficult to identify more patients according to our strict inclusion and exclusion criteria, and that is why we included all patients treated over 4 years in 3 regional hospitals. Other possible limitations were the placebo effect and the natural evolution. Placebo effect was avoided by repeated measures; before and 10 and 20 weeks after treatment. Natural evolution was controlled be the follow-up comparative control group without Gilbert’s syndrome. Although these results pertain solely to a region of the province of Jaen (Spain) and should not be considered generalizable, the methodology can be applied in different Dermatology Outpatient Clinics. Our results call for further investigation of hepatotoxicity of isotretinoin in patients with acne and Gilbert’s syndrome; future studies preferably should be performed on large prospective cohorts, to increase their internal validity.

By 10 weeks all patients with Gilbert’s syndrome had a significant decrease in total bilirubin levels and they maintained this decrease after 20 weeks.
The cause for decline in bilirubin levels in patients with Gilbert’s syndrome treated with isotretinoin is still unknown. Only few studies were published about this unexpected decline in bilirubin levels by a hepatotoxic drug like isotretinoin.[9-11]

Several drugs like phenobarbitals and corticosteroids induce microsomal enzymes as UDP-GT and stimulate the conjugation of bilirubin producing its decrease. In contrary, isotretinoin inhibits the inducer effect of other drugs and thus this does not seems to be its mechanism of action in patients with Gilbert’s syndrome.[12-13]

Currently, there are two hypotheses to explain this unexpected effect of isotretinoin in patients with Gilbert’s syndrome; reversible decrease in the serum levels of testosterone increasing the activity of UDP-GT or stimulating hepatocytes to generate transporting proteins that eliminate bilirubin.[14-15]

None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow-up. Of note, liver enzymes (AST, ALT and GGT) were maintained within normal levels in both studied groups.

Our study also explored the adverse effect of isotretinoin on lipid profile levels. Both studied groups did not show significant pathological increase in lipid profile levels (cholesterol, triglycerides, HDL and LDL). All lipid profile levels in Gilbert’s syndrome patients and control patients were maintained within the normal recommended levels. Recent follow-up study with 248 patients treated with isotretinoin because of acne vulgaris, showed that only 1.2% suffered impaired LFTs and 1.61% had high lipid profile levels after 16 weeks of treatment.[16] These findings agree with our results after 10 and 20 weeks of follow-up in both Gilbert’s syndrome and control patients.
LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Again, these findings are contradictory to what would be expected from hepatotoxic drug administrated in patients with hepatic dysfunction.

Previous studies showed that patients with Gilbert’s syndrome have lower prevalence of atherosclerosis and ischemic heart disease.[17-18] A systematic review confirmed that there is a reliable inverse and dose–response relationship between serum bilirubin and the atherosclerotic process.[19] Recent case control study showed that LDL, well known mediator of initial stages of atherosclerosis, was lower in patients with Gilbert’s syndrome when compared to healthy controls.[20] This agrees with the results of our study where basal levels of LDL and after isotretinoin treatment were lower in patients with Gilbert’s syndrome compared with control patients.

In conclusion, our preliminary results show that oral isotretinoin is not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment. Identification of the mechanism of action of oral isotretinoin in patients with Gilbert’s syndrome could help in elaborating new protective drugs against hepatotoxicity.
CONTRIBUTORSHIP STATEMENT:

Each author declares having participated in the activities described below and that they have seen and approved the final version. They also declare not having conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

Pablo Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

José Luis Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

Mohamed Farouk Allam: Data analysis, interpretation of results, decision making on content and paper write-up and revision of final draft.

Rafael Fernández-Crehuet Navajas: Author of original idea, study design, field work supervision, data analysis, decision making on content and paper write-up and revision of final draft.

Funding: none.

Conflicts of interest: none.

Data Sharing Statement: none
REFERENCES


Table 1. Follow-up and comparative study of liver function tests in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal bilirubin levels</td>
<td>1.78 ± 0.65</td>
<td>0.50 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 10 weeks</td>
<td>1.03 ± 0.18</td>
<td>0.52 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 20 weeks</td>
<td>1.38 ± 0.54</td>
<td>0.48 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal AST levels</td>
<td>22.1 ± 5.1</td>
<td>21.6 ± 14.7</td>
<td>0.14</td>
</tr>
<tr>
<td>AST levels after 10 weeks</td>
<td>23.8 ± 5.4</td>
<td>21.4 ± 7.8</td>
<td>0.11</td>
</tr>
<tr>
<td>AST levels after 20 weeks</td>
<td>21.5 ± 7.1</td>
<td>21.4 ± 7.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Basal ALT levels</td>
<td>20 ± 10.4</td>
<td>15.4 ± 9.0</td>
<td>0.12</td>
</tr>
<tr>
<td>ALT levels after 10 weeks</td>
<td>21.1 ± 7.9</td>
<td>15.8 ± 6.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT levels after 20 weeks</td>
<td>18.2 ± 7.3</td>
<td>15.5 ± 6.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Basal GGT levels</td>
<td>18.1 ± 9.9</td>
<td>14 ± 4.7</td>
<td>0.35</td>
</tr>
<tr>
<td>GGT levels after 10 weeks</td>
<td>24 ± 1.7</td>
<td>17.1 ± 7.9</td>
<td>0.23</td>
</tr>
<tr>
<td>GGT levels after 20 weeks</td>
<td>17.1 ± 4.5</td>
<td>18.4 ± 8.5</td>
<td>0.79</td>
</tr>
</tbody>
</table>

¹ Mann-Whitney Test
Table 2. Follow-up and comparative study of lipid profile in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cholesterol levels</td>
<td>143.6 ± 19.5</td>
<td>155.6 ± 24.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cholesterol levels after 10 weeks</td>
<td>164.4 ± 23.4</td>
<td>176.3 ± 30</td>
<td>0.32</td>
</tr>
<tr>
<td>Cholesterol levels after 20 weeks</td>
<td>162.7 ± 12.4</td>
<td>181.6 ± 30.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal triglycerides levels</td>
<td>71.2 ± 27.2</td>
<td>72.1 ± 43</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides levels after 10 weeks</td>
<td>84.3 ± 50.7</td>
<td>93.5 ± 46.4</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides levels after 20 weeks</td>
<td>67.2 ± 31</td>
<td>103.8 ± 56.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Basal HDL levels</td>
<td>53.5 ± 15.2</td>
<td>56.7 ± 11.4</td>
<td>0.33</td>
</tr>
<tr>
<td>HDL levels after 10 weeks</td>
<td>53.5 ± 14.8</td>
<td>56.7 ± 12.8</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL levels after 20 weeks</td>
<td>62.4 ± 17.5</td>
<td>54.8 ± 13.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Basal LDL levels</td>
<td>78.1 ± 13.9</td>
<td>82.3 ± 20.5</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL levels after 10 weeks</td>
<td>95.3 ± 19</td>
<td>99.4 ± 25.6</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL levels after 20 weeks</td>
<td>88.3 ± 14.1</td>
<td>107.3 ± 24.5</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mann-Whitney Test
<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| **Title and abstract** | 1  
(a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | 2  
Explain the scientific background and rationale for the investigation being reported |
| **Methods** | 3  
State specific objectives, including any prespecified hypotheses |
| **Participants** | 4  
Present key elements of study design early in the paper  
(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants  
(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed  
Case-control study—For matched studies, give matching criteria and the number of controls per case |
| **Variables** | 5  
Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| **Data sources/measurement** | 6  
For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| **Bias** | 7  
Describe any efforts to address potential sources of bias |
| **Study size** | 8  
Explain how the study size was arrived at |
| **Quantitative variables** | 9  
Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| **Statistical methods** | 10  
(a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) Cohort study—If applicable, explain how loss to follow-up was addressed  
Case-control study—If applicable, explain how matching of cases and controls was addressed  
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy  
(e) Describe any sensitivity analyses |

Continued on next page
### Results

#### Participants

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>13*</td>
<td>(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed</td>
</tr>
<tr>
<td></td>
<td>(b) Give reasons for non-participation at each stage</td>
</tr>
<tr>
<td></td>
<td>(c) Consider use of a flow diagram</td>
</tr>
</tbody>
</table>

#### Descriptive data

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14*</td>
<td>(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders</td>
</tr>
<tr>
<td></td>
<td>(b) Indicate number of participants with missing data for each variable of interest</td>
</tr>
<tr>
<td></td>
<td>(c) Cohort study—Summarise follow-up time (e.g., average and total amount)</td>
</tr>
</tbody>
</table>

#### Outcome data

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>15*</td>
<td>(a) Cohort study—Report numbers of outcome events or summary measures over time</td>
</tr>
<tr>
<td></td>
<td>Case-control study—Report numbers in each exposure category, or summary measures of exposure</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional study—Report numbers of outcome events or summary measures</td>
</tr>
</tbody>
</table>

#### Main results

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included</td>
</tr>
<tr>
<td></td>
<td>(b) Report category boundaries when continuous variables were categorized</td>
</tr>
<tr>
<td></td>
<td>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</td>
</tr>
</tbody>
</table>

#### Other analyses

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses</td>
</tr>
</tbody>
</table>

### Discussion

#### Key results

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Summarise key results with reference to study objectives</td>
</tr>
</tbody>
</table>

#### Limitations

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</td>
</tr>
</tbody>
</table>

#### Interpretation

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</td>
</tr>
</tbody>
</table>

#### Generalisability

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Discuss the generalisability (external validity) of the study results</td>
</tr>
</tbody>
</table>

### Other information

#### Funding

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based</td>
</tr>
</tbody>
</table>

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT’S SYNDROME: COMPARATIVE STUDY

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2013-004441.R2</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>27-Feb-2014</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Fernández-Crehuet, Pablo; Alto Guadalquivir Hospital, Dermatology Unit Serrano, José Luis; Alto Guadalquivir Hospital, Dermatology Unit Allam, Moahmed; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health Navajas, Rafael; Faculty of Medicine, University of Cordoba, Department of Preventive Medicine and Public Health</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Gastroenterology and hepatology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Dermatology, Gastroenterology and hepatology, Pharmacology and therapeutics</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Acne &lt; DERMATOLOGY, Hepatobiliary disease &lt; GASTROENTEROLOGY, Physiology &lt; BASIC SCIENCES, CLINICAL PHARMACOLOGY, Hepatology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME: COMPARATIVE STUDY

Pablo Fernández-Crehuet Serrano. MD, PhD
José Luis Fernández-Crehuet Serrano. MD, PhD
Mohamed Farouk Allam. MPH, PhD
Rafael Fernández-Crehuet Navajas. MD, PhD

1. Dermatology Unit. Alto Guadalquivir Hospital. Andújar, Jaén, Spain.

Correspondence: Mohamed Farouk Allam, Department of Preventive Medicine and Public Health, Faculty of Medicine, University of Cordoba. Avda. Menéndez Pidal, s/n Cordoba 14004, Spain.

Phone: +34 957 218 278; Fax. +34 957 218 573

E-mail: fm2faahm@uco.es

Funding: none.

Conflicts of interest: none.

Word count: 1944

Short Title: Hepatotoxicity of isotretinoin in patients with Gilbert's syndrome.
ABSTRACT

Objectives. The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne. Setting. Dermatology Outpatient Clinics of 3 regional hospitals of Jaen (Spain). Participants. Over 4 years, we included all patients diagnosed with severe acne. Only 37 patients were identified, of which 11 had Gilbert’s syndrome. Interventions. All patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks. Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Data and results of patients with severe acne and Gilbert’s syndrome were compared with these of the 26 patients with only severe acne (control group). Primary outcome. Blood analyses were repeated in the follow-up visits. Results. In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks. None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow up. Liver enzymes were maintained within normal levels in both groups. Both studied groups did not show significant pathological increase in lipid profile levels. LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Conclusion. Our preliminary results suggest that oral isotretinoin could be an effective safe treatment for patients with Gilbert’s syndrome, and may lower bilirubin levels in the first 10 weeks of treatment. Limitations of the study include the small numbers of participants and the fact that it is restricted to one region of Spain.
Keywords: Acne, bilirubin, Gilbert’s syndrome, triglycerides, isotretinoin.

Ethical Committee: Study approved by the Ethical Committee of Hospital de Andujar.

INTRODUCTION

The use of isotretinoin for the treatment of severe acne has been widely used over the last 30 years. Many adverse reactions to isotretinoin have been reported. Several studies have shown that hepatotoxicity could occur in about 10% and hyperlipidemia in 20-45%.[1-3]

Gilbert’s syndrome is a benign and inherited state characterized by intermittent unconjugated hyperbilirubinaemia with accompanying jaundice in the absence of
haemolysis or underlying liver disease.[4] Previous studies have shown that Gilbert's syndrome affects 5% to 7% of the population, mainly postpubertal male patients.[4-5]

In patients with hepatic dysfunction, like Gilbert’s syndrome, we would expect an increased sensitivity to isotretinoin that is metabolized by hepatic oxidation and biliary excretion.[6-7]

The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.

**METHODOLOGY**

All patients with severe acne who attended the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala la Real and Alcaudete (Jaen, Spain) over 4 years were eligible to be included in our study. Owning to the location of these 3 regional hospitals in the centre of Andalusia, they serve over one million persons. The study was approved the Ethical Committee of Alto Guadalquivir Hospital (Andújar, Spain).

Only patients with severe acne and treated with isotretinoin from September 1, 2008 to August 31, 2012 were included consecutively in the study.
Severe acne was diagnosed according to the European evidence-based (S3) guidelines for the treatment of acne.[8]

Inclusion criteria were age between 14 and 20 years old, severe acne, treatment with oral isotretinoin (0.5-0.8 mg/kg) and normal LFTs and lipid profiles.

Meanwhile, exclusion criteria were contradictions for isotretinoin, concomitant use before or during the study of other treatment inducer of Cytochrome P450, alcohol consumption, significant change in weight (>10%) and suspension of the treatment.

We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome.

All patients were informed about the aim of the study and signed an informed consent.

Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, liver function tests and lipid profiles. All questionnaire data were collected by the first author (P F-C S) in the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala la Real and Alcaudete, and the blood tests were done the laboratories of the same hospitals.

Basal levels of bilirubin in patients with Gilbert’s syndrome were measured in 3 consecutive mornings. Of the 3 measures, the mean basal level was calculated. Fasting levels of bilirubin in patients with Gilbert’s syndrome were >1.1 mg/dl.

All patients were followed up in our outpatient clinics after 10 and 20 weeks. Blood analyses were repeated in the follow-up visits. All participants were followed up over 20 weeks with no lost follow-up.
Data and results of patients with severe acne and Gilbert’s syndrome were compared with those of the 26 patients with only severe acne (control group).

No adverse effects of isotretinoin treatment were noted and in all patients the acne was in complete remission at the last follow-up visit.

**Statistical methods:**

First, the following descriptive analysis was done: frequency, percent, mean, standard deviation. Comparisons between patients with Gilbert’s syndrome and control patients were done using Mann-Whitney test for continuous variables and Pearson’s Chi square test for categorical variables. Comparisons of LFTs and lipid profile levels over the follow-up period were done using Friedman Test. Level of significance was set at p <0.05. All data variables were encoded and computerized. Data entry and statistical analysis were performed using the Statistical Package for Social Science (SPSS) version 15.0 (SPSS Inc., Chicago, Illinois).

**RESULTS**

Our follow-up study included 37 patients with severe acne; 11 patients with Gilbert’s syndrome and 26 control patients. No statistical significant differences were found between the 2 studied groups regarding age and gender.

In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks compared with the basal level (P
value <0.001). Meanwhile, AST, ALT and GGT levels over the 20 weeks follow-up did not vary significantly; P values 0.37, 0.77 and 0.46 respectively (Table 1).

In control patients, bilirubin and ALT levels did not vary significantly over the 20 weeks follow-up; P value 0.23 and 0.28 respectively. Meanwhile, AST and GGT varied significantly over the 20 weeks follow-up; P value 0.018 and 0.002 respectively (Table 1).

In Gilbert’s syndrome patients, LDL levels showed substantial increase over the 20 weeks follow-up, with more increase after 10 weeks compared with the basal level (P value <0.001). Meanwhile, cholesterol, triglycerides and HDL levels over the 20 weeks follow-up did not vary significantly; P values 0.18, 0.65 and 0.32 respectively (Table 2).

In control patients, cholesterol, triglycerides and LDL levels showed substantial increase over the 20 weeks follow-up; P values <0.001. Meanwhile, HDL levels over the 20 weeks follow up did not vary significantly; P values 0.34 (Table 2).

DISCUSSION

The main objective of the current follow-up study was to evaluate the hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome. In theory, hepatotoxic drugs like isotretinoin would increase bilirubin and liver enzymes levels in patients with hereditary hepatic disorders like Gilbert’s syndrome.[4]
Before reaching conclusions based on the present results, it is necessary to consider a number of potential objections to the methodology. Limitations such as the small sample size could affect the validity of the results. Our study included only 37 patients with severe acne and treated with oral isotretinoin. It was difficult to identify more patients according to our strict inclusion and exclusion criteria, and that is why we included all patients treated over 4 years in 3 regional hospitals. Other possible limitations were the placebo effect and the natural evolution. Placebo effect was avoided by repeated measures; before and 10 and 20 weeks after treatment. Natural evolution was controlled by the follow-up comparative control group without Gilbert’s syndrome. Although these results pertain solely to a region of the province of Jaen (Spain) and should not be considered generalizable, the methodology can be applied in different Dermatology Outpatient Clinics. Our results call for further investigation of hepatotoxicity of isotretinoin in patients with acne and Gilbert's syndrome; future studies preferably should be performed on large prospective cohorts, to increase their internal validity.

By 10 weeks all patients with Gilbert’s syndrome had a significant decrease in total bilirubin levels and they maintained this decrease after 20 weeks.

The cause for decline in bilirubin levels in patients with Gilbert’s syndrome treated with isotretinoin is still unknown. Only few studies were published about this unexpected decline in bilirubin levels by a hepatotoxic drug like isotretinoin.[9-11]

Several drugs like phenobarbitals and corticosteroids induce microsomal enzymes as UDP-GT and stimulate the conjugation of bilirubin producing its decrease. In contrary,
isotretinoin inhibits the inducer effect of other drugs and thus this does not seems to be its mechanism of action in patients with Gilbert’s syndrome.[12-13]

Currently, there are two hypotheses to explain this unexpected effect of isotretinoin in patients with Gilbert’s syndrome; reversible decrease in the serum levels of testosterone increasing the activity of UDP-GT or stimulating hepatocytes to generate transporting proteins that eliminate bilirubin.[14-15]

None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow-up. Of note, liver enzymes (AST, ALT and GGT) were maintained within normal levels in both studied groups.

Our study also explored the adverse effect of isotretinoin on lipid profile levels. Both studied groups did not show significant pathological increase in lipid profile levels (cholesterol, triglycerides, HDL and LDL). All lipid profile levels in Gilbert’s syndrome patients and control patients were maintained within the normal recommended levels. Recent follow-up study with 248 patients treated with isotretinoin because of acne vulgaris, showed that only 1.2% suffered impaired LFTs and 1.61% had high lipid profile levels after 16 weeks of treatment.[16] These findings agree with our results after 10 and 20 weeks of follow-up in both Gilbert’s syndrome and control patients.

LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Again, these findings are contradictory to what would be expected from hepatotoxic drug administrated in patients with hepatic dysfunction.

Previous studies showed that patients with Gilbert’s syndrome have lower prevalence of atherosclerosis and ischemic heart disease.[17-18] A systematic review confirmed that
there is a reliable inverse and dose–response relationship between serum bilirubin and the atherosclerotic process.[19] Recent case control study showed that LDL, well known mediator of initial stages of atherosclerosis, was lower in patients with Gilbert’s syndrome when compared to healthy controls.[20] This agrees with the results of our study where basal levels of LDL and after isotretinoin treatment were lower in patients with Gilbert’s syndrome compared with control patients.

In conclusion, our preliminary results suggest that oral isotretinoin is not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment. Identification of the mechanism of action of oral isotretinoin in patients with Gilbert’s syndrome could help in elaborating new protective drugs against hepatotoxicity.

**CONTRIBUTORSHIP STATEMENT:**

Each author declares having participated in the activities described below and that they have seen and approved the final version. They also declare not having conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

Pablo Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design,
data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

José Luis Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

Mohamed Farouk Allam: Data analysis, interpretation of results, decision making on content and paper write-up and revision of final draft.

Rafael Fernández-Crehuet Navajas: Author of original idea, study design, field work supervision, data analysis, decision making on content and paper write-up and revision of final draft.

**Data sharing**

No additional data available.

**Competing Interests**

None

**REFERENCES**


Table 1. Follow-up and comparative study of liver function tests in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal bilirubin levels</td>
<td>1.78 ± 0.65</td>
<td>0.50 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 10 weeks</td>
<td>1.03 ± 0.18</td>
<td>0.52 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 20 weeks</td>
<td>1.38 ± 0.54</td>
<td>0.48 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal AST levels</td>
<td>22.1 ± 5.1</td>
<td>21.6 ± 14.7</td>
<td>0.14</td>
</tr>
<tr>
<td>AST levels after 10 weeks</td>
<td>23.8 ± 5.4</td>
<td>21.4 ± 7.8</td>
<td>0.11</td>
</tr>
<tr>
<td>AST levels after 20 weeks</td>
<td>21.5 ± 7.1</td>
<td>21.4 ± 7.6</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Table 2. Follow-up and comparative study of lipid profile in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cholesterol levels</td>
<td>143.6 ± 19.5</td>
<td>155.6 ± 24.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cholesterol levels after 10 weeks</td>
<td>164.4 ± 23.4</td>
<td>176.3 ± 30</td>
<td>0.32</td>
</tr>
<tr>
<td>Cholesterol levels after 20 weeks</td>
<td>162.7 ± 12.4</td>
<td>181.6 ± 30.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal triglycerides levels</td>
<td>71.2 ± 27.2</td>
<td>72.1 ± 43</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides levels after 10 weeks</td>
<td>84.3 ± 50.7</td>
<td>93.5 ± 46.4</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides levels after 20 weeks</td>
<td>67.2 ± 31</td>
<td>103.8 ± 56.9</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

\(^1\) Mann-Whitney Test
<table>
<thead>
<tr>
<th></th>
<th>Basal HDL levels</th>
<th>HDL levels after 10 weeks</th>
<th>HDL levels after 20 weeks</th>
<th>Basal LDL levels</th>
<th>LDL levels after 10 weeks</th>
<th>LDL levels after 20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>53.5 ± 15.2</td>
<td>53.5 ± 14.8</td>
<td>62.4 ± 17.5</td>
<td>78.1 ± 13.9</td>
<td>95.3 ± 19</td>
<td>88.3 ± 14.1</td>
</tr>
<tr>
<td></td>
<td>56.7 ± 11.4</td>
<td>56.7 ± 12.8</td>
<td>54.8 ± 13.6</td>
<td>82.3 ± 20.5</td>
<td>99.4 ± 25.6</td>
<td>107.3 ± 24.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
<td></td>
<td>0.54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mann-Whitney Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HEPATOTOXICITY OF ISOTRETINOIN IN PATIENTS WITH ACNE AND GILBERT'S SYNDROME: COMPARATIVE STUDY

Pablo Fernández-Crehuet Serrano. MD, PhD

José Luis Fernández-Crehuet Serrano. MD, PhD

Mohamed Farouk Allam. MPH, PhD

Rafael Fernández-Crehuet Navajas. MD, PhD

1. Dermatology Unit. Alto Guadalquivir Hospital. Andújar, Jaén, Spain.


Correspondence: Mohamed Farouk Allam, Department of Preventive Medicine and Public Health, Faculty of Medicine, University of Cordoba.

Avda. Menéndez Pidal, s/n Cordoba 14004, Spain.

Phone: +34 957 218 278; Fax. +34 957 218 573

E-mail: fm2faahm@uco.es

Funding: none.

Conflicts of interest: none.

Word count: 1944

Short Title: Hepatotoxicity of isotretinoin in patients with Gilbert's syndrome.
CONTRIBUTORSHIP STATEMENT:

Each author declares having participated in the activities described below and that they have seen and approved the final version. They also declare not having conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

Pablo Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

José Luis Fernández-Crehuet Serrano: Field work supervision, analysis strategy and design, data management, data analysis and interpretation of results, decision making on content and paper write-up and revision of final draft.

Mohamed Farouk Allam: Data analysis, interpretation of results, decision making on content and paper write-up and revision of final draft.

Rafael Fernández-Crehuet Navajas: Author of original idea, study design, field work supervision, data analysis, decision making on content and paper write-up and revision of final draft.
ABSTRACT

Objectives. The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne. Setting. Dermatology Outpatient Clinics of 3 regional hospitals of Jaen (Spain). Participants. Over 4 years, we included all patients diagnosed with severe acne. Only 37 patients were identified, of which 11 had Gilbert’s syndrome. Interventions. All patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks. Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Data and results of patients with severe acne and Gilbert’s syndrome were compared with those of the 26 patients with only severe acne (control group). Primary outcome. Blood analyses were repeated in the follow-up visits. Results. In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks. None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow up. Liver enzymes were maintained within normal levels in both groups. Both studied groups did not show significant pathological increase in lipid profile levels. LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Conclusion. Our preliminary results suggest that oral isotretinoin could be an effective safe treatment for patients with Gilbert’s syndrome, and may lower bilirubin levels in the first 10 weeks of treatment. Limitations of the study include the small numbers of participants and the fact that it is restricted to one region of Spain.

Keywords: Acne, bilirubin, Gilbert’s syndrome, triglycerides, isotretinoin.
Ethical Committee: Study approved by the Ethical Committee of Hospital de Andjuar.

Article summary

'Strengths and limitations of this study'

- All patients with severe acne who attended the Dermatology Outpatient Clinics over 4 years were eligible to be included in our study.

- We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome. Patients were treated with isotretinoin and followed-up in our outpatient clinics after 10 and 20 weeks.

- Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, LFTs and lipid profiles. Blood analyses were repeated in the follow-up visits.

- Oral isotretinoin was not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment.

- Lipid profile levels in Gilbert’s syndrome patients were maintained within the normal recommended levels.

- Limitations of the study include the small numbers of participants and the fact that it is restricted to one region of Spain.
INTRODUCTION

The use of isotretinoin for the treatment of severe acne has been widely used over the last 30 years. Many adverse reactions to isotretinoin have been reported. Several studies have shown that hepatotoxicity could occur in about 10% and hyperlipidemia in 20-45%.[1-3]

Gilbert’s syndrome is a benign and inherited state characterized by intermittent unconjugated hyperbilirubinaemia with accompanying jaundice in the absence of haemolysis or underlying liver disease.[4] Previous studies have shown that Gilbert's syndrome affects 5% to 7% of the population, mainly postpubertal male patients.[4-5]

In patients with hepatic dysfunction, like Gilbert’s syndrome, we would expect an increased sensitivity to isotretinoin that is metabolized by hepatic oxidation and biliary excretion.[6-7]

The objective of our follow-up study is to evaluate liver function tests (LFTs) and lipid profiles in patients with Gilbert’s syndrome treated with isotretinoin because of severe acne.
METHODOLOGY

All patients with severe acne who attended the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala la Real and Alcaudete (Jaen, Spain) over 4 years were eligible to be included in our study. Owning to the location of these 3 regional hospitals in the centre of Andalusia, they serve over one million persons. The study was approved the Ethical Committee of Alto Guadalquivir Hospital (Andújar, Spain).

Only patients with severe acne and treated with isotretinoin from September 1, 2008 to August 31, 2012 were included consecutively in the study.

Severe acne was diagnosed according to the European evidence-based (S3) guidelines for the treatment of acne.[8]

Inclusion criteria were age between 14 and 20 years old, severe acne, treatment with oral isotretinoin (0.5-0.8 mg/kg) and normal LFTs and lipid profiles.

Meanwhile, exclusion criteria were contradictions for isotretinoin, concomitant use before or during the study of other treatment inducer of Cytochrome P450, alcohol consumption, significant change in weight (>10%) and suspension of the treatment.

We identified 37 patients with severe acne during the study period, of which 11 had Gilbert’s syndrome.

All patients were informed about the aim of the study and signed an informed consent.

Patients were subjected to an interview questionnaire which included data on age, gender, complete blood count, coagulation profile, fasting blood glucose, liver function tests and lipid profiles. All questionnaire data were collected by the first author (P F-C S) in the Dermatology Outpatient Clinics of the 3 regional hospitals of Andujar, Alcala
la Real and Alcaudete, and the blood tests were done the laboratories of the same hospitals.

Basal levels of bilirubin in patients with Gilbert’s syndrome were measured in 3 consecutive mornings. Of the 3 measures, the mean basal level was calculated. Fasting levels of bilirubin in patients with Gilbert’s syndrome were >1.1 mg/dl.

All patients were followed up in our outpatient clinics after 10 and 20 weeks. Blood analyses were repeated in the follow-up visits. All participants were followed up over 20 weeks with no lost follow-up.

Data and results of patients with severe acne and Gilbert’s syndrome were compared with these of the 26 patients with only severe acne (control group).

No adverse effects of isotretinoin treatment were noted and in all patients the acne was in complete remission at the last follow-up visit.

**Statistical methods:**

First, the following descriptive analysis was done: frequency, percent, mean, standard deviation. Comparisons between patients with Gilbert’s syndrome and control patients were done using Mann-Whitney test for continuous variables and Pearson’s Chi square test for categorical variables. Comparisons of LFTs and lipid profile levels over the follow-up period were done using Friedman Test. Level of significance was set at $p<0.05$. All data variables were encoded and computerized. Data entry and statistical analysis were performed using the Statistical Package for Social Science (SPSS) version 15.0 (SPSS Inc., Chicago, Illinois).
RESULTS

Our follow-up study included 37 patients with severe acne; 11 patients with Gilbert’s syndrome and 26 control patients. No statistical significant differences were found between the 2 studied groups regarding age and gender.

In Gilbert’s syndrome patients, bilirubin levels showed substantial decrease over the 20 weeks follow-up, with more decrease after 10 weeks compared with the basal level (P value <0.001). Meanwhile, AST, ALT and GGT levels over the 20 weeks follow-up did not vary significantly; P values 0.37, 0.77 and 0.46 respectively (Table 1).

In control patients, bilirubin and ALT levels did not vary significantly over the 20 weeks follow-up; P value 0.23 and 0.28 respectively. Meanwhile, AST and GGT varied significantly over the 20 weeks follow-up; P value 0.018 and 0.002 respectively (Table 1).

In Gilbert’s syndrome patients, LDL levels showed substantial increase over the 20 weeks follow-up, with more increase after 10 weeks compared with the basal level (P value <0.001). Meanwhile, cholesterol, triglycerides and HDL levels over the 20 weeks follow-up did not vary significantly; P values 0.18, 0.65 and 0.32 respectively (Table 2).

In control patients, cholesterol, triglycerides and LDL levels showed substantial increase over the 20 weeks follow-up; P values <0.001. Meanwhile, HDL levels over the 20 weeks follow up did not vary significantly; P values 0.34 (Table 2).
DISCUSSION

The main objective of the current follow-up study was to evaluate the hepatotoxicity of isotretinoin in patients with Gilbert’s syndrome. In theory, hepatotoxic drugs like isotretinoin would increase bilirubin and liver enzymes levels in patients with hereditary hepatic disorders like Gilbert’s syndrome. [4]

Before reaching conclusions based on the present results, it is necessary to consider a number of potential objections to the methodology. Limitations such as the small sample size could affect the validity of the results. Our study included only 37 patients with severe acne and treated with oral isotretinoin. It was difficult to identify more patients according to our strict inclusion and exclusion criteria, and that is why we included all patients treated over 4 years in 3 regional hospitals. Other possible limitations were the placebo effect and the natural evolution. Placebo effect was avoided by repeated measures; before and 10 and 20 weeks after treatment. Natural evolution was controlled by the follow-up comparative control group without Gilbert’s syndrome. Although these results pertain solely to a region of the province of Jaen (Spain) and should not be considered generalizable, the methodology can be applied in different Dermatology Outpatient Clinics. Our results call for further investigation of hepatotoxicity of isotretinoin in patients with acne and Gilbert's syndrome; future studies preferably should be performed on large prospective cohorts, to increase their internal validity.

By 10 weeks all patients with Gilbert’s syndrome had a significant decrease in total bilirubin levels and they maintained this decrease after 20 weeks.
The cause for decline in bilirubin levels in patients with Gilbert’s syndrome treated with isotretinoin is still unknown. Only few studies were published about this unexpected decline in bilirubin levels by a hepatotoxic drug like isotretinoin.[9-11]

Several drugs like phenobarbitals and corticosteroids induce microsomal enzymes as UDP-GT and stimulate the conjugation of bilirubin producing its decrease. In contrary, isotretinoin inhibits the inducer effect of other drugs and thus this does not seems to be its mechanism of action in patients with Gilbert’s syndrome.[12-13]

Currently, there are two hypotheses to explain this unexpected effect of isotretinoin in patients with Gilbert’s syndrome; reversible decrease in the serum levels of testosterone increasing the activity of UDP-GT or stimulating hepatocytes to generate transporting proteins that eliminate bilirubin.[14-15]

None of the control group patients had significant increase in total bilirubin levels after 10 and 20 weeks follow-up. Of note, liver enzymes (AST, ALT and GGT) were maintained within normal levels in both studied groups.

Our study also explored the adverse effect of isotretinoin on lipid profile levels. Both studied groups did not show significant pathological increase in lipid profile levels (cholesterol, triglycerides, HDL and LDL). All lipid profile levels in Gilbert’s syndrome patients and control patients were maintained within the normal recommended levels. Recent follow-up study with 248 patients treated with isotretinoin because of acne vulgaris, showed that only 1.2% suffered impaired LFTs and 1.61% had high lipid profile levels after 16 weeks of treatment.[16] These findings agree with our results after 10 and 20 weeks of follow-up in both Gilbert’s syndrome and control patients.
LDL levels were increased in the 2 studied groups, but this increase was less substantial in Gilbert’s syndrome patients. Again, these findings are contradictory to what would be expected from hepatotoxic drug administrated in patients with hepatic dysfunction.

Previous studies showed that patients with Gilbert’s syndrome have lower prevalence of atherosclerosis and ischemic heart disease.[17-18] A systematic review confirmed that there is a reliable inverse and dose–response relationship between serum bilirubin and the atherosclerotic process.[19] Recent case control study showed that LDL, well known mediator of initial stages of atherosclerosis, was lower in patients with Gilbert’s syndrome when compared to healthy controls.[20] This agrees with the results of our study where basal levels of LDL and after isotretinoin treatment were lower in patients with Gilbert’s syndrome compared with control patients.

In conclusion, our preliminary results suggest that oral isotretinoin is not only an effective safe treatment for patients with Gilbert’s syndrome, but also it lowers bilirubin levels in the first 10 weeks of treatment. Identification of the mechanism of action of oral isotretinoin in patients with Gilbert’s syndrome could help in elaborating new protective drugs against hepatotoxicity.
REFERENCES


Table 1. Follow-up and comparative study of liver function tests in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal bilirubin levels</td>
<td>1.78 ± 0.65</td>
<td>0.50 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 10 weeks</td>
<td>1.03 ± 0.18</td>
<td>0.52 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin levels after 20 weeks</td>
<td>1.38 ± 0.54</td>
<td>0.48 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal AST levels</td>
<td>22.1 ± 5.1</td>
<td>21.6 ± 14.7</td>
<td>0.14</td>
</tr>
<tr>
<td>AST levels after 10 weeks</td>
<td>23.8 ± 5.4</td>
<td>21.4 ± 7.8</td>
<td>0.11</td>
</tr>
<tr>
<td>AST levels after 20 weeks</td>
<td>21.5 ± 7.1</td>
<td>21.4 ± 7.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Basal ALT levels</td>
<td>20 ± 10.4</td>
<td>15.4 ± 9.0</td>
<td>0.12</td>
</tr>
<tr>
<td>ALT levels after 10 weeks</td>
<td>21.1 ± 7.9</td>
<td>15.8 ± 6.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT levels after 20 weeks</td>
<td>18.2 ± 7.3</td>
<td>15.5 ± 6.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Basal GGT levels</td>
<td>18.1 ± 9.9</td>
<td>14 ± 4.7</td>
<td>0.35</td>
</tr>
<tr>
<td>GGT levels after 10 weeks</td>
<td>24 ± 1.7</td>
<td>17.1 ± 7.9</td>
<td>0.23</td>
</tr>
<tr>
<td>GGT levels after 20 weeks</td>
<td>17.1 ± 4.5</td>
<td>18.4 ± 8.5</td>
<td>0.79</td>
</tr>
</tbody>
</table>

\(^1\) Mann-Whitney Test
Table 2. Follow-up and comparative study of lipid profile in controls and patients with Gilbert’s syndrome treated with isotretinoin.

<table>
<thead>
<tr>
<th>Case/Variable</th>
<th>Gilbert’s Syndrome</th>
<th>Control</th>
<th>P value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cholesterol levels</td>
<td>143.6 ± 19.5</td>
<td>155.6 ± 24.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cholesterol levels after 10 weeks</td>
<td>164.4 ± 23.4</td>
<td>176.3 ± 30</td>
<td>0.32</td>
</tr>
<tr>
<td>Cholesterol levels after 20 weeks</td>
<td>162.7 ± 12.4</td>
<td>181.6 ± 30.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal triglycerides levels</td>
<td>71.2 ± 27.2</td>
<td>72.1 ± 43</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides levels after 10 weeks</td>
<td>84.3 ± 50.7</td>
<td>93.5 ± 46.4</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides levels after 20 weeks</td>
<td>67.2 ± 31</td>
<td>103.8 ± 56.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Basal HDL levels</td>
<td>53.5 ± 15.2</td>
<td>56.7 ± 11.4</td>
<td>0.33</td>
</tr>
<tr>
<td>HDL levels after 10 weeks</td>
<td>53.5 ± 14.8</td>
<td>56.7 ± 12.8</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL levels after 20 weeks</td>
<td>62.4 ± 17.5</td>
<td>54.8 ± 13.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Basal LDL levels</td>
<td>78.1 ± 13.9</td>
<td>82.3 ± 20.5</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL levels after 10 weeks</td>
<td>95.3 ± 19</td>
<td>99.4 ± 25.6</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL levels after 20 weeks</td>
<td>88.3 ± 14.1</td>
<td>107.3 ± 24.5</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mann-Whitney Test
### STROBE Statement—checklist of items that should be included in reports of observational studies

<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| **Title and abstract** | 1 | (a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | 2 | Explain the scientific background and rationale for the investigation being reported |
| **Objectives** | 3 | State specific objectives, including any prespecified hypotheses |
| **Methods** | 4 | Present key elements of study design early in the paper |
| | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| **Participants** | 6 | (a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants  
(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed  
Case-control study—For matched studies, give matching criteria and the number of controls per case |
| **Variables** | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| **Data sources/measurement** | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| **Bias** | 9 | Describe any efforts to address potential sources of bias |
| **Study size** | 10 | Explain how the study size was arrived at |
| **Quantitative variables** | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| **Statistical methods** | 12 | (a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) Cohort study—If applicable, explain how loss to follow-up was addressed  
Case-control study—If applicable, explain how matching of cases and controls was addressed  
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy  
(e) Describe any sensitivity analyses |

Continued on next page
Results

Participants
(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
(b) Give reasons for non-participation at each stage
(c) Consider use of a flow diagram

Descriptive data
(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders

(b) Indicate number of participants with missing data for each variable of interest

(c) Cohort study—Summarise follow-up time (e.g. average and total amount)

Outcome data
(Cohort study)—Report numbers of outcome events or summary measures over time
(Case-control study)—Report numbers in each exposure category, or summary measures of exposure
(Cross-sectional study)—Report numbers of outcome events or summary measures

Main results
(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included
(b) Report category boundaries when continuous variables were categorized
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses
Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses

Discussion
Key results
Summarise key results with reference to study objectives

Limitations
Discuss limitations of the study, taking into account sources of potential bias or imprecision.
Discuss both direction and magnitude of any potential bias

Interpretation
Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

Generalisability
Discuss the generalisability (external validity) of the study results

Other information
Funding
Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.