ABOUT JCMR

The Journal of Clinical Medicine and Research (JCMR) is published monthly (one volume per year) by Academic Journals.

Journal of Clinical Medicine and Research (JCMR) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as cardiology, critical care medicine, Family Medicine, geriatrics, pediatrics etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JCMR are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jcmr@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

For all other correspondence that cannot be sent by e-mail, please contact the editorial office (at jcmr@academicjournals.org).

The Journal of Clinical Medicine and Research will only accept manuscripts submitted as e-mail attachments.

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author.
Editors

Prof. Neveen Helmy Ahmed Aboelsoud,
Complementary Medicine Researches & Applications (CAM),
National Research Center,
Research St (Tahrir),
Dokki, Cairo,
Egypt.

Prof. Bodh Raj Panhotra,
Department of Medical Microbiology,
Medical Laboratory Technology & Clinical Sciences,
Sardar Bhagwan Singh Postgraduate Institute of Biomedical Sciences & Research,
Balawala, Dehradun,
India.

Editorial Board

Prof. Ahmed BaHammam,
King Saud University,
Saudi Arabia.

Dr. Ellen Roskam,
Senior Scholar, Woodrow Wilson International Center for Scholars,
Washington, D.C.,
Adjunct Professor, University of Massachusetts, Lowell,
Visiting Senior Fellow, University of Surrey,
Faculty of Health and Medical Sciences, England, Switzerland.

Dr. Philippe Connes,
National Institute of Health and Medical Research (763),
Academic Hospital of Pointe a Pitre,
Guadeloupe (French West Indies),
Guadeloupe.

Dr. Robert G Bota,
University of Missouri,
Kansas City,
USA.

Dr. Haiyang Zhou,
Department of General Surgery,
Changzheng Hospital,
Second Military Medical University.
China.

Dr. Jimmy Jose,
SAC College of Pharmacy, Karnataka,
India.

Dr. Carlos A. Feldstein,
Hospital de Clinicas Jose de San Martin,
Av. Cordoba 2351 Buenos Aires 1120,
Argentina.

Dr. Fadia Mostafa Attia,
Faculty of Medicine,
Suez Canal University,
Egypt.

Dr. Hamza Mujagic,
Massachusetts General Hospital,
USA.

Dr. O.U.J. Umeora,
Ebonyi State University/Teaching Hospital,
Nigeria.
Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JCMR to publish manuscripts within weeks after submission.

Regular articles
All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page. The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.
**Results** should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The **Acknowledgments** of people, grants, funds, etc should be brief.

**Tables** should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

**Figure legends** should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

**References**: In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al.’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

**Examples:**

Nishimura (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 2001), (Chege, 1998; Stein, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

**Examples:**


Case Studies

Case Studies include original case reports that will deepen the understanding of general medical knowledge.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml).

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines. The presentation of the case study should include the important information regarding the case. This must include the medical history, demographics, symptoms, tests etc. Kindly note that all information that will lead to the identification of the particular patient(s) must be excluded.

The conclusion should highlight the contribution of the study and its relevance in general medical knowledge.

The Acknowledgments of people, grants, funds, etc should be brief.

References: Same as in regular articles

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage. Because IJMMS will be published freely online to attract a wide audience), authors will have free electronic access to the full text (in both HTML and PDF) of the article. Authors can freely download the PDF file from which they can print unlimited copies of their articles.
**Fees and Charges:** Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Clinical Medicine and Research is not contingent upon the author’s ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

**Copyright:** © 2012, Academic Journals. All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

**Disclaimer of Warranties**

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JCMR, whether or not advised of the possibility of damage, and on any theory of liability. This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
ARTICLES

Research Articles

Body mass index (BMI) related insulin resistance in polycystic ovarian syndrome among patients referred to gynecology clinic of Imam Reza Hospital, Tehran, Iran
Khayyat Khameneie Maryam, Nahid Arian pour, Aghdas Safari and Rasool Roozegar

The effect of furosemide on experimentally-induced seizures in mice
S. E. Oriaifo, I. Otokiti and E. K. Omogbai
Body mass index (BMI) related insulin resistance in polycystic ovarian syndrome among patients referred to gynecology clinic of Imam Reza Hospital, Tehran, Iran

Khayyat Khameneie Maryam¹, Nahid Arian pour²*, Aghdas Safari¹ and Rasool Roozegar³

¹Department of Obstetrics and Gynecology, Imam Reza Hospital, Tehran, Iran.
²Department of Microbiology, AJA University of Medical Sciences, Tehran, Iran.
³Department of Statistics, Yazd University, Yazd, 89195-741, Iran.

Accepted 18 September, 2012

Polycystic ovarian syndrome (PCOs) is one of the commonest endocrinopathies of women. The present work has been undertaken with an aim to study insulin resistance rate among army personnel families. Out of 108 women suffering from oligomenorrhea and hirsutism referred to gynecology clinic of Imam Reza hospital from 2009 to 2010, 58 patients with PCOs were evaluated for insulin resistance. Their disease was diagnosed clinically and confirmed by laboratory findings. The patients fasting blood sugar (FBS) was measured to be 81 to 103 with mean of 91.44 ± 6.52. Insulin level of their fasting blood (FBI) was 3.1 to 33 with mean of 10.95 ± 7.53. FBS/FBI ratio ranged from 2.87 to 31.6 with mean of 12.54 ± 7.98. The co-relational analysis reveals a positive relationship between insulin resistance and body mass index (BMI; normal, overweight and obese). There was a significant association between these two ordinal variables (Spearman’s Correlation Coefficient is 0.59, P < 0.01). Leutinizing hormone/follicle stimulating hormone (LH/FSH) ratio is not significantly related with BMI (P = 0.65) and no significant relationship was observed between age groups and LH/FSH ratio (P = 0.76). The results achieved by two-way analysis of variance (ANOVA), that is, the F-values given in the Tests of Between-Subjects Effects indicate that the contribution of age group to ANOVA is not significant (F = 0.160, P = 0.852). The level of significance between BMI and FBS/FBI is 0.699 (F = 0.360). In addition to the main effects of both variables, there is no significant interaction. 6% of overweight and 39% of our obese patients were resistant to insulin, while no resistance to insulin was observed among cases with normal BMI.

Key words: Polycystic ovarian syndrome (PCOs), insulin resistance, diabetes, body mass index (BMI), infertility.

INTRODUCTION

Polycystic ovarian syndrome (PCOs) is one of the commonest endocrinopathies of women which is the most widely studied and is a controversial area in gynecologic endocrinology (Battaglia et al., 2008). The current estimates suggest that PCOs affects 5 to 10% of reproductive age women (Fleischman and Mansfield, 2005; Chang and Coffler, 2007; Lam et al., 2004; Pfeifer, 2005). It is also the most common cause of female infertility (Legro et al., 2007) accounting for more than 40% of all cases. Some (Mastorakos et al., 2006; Aziz et al., 2005) believe that PCOs has substantial psychological, social and economic consequences and is associated with the development of a number of sequelae, including increased risk of glucose intolerance (gestational and type II diabetes).
According to Angioni et al. (2008), approximately 40 to 50% of women affected by PCOs are overweight or obese, frequently presenting high insulin levels and reduced glucose-induced insulin metabolism, while Lam et al. (2004) stated that women with PCOs, both lean and obese, may be insulin resistant as reflected by fasting glucose/insulin ratio < 4.5. Insulin resistance in women with PCOs seem to be common in both obese and non obese women and there is strong evidence that women with PCOs are at an increased (3 to 7 times) risk of developing type 2 diabetes and possibly cardiovascular complications (Yavasoglu et al., 2009; Bener et al., 2007), abnormal gonadotropin secretion, hyperandrogenism or excessive production of androgens and insulin resistance (Chang and Coffler, 2007; Eisenhardt et al., 2006) and secondary oligomenorrhea or amenorrhea (Bartoszek, 2009).

Since majority of the patients referred to Imam Reza hospital, Tehran, Iran are army personnel or their families, this work has been undertaken with an aim to study insulin resistance rate among this group of patients.

### MATERIALS AND METHODS

**Case selection**

Women (108) suffering from oligomenorrhea and hirsutism referred to gynecology clinic of Imam Reza hospital from 2009 to 2010 were the study cases.

**Inclusion criteria**

Patients were included in the study on the basis of criteria such as presence of hyperandrogenism (hirsutism or hyperandrogenemia) with either oligo/amenorrhea or polycystic ovaries (≥12 small cyst with 9 to 2 mm in diameter or an increased ovarian volume >10 ml or both) and missing exclusion criteria.

**Exclusion criteria**

Patients were excluded from the study on the basis of the presence of hyper prolactinemia, thyroid abnormalities, late onset congenital adrenal hyperplasia (CAH), androgen secreting tumors and treatment in past 6 months for PCOs.

Patients (50) were excluded from the study on the basis of our exclusion criteria. At last, the study was carried out with 58 patients with PCOs who were evaluated for insulin resistance related to their BMI.

Patients were checked for hirsutism, hyperandrogenemia (total testosterone concentration and dehydroepiandrosterone sulfate (DHEAS)) and oligomenorrhea or polycystic ovaries. Sonography was performed as a diagnostic tool for determining the specificities of ovarian cyst, if present, on the basis of criteria like ≥12 cysts (2 to 9 mm in diameter) or an increased ovarian volume (>10 ml) or both (Cunningham et al., 2010).

**Experimental**

The height and weight of every patient was measured and recorded on their first visit and BMI was calculated. Patients were grouped according to their BMI into 3 groups of normal with BMI of 20 to 24.9, overweight (25 to 29.9) and obese (≥30). A questionnaire was filled by every patient, which included information regarding history of menstruation like interval and duration, history of infertility, presence of galactorrhea and weight changes. Laboratory investigations were requested for every patient on third day of their period including total testosterone concentration, leutinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH ratio, fasting blood sugar (FBS), fasting blood insulin (FBI), 17-Hydroxyprogesterone (17-OHP), DHEAS, thyroid stimulating hormone (TSH) tests and the results were recorded in their files.

All the patients' laboratory tests were done in the hospital laboratory. Patients with increased or decreased TSH, patients with 17-OHP level more than 200 ng/dl or those with testosterone level higher than 200 ng/dl and DHEAS more than 700 µg/dl were excluded from the study.

The insulin resistance was diagnosed according to the criteria laid down in William's Gynecology (Cunningham et al., 2010). Multiple testing and screening approaches have been proposed to assess the presence of insulin resistance (Fritz and Speroff, 2011). The gold standard for evaluating insulin resistance has been hyperinsulinemic euglycemic clamp. Homeostatic model assessment-insulin resistance (HOMA-IR) [glucose (mg/dl)] × [insulin (µU/ml)/405] is another method of measuring insulin resistance (Speroff, 2011). As hyperinsulinemic euglycemic clamp is not practical in a clinical setting and HOMA-IR is used in larger epidemiologic studies, so we used fasting serum glucose to insulin ratio to calculate insulin resistance. We also considered values less than 4.5 as insulin resistant cases. A consent form was signed by every patient.

Statistical Package for Social Sciences (SPSS) 17 was employed for analytical purposes. In order to statistically analyze our data, we applied both descriptive and inferential statistics. Descriptive statistics contains minimum, maximum, mean and standard deviation for each variable. In the inferential part, we used Chi-Square test, Spearman and Pearson correlation coefficients, and Phi and Cramer V and linear regression to find out the relationship between all the variables. A two-way analysis of variance (ANOVA) test is also used to find out the effects of BMI and age group on FBS/FBI. The level of significance was considered as 0.01.

### RESULTS

In this study, our patient’s age varied from 14 to 38 years with mean of 23.67 ± 6.34. Patients BMI was calculated which ranged from 23 to 36. Most of our cases, that is, 35 cases (60.34%), aged 20 to 27 years. They had the highest mean BMI (38 kg/m²) as well. The lowest study cases belong to age group ≥28, that is, 18.96% of all study cases with mean BMI of 33 kg/m² (Table 1).

As regard the relationship between BMI (normal, overweight and obese) and age, no significant relationship was observed ($P = 0.63$). Patients were checked for hyperandrogenism in the clinic. Hirsutism was observed in 32 (55%) patients, while hyperandrogenemia was noticed in 45% of the cases.

To find out the association between hyperinsulinemia and hyperandrogenemia, as these two variables are closely linked, we used Phi and Cramer's V test. Our analysis reveals $P > 0.05$, indicating that their relationship is not significant.

Amongst patients with hyperandrogenism, amenorrhea and/or oligomenorrhea was noticed in 94% of the cases.
Table 1. Age distribution, BMI, FBS and FBI of the study cases.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. in each group</th>
<th>Mean BMI</th>
<th>Mean FBS</th>
<th>Mean FBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤19</td>
<td>12</td>
<td>28</td>
<td>81.69 ± 3.81</td>
<td>5.14 ± 3.12</td>
</tr>
<tr>
<td>20 - 27</td>
<td>35</td>
<td>38</td>
<td>138.73 ± 8.93</td>
<td>19.29 ± 2.30</td>
</tr>
<tr>
<td>≥28</td>
<td>11</td>
<td>33</td>
<td>78.46 ± 4.91</td>
<td>8.43 ± 2.11</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>33</td>
<td>91.44 ± 6.52</td>
<td>10.95 ± 7.53</td>
</tr>
</tbody>
</table>

Figure 1. Linear regression scattered plot for all correlations.

Sonographic findings indicate that 45 out of 58 (77.58%) cases had polycystic ovaries. Insulin resistance is defined as a reduced glucose response to a given amount of insulin. We used fasting glucose/insulin ratio as an index of insulin resistance in women with PCOs. The patients FBI level was 3.1 to 33 with mean of 10.95 ± 7.53. Sugar level of their fasting blood was measured to be 81 to 103 with mean of 91.44 ± 6.52. FBS/FBI ratio ranged from 2.87 to 31.6 (Table 1).

As some of our data are quantitative like FBS/FBI ratio and LH/FSH ratio, we used Pearson’s correlation and for qualitative readings we used Spearman’s linear regression correlation (Figure 1).

Resistance to insulin on the basis of FBS/FBI ratio showed that 6% of the overweight and 39% of the obese cases were resistant to insulin; none of our studied cases with normal BMI were resistant to insulin and none of our cases who were resistant to insulin had type II diabetes mellitus.

The co-relational analysis reveals a positive relationship between insulin resistance and BMI (normal, overweight and obese). There was a significant association between these two ordinal variables (Spearman’s Correlation Coefficient is 0.59, P < 0.01). Pearson’s correlation between BMI (Scale variable) and FBS/FBI ratio indicates that the relationship is significant.
(P = 0.013) and as Pearson’s correlation coefficient is -0.4 indicating a reverse relationship between these two variables with medium intensity.

The LH/FSH ratio is not significantly correlated with BMI (P = 0.65) and no significant relationship was observed between age and LH/FSH ratio (P = 0.76).

The results achieved by two-way ANOVA, that is, the F-values given in the Tests of Between-Subjects Effects indicate that the contribution of age group to ANOVA is not significant (F = 0.160, P = 0.852). The level of significance between BMI (normal, overweight and obese) and FBS/FBI is 0.699 (F = 0.360). In addition to the main effects of both variables, there is no significant interaction.

DISCUSSION

PCOs is one of the common causes of infertility due to anovulation in 35 to 94% of women (Yavasoglu et al., 2009). In our study also, 54 (93.1%) patients missed regular menstruations and only 6.89% (4 cases) had regular menstruations.

It has been inferred that hyperinsulinemia, compensatory to insulin resistance, contributes to the hyperandrogenism, because most women with PCOs appear to have increased insulin resistance. We observed clinical hyperandrogenism signs like acne, hirsutism, and hair fall in 33 out of 58 (56.89%) patients. Sonographic findings of our cases revealed 45 out of 58 (77.58%) had polycystic ovaries and no PCOs was detected in 13 (22.41%) cases.

Many researchers (Frizzetti et al., 2008; Diamanti-Kandarakis et al., 2007; Berneis et al., 2007; Cupisti et al., 2008) believe that obesity is more prevalent in women suffering from PCOS. According to Angioni et al. (2008), a high proportion of women with PCOS are obese, while in this study only 10 (17.24 %) patients were obese.

The fasting glucose/insulin (G/I) ratio has been widely used as an index of insulin resistance (Fritz and Speroff, 2011; Angioni et al., 2008). According to Angioni et al. (2008), G/I ratio lower than 4.5 implies a 95% sensitivity and 84% specificity for insulin resistance.

Ketel et al. (2009) reported that androgen and LH concentrations were increased in both normal-weight and obese women suffering from PCOS, while FSH was slightly lower in the normal weight women with PCOS as compared to the normal weight controls. In their study, LH/FSH ratio of 33 (56.89%) patients was above 2 and in 25 (43.1%) cases the ratio was less than 2. In our study, no relationship was found between LH and BMI in cases with LH/FSH ratio greater than 2. Also, no relationship was found between hirsutism and insulin resistance in cases with LH/FSH ratio greater than 2.

Areej and Catherine (2008) reported insulin resistance accompanied by compensatory hyperinsulinemia, a common finding in both lean and obese women with PCOs. According to them, insulin resistance is most marked in obese cases with 70% incidence. In our study also, 45% of patients with BMI more than normal (39% of the obese and 6% of overweight patients) were resistant to insulin.

Conclusions

Considering the side effects of insulin resistance in patients with PCOs and significant relationship between BMI and insulin resistance, weight loss of obese and overweight patients with PCOS is strongly recommended. As good sum of money is to be spent for treatment, it is wise to screen for impaired insulin tolerance in women with PCOs, especially those who are obese or overweight.

REFERENCES


Frizzetti F, Perini D, Lazzarini V (2008). Adolescent girls with Polycystic Ovary Syndrome showing different phenotypes have a different metabolic profile associated with increasing androgen levels.


Full Length Research Paper

The effect of furosemide on experimentally-induced seizures in mice

S. E. Oriaifo¹*, I. Otokiti² and E. K. Omogbai²

¹Department of Pharmacology, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
²Department of Pharmacology, University of Benin, Benin-City, Edo State, Nigeria.

Accepted 10 April, 2012

The aim of this study was to investigate the effect of the loop diuretic, furosemide, on maximal electroshock- and leptazol-induced seizures in mice. Mice were given furosemide, phenytoin and diazepam intraperitoneally and after 30 min they were challenged with maximal electroshock (MES) or leptazol. In MES, furosemide at doses of 25 and 50 mg/kg did not protect against hind limb tonic extension (HLTE) while phenytoin (25 mg/kg) provided protection against HLTE. Furosemide at doses of 25 and 50 mg/kg afforded protection against leptazol-induced Racine stage 4 seizures (P < 0.05), but 100% (all the animals in a group surviving) protection was only provided at 30 min by the 50 mg/kg dose. Diazepam at 2 mg/kg did not offer 100% protection at 30 min. Results provide evidence that furosemide is effective against leptazol-induced seizures, but not against maximal electroshock-induced seizures; and the present results suggest that furosemide is more potent than diazepam against leptazol-induced seizures.

Key words: Furosemide, phenytoin, diazepam, maximal electroshock (MES), leptazol, seizures.

INTRODUCTION

The maximal electroshock (MES) is a useful tool for evaluating generalized tonic-clonic (grand mal) seizures (Territo et al., 2007). In mice, MES-induced seizures consist of initial tonic flexion, then hind limb tonic extension (HLTE), followed by the stage of clonus and terminal stupor. The endpoint of efficacy is taken as inhibition or abolition of HLTE (Naveen et al., 2011; Porter et al., 1984). Pentylenetetrazol (also known as leptazol or PTZ) at high doses interacts with the picrotoxin site of the gamma-aminobutyric acidA (GABAₐ) receptor to inhibit the specific binding of GABA and cause convulsions. Leptazol-induced convulsion is now known to lead to widespread hippocampal apoptotic neuronal cell death by activation of caspase-3 (Nasser et al., 2009). Though leptazol-induced convulsions may be considered as more characteristic of grand-mal, the leptazol (PTZ) seizure model is also considered valid for human generalized myoclonic and absence seizures (Kumar and Madhab, 2011; Kent and Webster, 1983). Four stages of PTZ-induced seizures (Racine, 1972) are generally recognized: (0) no seizure; (1) stage of facial automatisms; (2) stage of head nodding and jerks; (3) stage of forelimb clonus; (4) stage of rearing and falling with forelimb clonus (generalized motor seizures) which may be taken to be the endpoint of efficacy. Phenytoin, by its stabilization of the sodium channel in the inactive state and by its inhibition of the calcium channel, blocks and prevents post-tetanic potentiation, limits development of maximal seizure activity and reduces the spread of seizures (Sankar and Holmes, 2004); attributes that have made it useful also for leptazol-induced seizures (Leach et al., 1991). Phenytoin can also cause reciprocal

*Corresponding author. E-mail: pravee.21msc@gmail.com.

Abbreviations: MES, maximal electroshock; GABA, gamma-aminobutyric acid; NKCC₁, isoform 1 of the Na⁺-K⁺-2Cl⁻ co-transporter; KCC₂, isoform 2 of the K⁺-2Cl⁻ co-transporter; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin-related tyrosine kinase receptor B; NMDA, N-methyl-D-aspartate; ECS, extra-cellular space; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; SD, standard deviation; ANOVA, analysis of variance.
The aim of this study was to evaluate the effect of the loop diuretic, furosemide, in the PTZ and MES models of epilepsy in mice and to compare its anticonvulsant effect with that of diazepam in the PTZ model and with phenytoin in the MES model.

MATERIALS AND METHODS

Male albino mice (20 to 40 g) were allowed to acclimatize in the University's Animal House for two weeks before they were divided into groups of 6 mice each in separate labeled cages for control (0.2 ml of 10% Tween 80), furosemide at doses of 25 and 50 mg/kg; phenytoin at a dose of 25 mg/kg and diazepam, at dose of 2 mg/kg. They were then transported to the Laboratory and one hour elapsed before the injections were given intra-peritoneally (ip).

PTZ-induced seizures

In this model, the effects of the control group, the diazepam group and the furosemide groups were compared. The injections were given intra-peritoneally as stated earlier. After 30 min, 70 mg/kg of pentylenetetrazol (leptazol) was administered ip. Animals were observed for onset and character of myoclonic spasms and tonic-clonic convulsions (Racine stage 4 generalized motor seizures) up to 60 min after leptazol injection. The animals could convulse and recover (when protection is assumed) or convulse and die. 100% protection is assumed if no animal in the group convulses and dies. Arumugam et al. (2009) reported that the percentage of prevention of mortality in a group of rodents (n = 6) was a useful index of protection by antiseizure agents. Latency was determined by the time needed for the development of unequivocal sustained seizure activity which is Racine stage 4 (Khosla and Pandhi, 2001).

MES-induced seizures

In another related experiment, groups of mice with 6 in each group were given same doses of control injection, Furosemide and phenytoin. After 30 min, maximal electroshock was delivered by a Rodent Shocker convulsimetry through ear clips. Seizure induction was by alternating current of 50 mA and stimulus duration was 0.2 s. Animals were observed closely for 2 min for duration or abolition of HLTE which was the endpoint of efficacy and an indication of seizure prevention (Browning, 1992). Drugs were purchased from Sigma-Aldrich via Rovet Chemicals, Benin-City.

Statistical analysis

Results are expressed in seconds (s) ± standard deviation (SD). Mann-Whitney non-parametric test was used to compare two groups for significant difference and was considered significant if \( P < 0.05 \). Analysis of variance (ANOVA) was used for multiple sample analysis followed by post-hoc Tukey's test and \( P < 0.05 \) was taken to be significant difference.

RESULTS

Table 1 shows that there is a significant dose-response relationship to furosemide \( F(2, 15) = 14.35, (P < 0.05) \). Effect was maximal with the 50 mg/kg dose against leptazol-induced seizures which provided 100% protection as compared to the 66.6% protection provided by diazepam. Period of onset of Racine stage 4 convulsions with furosemide was dose-dependent.

In the MES model of epileptic seizures, none of the furosemide dosage provided protection while the phenytoin dose provided 100% protection against MES-induced seizures; but the furosemide doses reduced the duration of HLTE significantly (\( P < 0.05 \)) as compared to controls (Table 2).
Table 1. Effect of Furosemide and Diazepam on Leptazol-Induced Seizures at 30 min.

<table>
<thead>
<tr>
<th>Group</th>
<th>Seizure onset (s ± SD)</th>
<th>P</th>
<th>Protection (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml</td>
<td>20.5 ± 2.3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Furosemide</td>
<td>25 mg/kg</td>
<td>82.0 ± 1.8</td>
<td>&lt;0.05</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>50 mg/kg</td>
<td>100.00 ± 3.00</td>
<td>&lt; 0.05</td>
<td>100</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2 mg/kg</td>
<td>100.5 ± 3.1</td>
<td>&lt; 0.05</td>
<td>66.6</td>
</tr>
</tbody>
</table>

Order of magnitude of response was furosemide > diazepam. The effect of furosemide, maximal at 50 mg/kg, was dose-dependent and the difference in period of onset of seizures (latency) between test and control groups was statistically significant (P < 0.05). 100% protection is assumed if no animal in a group convulses and dies.

Table 2. Effect of Furosemide and Phenytoin on MES-Induced Seizures at 30 min.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean duration of HLTE (s ± SD)</th>
<th>Protection (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml</td>
<td>16.5 ± 3.5</td>
<td>0</td>
</tr>
<tr>
<td>Furosemide</td>
<td>25 mg/kg</td>
<td>12.0 ± 4.6 (P &lt; 0.05)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50 mg/kg</td>
<td>6.3 ± 3.9 (P &lt; 0.05)</td>
<td>0</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>20 mg/kg</td>
<td>0 (P &lt; 0.05)</td>
<td>100</td>
</tr>
</tbody>
</table>

At the chosen doses, furosemide offered no protection to mice against MES-induced seizures; but reduced the duration of HLTE significantly compared to control (P < 0.05) which was dose-dependent. 0% protection is assumed if no animal in a group survives the convulsive episodes.

DISCUSSION

The experimental results confirm the only previous work on the protective action of furosemide against leptazol-induced seizures by Kielczewska-Mrozikiewicz (1968). The present result, also, is in agreement with the previous report (Luszczki et al., 2007) that furosemide did not protect mice against MES-induced convulsions and is at variance with the report of Hesdorffer et al. (2001) that the diuretic, furosemide, provided protection against MES-induced seizures. Our results showed that furosemide is, nevertheless, able to reduce the duration of HLTE of MES-induced seizures and this may be responsible for its potentiation of valproate against MES-induced seizures reported by Luszczki et al. (2007). Further work may be necessary to determine the differential effect between furosemide and bumetanide, another loop diuretic, and between furosemide and a calcium channel blocker, such as nifedipine, that modulates NMDA receptor function so as to shed more light on the mechanism of action of furosemide in protecting mice against leptazol-induced convulsions.

The sodium-potassium-chloride co-transporter inhibitor, furosemide, has been found recently to possess a wide spectrum of activity which includes anti-convulsant activity. An emerging body of evidence points to the efficacy of furosemide as a neurochemical with neuroprotective effects (Ahmad et al., 1976, 1977). A primary mechanistic explanation for furosemide’s ability to terminate seizures may be its ability to prevent sustained Ca\(^{2+}\) intracellular increases due to hyperglutamatergic excitotoxicity (Sanchez-Gomez et al., 2011), a property it now seems to share with other NMDA receptor antagonists, such as the calcium channel blockers (Palmer et al., 1993). Besides, furosemide, which induces BDNF release (Szekeres et al., 2010), may enhance the seizure-terminating effect of neuropeptide Y (Binder et al., 2001) which is upregulated by BDNF. Furthermore, changes in chloride transporter expression contribute to human epileptiform activity (Huberfeld et al., 2007) with increased sodium-potassium-chloride cotransporter (NKCC\(_1\)) and altered
distribution of the neuron-specific potassium-chloride cotransporter (KCC2) (Reid et al., 2000; Aronica et al., 2007), and molecules such as furosemide acting on these transporters may be useful antiepileptic drugs. Reid et al. (2000) had suggested that the KCC2 co-transporter may be upregulated in those instances, such as global ischaemia when the loop diuretics which are blockers of the co-transporter may be effective in terminating seizures. Modulation of electrical field interactions via the extra-cellular space (ECS) might also contribute to neuronal hypersynchrony and epileptogenicity and present evidence suggesting that non-synaptic mechanisms play a critical role in modulating the epileptogenicity of the human brain. Furosemide and other drugs that modulate the extra-cellular space (Gutschmidt et al, 1999; Hochman et al, 1999; Hochman et al, 1995) might possess clinically useful antiepileptic properties, while avoiding the side-effects associated with the suppression of neuronal excitability (Haglund and Hochman, 2005) being exhibited by drugs, such as the 1, 4-benzodiazepines.

Studies have also shown that furosemide can reversibly suppress low Ca\(^{2+}\)-induced and low Mg\(^{2+}\)-induced epileptiform activity. Amplitudes of evoked field potentials underwent an initial slight increase followed by a significant reduction after prolonged furosemide treatment. Furosemide more potently blocks leptazol-induced epileptiform activity in our experiments than diazepam. Endogeneous field effects in the CNS play functional roles and they are thought to contribute to epileptogenesis (Weiss and Faber, 2010) and there is evidence for a role of field effects (epaptic transmission) in rhythmogenesis in cortex and hippocampus (Buzsaki, 2002). The administration of furosemide suppresses leptazol-induced epileptic activity potentily in the human cortex probably by also reducing field effect interactions (epaptic transmission) (Haglund and Hochman, 2005; Weiss and Faber, 2010; Dudek et al., 1989) more than diazepam.

Recent studies have shown evidence that the persistently high levels of brain-derived neurotrophic factor (BDNF) engendered by ictal activity may be pro-nectrotic through activation of NADPH oxidase (Kim et al., 2002; Park et al., 2006) and that the Bcl-2-associated protein X (Bax) blocker furosemide (Lin et al., 2005) which is anti-apoptotic may serve in this instance as a neuroprotective. Also, since oxidative stress (Ikonomidou, 2002), inflammatory mediators and hyperglutamatergic excitotoxicity too underlie epileptogenesis and epileptic brain injury, antioxidants such as furosemide (Hamelinck et al., 2005) may play a greater role in future in preventing neurodegeneration from being a cause of and sequel of epilepsy (Vercueil, 2004; Koyama and Ikegaya, 2005).

In conclusion, the present experimental results show that furosemide significantly and dose-dependently suppressed leptazol-induced convulsions in mice and displayed no protective effect against MES-induced seizures in this in vivo study.

REFERENCES


Kent AP, Webster RA (1983). The evaluation of different types of anti-
convulsant drug activity against lepitol-induced epileptogenic

Khosla P, Pandi P (2001). Anticonvulsant effect of nimodipine alone and
in combination with diazepam on pentylenetetrazol-induced status
epilepticus. Indian J. Pharmacol. 33:208-211.

Kieczezewa-Mrozikiewicz D (1968). Experimental studies on the effect
of lasix on the occurrence of cardiac convulsions. Przegląd Lekarski
24:716-718.

BDNF can act as a pro-neurotic factor through transcriptional and
translational activation of NADPH oxidase. JCB., 159(5):821-831.

Koyama R, Ikegaya Y (2005). To BDNF or not to BDNF: That is the

Kumar RS, Madhab KG (2011). An experimental evaluation of
anticonvulsant activity of Nerium oleander leaf extract. Int. Res. J.
Pharmacol. 2(10):73-75.

49(10):1651-1664.

Leach MJ, Baxter MG, Critchley MA (1991). Neurochemical and
behavioral aspects of lamotrigine. Epilepsia 32(Suppl. 2):S4-8.

mitochondrial translocation is responsible for the in-vitro ischaemia-
induced neuronal cell death of Sprague-Dawley rats. Neurosci. lett.

Furosemide potentiates the anticonvulsant action of valproate in the

co-transport blocking diuretics in a rat hippocampal slice model of

Murthy JMK (2011). Seizure aggravation with antiepileptic drugs in

induced by pentylenetetrazol: apoptotic neurodegeneration and
decreased GABA_A receptor expression in prenatal rat brain.

Anticonvulsant activity of flupirtine in albino mice. Pharmacol. online
3:860-867.

Palmer GC, Stagnitto ML, Ray RK, Knowles MA, Harvey R, Garske GE
(1993). Anticonvulsant properties of calcium channel blockers in
mice: N-methyl-D-L-aspartate and Bay K 8644-induced convulsions
are potently blocked by the dihydropyridines. Epilepsia

seizures by pentylenetetrazole cause neurodegeneration and
promote neurogenes in discrete brain regions of freely moving


Racine RJ (1972). Modification of seizure activity by electrical
stimulation. II. Motor Seizure. Electroencephalography and Clinical

Reid KH, Shang ZG, Vasudeva GI (2000). Agents which block
potassium-chloride cotransport prevent sound-triggered
seizures in post-ischaeamic audiogenic seizure-prone rats.
Brain Res. 864:134-137.

Sanchez-Gomez MV, Alberdi E, Perez-Navarro E, Alberjch J, Matute
C (2011). Bax and calpain mediate excitotoxic oligodendrocyte
death induced by activation of both AMPA and Kainate receptors. J.
Neurosci. 31(8):2996-3006.

used antiepileptic drugs: relevance to antiepileptic drug-associated
neurobehavioral adverse effects. J. Child Neurol. 19(S1):S6-14.

Srivastava AK, Gupta YK (2001). Aspirin modulates the anticonvulsant
effect of diazepam and sodium valproate in
pentylenetetrazol and maximal electroshock induced seizures in

Staley KJ (2002). Diuretics as antiepileptic drugs: should we go with the
flow? Review Article. Epilepsy Curr. 2(2):35-38 doi: 10.1046/i.1535-
7597.2002.00018.x


Stringer JL, Pan E (1997). Effect of seizures and diuretics on the

Szekeress M, Nadaey GL, Turu G, Supeki K, Svidonya L, Buday L,
expression of BDNF in human and rat adrenocortical cells.
Endocrinology 151(4):1695-1703.

Torrillo PR, Freise KJ, Newhall K, Barnhart SD, Peters SC, Engleking
DR, Burnett TJ, Abdul-Karim B, Shannon HE (2007). Development
and validation of the maximal electroshock seizure model in dogs. J.

Epileptic Disord. 6(1):47-57.

Weiss SA, Faber DS (2010). Field effects in the CNS play functional
UPCOMING CONFERENCES

Hawaii Heart 2013: Echocardiography & Multimodality Imaging, Case Based Clinical Decision Making, Kauai, USA, 4 Feb 2013

14th Annual Clinical Trial Supply Europe, Berlin, Germany, 26 Feb 2013

9th International Conference on Clinical Ethics Consultation, Munich, Germany, 14 Mar 2013
Conferences and Advert

**March 2013**
11th International Conference of Chemistry & its Role in Development, ElSheikh, Egypt, 11 Mar 2013

**April 2013**
23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany, 27 Apr 2013

3rd International Conference on Clinical and Experimental Cardiology, Chicago, USA, 15 Apr 2013
Journal of Clinical Medicine and Research

Related Journals Published by Academic Journals

- Journal of Metabolomics and Systems Biology
- Journal of Neuroscience and Behavioral Health
- Journal of Physiology and Pathophysiology
- Journal of Public Health and Epidemiology
- Medical Case Studies
- Medical Practice and Reviews
- Journal of General and Molecular Virology
- Research in Pharmaceutical Biotechnology