Aim of Work: To evaluate the relationship between estimated serum levels of adipocytokines and insulin resistance (IR) parameters in women with definite gynecological disorders.

Patients & Methods: The study included 75 patients; 31 had uterine myoma (UM), 19 had endometrial carcinoma (EC) and 25 had endometriosis (EM) as diagnosed by US examination and pathological examination of hysteroscopic biopsy for EM and EC. All women gave two fasting blood samples for colorometric estimation of fasting blood glucose (FBG) and ELISA estimation of serum insulin (FSI), adiponectin (APN), leptin and YKL-40 levels. Homeostasis model assessment index of IR (HOMA-IR) and leptin/adiponectin (L/A) ratio were calculated.

Results: FBG and FSI levels and HOMA-IR index were significantly higher in patients than controls and in EC patients than other patients. Serum YKL-40 and leptin were significantly higher with significantly lower APN levels in patients than controls. Serum YKL-40 levels in EM patients were significantly higher than other patients, but were significantly higher in EC than UM patients. Serum leptin levels were significantly lower in UM patients than other patients. Serum APN levels were significantly lower in EC patients than other patients, but were significantly lower in EM than UM patients. Calculated L/A ratio was significantly lower in UM than in other patients and was non-significantly lower in EM than EC patients. ROC curve and Regression analyses showed that lower levels of APN and disturbed L/A ratio can predict the presence of EC, while elevated levels of YKL-40 and leptin can characterize patients with EM, but high HOMA-IR index can predict UM presence.

Conclusion: Elevated serum YKL-40 could be used for discrimination of EM out of other pathologies. Lower serum APN levels and high L/A ratio could be used as a significant predictor for presence of EC. High HOMA-IR index can predict the presence of UM. A diagnostic panel of estimation of serum YKL-40 and APN could define and differentiate between EC and EM patients.
INTRODUCTION

Endometrial carcinoma (EC) is the most common gynecological cancer (Ramesh et al., 2013) that showed progressive increase in incidence reached up to 21% incidence-rate since 2008 and is associated with increased mortality rate by more than 100% over the past two decades (Sorosky et al., 2012). The mainstay of the initial treatment for EC is surgery with total hysterectomy and bilateral salpingo-oophorectomy with or without pelvic-aortic lymphadenectomy (Loukovaara et al., 2014). Precursor lesions of complex hyperplasia with atypia are associated with EC in more than 40% of cases (Sorosky et al., 2012).

Obesity is an established risk factor for several cancers (Goday et al., 2015) and is increasingly a public health concern (O’Flanagan et al., 2015). Obesity is also associated with a high risk of recurrence (Benedetto et al., 2015). Moreover, obese cancer patients have poorer prognoses, reduced response to standard treatments, and are more likely to develop metastatic disease than normo-weight individuals (O’Flanagan et al., 2015). Chronic inflammation, hyperinsulinaemia, insulin resistance, and raised leptin and estrogens were implicated in association between cancer and obesity (Goday et al., 2015).

Adiponectin (APN) is insulin-sensitizing protein that is secreted from adipose tissue (Erdogan et al., 2013). APN dysregulation is postulated to affect cancer risk via modulation of insulin resistance and inflammation (Beg et al., 2015) and its lower plasma concentration was related to an increased incidence and may act as an independent predictor for malignancy in variant tissues (Liao et al., 2013).

Leptin is a single-chain proteohormone (Webber et al., 2003) that is produced by differentiated adipocytes (Baratta et al., 2002) and plays a key role in the regulation of body weight (Webber et al., 2003). Leptin was found to be produced by other tissues than adipose tissue (Baratta et al., 2002) and its receptors are found ubiquitously in the body (Tsiotra et al., 2000), but a circulating form of leptin receptor exists (Lammert et al., 2001).

YKL-40, a 40 kDA glycoprotein (Metllinos-Katsaras et al., 2012) produced by immunologically active cells and is a highly conserved serum protein (Elshorbagy et al., 2012). YKL-40 exerts anti-apoptosis action, promotes angiogenesis and stimulates cellular (Maggio et al., 2012). High YKL-40 plasma concentrations were detected in diseases characterized by increased tissue remodeling or with cancer (Zhu et al., 2002).

Hypothesis

The current study hypothesized the possibility of a correlation between disturbed adipocytokine system, obesity and development of gynecological pathologies and such disturbance may underlie development of gynecological neoplastic lesions.

Aim of Work

This study aimed to evaluate the relationship between estimated serum levels of adipocytokines and insulin resistance (IR) parameters in women with definite gynecological disorders.

Design

Prospective Observational comparative study

Setting

Prince Sultan Armed Forces Hospital, Madenah, KSA
PATIENTS & METHODS

The current was conducted at departments of Obstetrics and Gynecology, and Medical Biochemistry since April 2014 till August 2015. The study protocol was approved by Local Ethical Committee. Written fully informed consent for study participation was signed by all patients attending the Gynecology outpatient clinic complaining of symptoms suggestive of having intrauterine mass lesion.

All patients with symptoms suggestive of having endometrial carcinoma (EC), endometriosis (EM) or uterine myoma (UM) underwent clinical evaluation including full gynecological examination and underwent abdominal and transvaginal ultrasound examination. Patients with US imaging suggestive of endometrial lesion were subjected to hysteroscopic biopsy taking for histological examination and staging in case of EC. Patients with definite EC underwent chest X-ray and MRI imaging for detection of metastasis.

Exclusion criteria included presence of lesions other than EC, EM or UM, obesity-inducing endocrinopathy, diabetes, liver, vascular or neoplastic disorders were excluded from the study. The study also included 15 age-and BMI-matched women free of malignancy and other morbidities who gave blood samples as control group for the results of laboratory investigations.

All women had complete history taking and full clinical examination including body height and weight, and body mass index (BMI) was calculated as weight (kg)/height (m$^2$) (Khosla & Lowe, 1967). Insulin resistance (IR) was measured by homeostasis model assessment (HOMA). The HOMA-IR score was calculated as (fasting serum insulin (µU/ml) x [fasting plasma glucose (mg/ml)/18])/22.5 (Matthews et al., 1985) considering an abnormal HOMA-index >2 (Ascaso et al., 2001).

Laboratory investigations

Two fasting venous blood samples were obtained under complete aseptic conditions from the antecubital vein:

1. The 1$^{\text{st}}$ blood sample was collected in EDTA containing tube for colorometric estimation of fasting blood glucose.

2. The 2$^{\text{nd}}$ blood sample was put in a plane container and left to clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000g. Freshly prepared serum was stored at –20°C till be assayed.

Investigations:

1. Estimations of serum insulin levels using a commercial human ELISA kit (Mercodia ELISA; ALPCO Diagnostics, Uppsala, Sweden) (Gordon et al., 1985).

2. Estimation of serum adiponectin levels using a commercial human ELISA kit (Abcam’s Human Adiponectin ELISA, San Francisco, USA) (Yokota et al., 2000).


4. Estimation of serum YKL-40 levels using a commercial human ELISA kit (Human Chitinase 3-like 1/YKL-40 PicoKine™ ELISA Kit, Valley Ave, Pleasanton, USA) (Shackelton et al., 1995).

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Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and percentages. Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test ($X^2$ test). Possible relationships were investigated using Spearman linear regression. Sensitivity & specificity of estimated parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Regression analysis (Stepwise method) was used for stratification of studied parameters as significant predictors for the target disease. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

The study included 91 patients eligible for evaluation, 16 patients were excluded and 75 patients were enrolled in the study; 31 women had UM, 19 had EC and 25 women had EM as diagnosed by US examination and pathological examination of hystroscopic biopsy for EM and EC (Fig. 1). Women had EC were significantly older than women had other pathologies, with non-significant difference between women had UM and EM. Body mass index data showed non-significant difference between studied patients. Enrolled control women showed non-significant difference as regards age and BMI compared to total patients' population (Table 1).

Table (1): Patients' enrolment data

<table>
<thead>
<tr>
<th>Data</th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.3±10.7</td>
<td>54.3±9.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>88.5±7.7</td>
<td>87.3±8.2</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>171±3.1</td>
<td>169±3.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.3±2.6</td>
<td>30.5±2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.3±9.6</td>
<td>48.4±8.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>87.3±8.2</td>
<td>91.6±7.4</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>169±3.6</td>
<td>169.5±2.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.5±2.3</td>
<td>31.9±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.4±3.4†‡</td>
<td>30.3±2.1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>87.9±5.8</td>
<td>30.9±2.2</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>170.4±2.3</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.9±2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.4±3.4†‡</td>
<td>55±10</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>87.9±5.8</td>
<td>88.9±7.5</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>170.4±2.3</td>
<td>169.5±3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.9±2</td>
<td>30.9±2.2</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; †: significant difference versus UM patients; ‡:significant difference versus EM patients
Estimated FBG and FSI levels and calculated HOMA-IR index were significantly higher in patients than in controls and in EC patients compared to both UM and EM patients who showed non-significant difference despite being higher in EM patients (Fig. 2). Similarly, serum YKL-40 and leptin were significantly higher with significantly lower serum adiponectin levels in patients compared to controls. Serum adipokines showed discrepant results among studied patients where serum YKL-40 levels in UM patients were significantly lower than in EM and EC patients, but were significantly higher in EM patients than EC patients. Serum leptin levels were significantly lower in UM patients than in other patients with non-significantly lower levels in EC patients than in EM patients. On the other hand, serum adiponectin levels were significantly lower in EC patients compared to other patients with significantly lower levels in EM patients compared to UM patients (Table 2, Fig. 3).
Table (2): Laboratory data and calculated ratios of studied patients

<table>
<thead>
<tr>
<th>Data</th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UM</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>110.2±3.2</td>
<td>135.6±13.7*</td>
</tr>
<tr>
<td>FSI (IU/ml)</td>
<td>4.2±0.8</td>
<td>4.9±1.09*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.14±0.22</td>
<td>1.64±0.37*</td>
</tr>
<tr>
<td>YKL-40 (pg/ml)</td>
<td>22.5±7.97</td>
<td>109.1±52.7*</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.72±2.37</td>
<td>6.74±1.83*</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>16.62±2.33</td>
<td>13.39±2.39*</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; *: significant difference versus controls, †: significant difference versus UM patients; ‡: significant difference versus EM patients.

Calculated leptin/adiponectin ratio (L/A) was significantly lower in UM patients (0.52±0.18) compared to L/A ratio in EM patients (1.49±0.69) and EC patients (1.55±0.78) with non-significantly lower L/A ratio in EM patients than in EC patients (Fig. 4).

Fig. (2): Mean fasting blood glucose and serum insulin levels and HOMA-IR index

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ROC curve analysis of HOMA-IR index, serum parameters and calculated L/A ratio in UM patients defined high serum leptin and high L/A ratio as the highly significant predictors for presence of UM, followed by high HOMA-IR index, low serum adiponectin and high serum YKL-40; in decreasing order of significance. In EM patients, ROC curve analysis defined high serum YKL-40 and leptin as the highly significant predictors for presence of EM, followed by high L/A ratio; in decreasing order of significance, while HOMA-IR index and serum adiponectin were non-significant predictors. In EC patients, ROC curve analysis, defined high L/A ratio and high HOMA-IR index as the highly significant predictors for presence of EC, followed by high serum leptin and low serum adiponectin; in decreasing order of significance, while serum YKL-40 levels were non-significant predictor (Fig. 6).

Fig. (3): Mean serum levels of studied adipocytokines in patient’s groups
Fig. (4): Mean (±SD) L/A ratio in studied patients

Table (3): ROC curve analysis of HOMA-IR index, serum parameters and calculated L/A ratio for differentiation between patients with UM, EM or EC

<table>
<thead>
<tr>
<th>Disease state Parameter</th>
<th>UM AUC (±SE)</th>
<th>Sig.</th>
<th>95% CI</th>
<th>EM AUC (±SE)</th>
<th>Sig.</th>
<th>95% CI</th>
<th>EC AUC (±SE)</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>0.265 (0.058)</td>
<td>0.00</td>
<td>0.151-0.379</td>
<td>0.445 (0.071)</td>
<td>0.441</td>
<td>0.307-0.583</td>
<td>0.866 (0.052)</td>
<td>0.000</td>
<td>0.764-0.967</td>
</tr>
<tr>
<td>YKL-40</td>
<td>0.337 (0.063)</td>
<td>0.01</td>
<td>0.213-0.461</td>
<td>0.758 (0.055)</td>
<td>0.000</td>
<td>0.650-0.867</td>
<td>0.527 (0.077)</td>
<td>0.724</td>
<td>0.377-0.678</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.068 (0.026)</td>
<td>0.00</td>
<td>0.016-0.120</td>
<td>0.708 (0.048)</td>
<td>0.000</td>
<td>0.644-0.872</td>
<td>0.750 (0.054)</td>
<td>0.001</td>
<td>0.645-0.856</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.707 (0.059)</td>
<td>0.00</td>
<td>0.590-0.823</td>
<td>0.450 (0.071)</td>
<td>0.486</td>
<td>0.310-0.590</td>
<td>0.293 (0.080)</td>
<td>0.007</td>
<td>0.137-0.450</td>
</tr>
<tr>
<td>L/A ratio</td>
<td>0.051 (0.022)</td>
<td>0.00</td>
<td>0.008-0.093</td>
<td>0.655 (0.065)</td>
<td>0.030</td>
<td>0.527-0.783</td>
<td>0.527 (0.077)</td>
<td>0.000</td>
<td>0.663-0.882</td>
</tr>
</tbody>
</table>

AUC: Area under curve; SE: Standard error; CI: Confidence interval

Regression analysis of studied parameters defined high serum YKL-40 and leptin levels as significant parameters for differentiating patients with EM out of other patients, while high L/A ratio can identify EC patients with high significance (Table 4).
DISCUSSION

The hypothesis of the current study is that a correlation between disturbed adipocytokine system, obesity and development of gynecological pathologies is possible. Such hypothesis go in hand with multiple previous studies detected certain relations between disturbed serum levels of adipocytokines and variant gynecological and obstetric problems, where Al-Kholy et al. (2010) and Al-Nory et al. (2011) detected a relation between development of pre-eclampsia (PE) and serum retinol binding protein-4 and soluble endoglin, respectively and reported that high retinol binding protein-4 can predict PE severity clinical markers and endoglin could be used as a sensitive screening test for liability to develop PE. Also, Wickham et al. (2011) found women with polycystic ovary syndrome (PCOS) have lower total and high-molecular weight adiponectin (APN) levels than control women and Chen et al. (2015) suggested that adipose tissue might play an important role in the metabolic complications in PCOS women.

The obtained results assured the study hypothesis; as estimated levels of fasting blood glucose and serum insulin, leptin, YKL-40 were significantly higher, while serum levels of APN were significantly lower in total studied patients than control women. Also, the calculated HOMA-IR index and leptin/adiponectin (L/A) ratio were significantly higher in patients than in controls. These findings indicated a relationship between dis-
turbed serum levels of estimated adipocytokines on one angle, obesity on the other angle and development of gynecological disorders closed the triangle.

Moreover, ROC curve and Regression analyses of estimated levels of studied adipocytokines allowed blinded prediction of type of lesion; where lower levels of APN and disturbed L/A ratio can predict the presence of EC, while elevated serum levels of YKL 40 and leptin can characterize patients with EM from those with EC and UM, but high insulin resistance markers and HOMA-IR index can predict the presence of UM.

These findings spot light on the possibility to categorize patients with gynecological neoplasia and go in hand with previous studies evaluated the role played by disturbed adipocytokines in pathogenesis of gynecological disorders; where Soliman et al. (2006), Cust et al. (2007), Erdogan et al. (2013) and Zheng et al. (2015) found women with EC were more likely to have low APN levels than controls and APN level, independent of other obesity-related risk factors, are associated with reduced EC risk. Also, Dallal et al. (2013) and Wang et al., (2014) reported that leptin-BMI axis might increase EC risk through mechanisms other than estrogen-driven proliferation and high leptin level is an independent risk factor of EC.

In line with the diagnostic value of high L/A ratio, Gong et al. (2015) suggested that increased circulating APN or decreased leptin concentrations were associated with reduced risk of EC. Moreover, Lin et al. (2015) and Zeng et al. (2015) suggested that higher APN levels might have a protective effect against EC, especially in postmenopausal women.

Statistical analyses defined high serum YKL-40 and leptin as the highly significant predictors for presence of EM and high serum YKL-40 showed significant discriminative ability over leptin, while serum APN failed to be discriminative parameter despite of its low levels. In line with these findings, Kim et al. (2010) using immuno-histochemistry reported that YKL-40 is related to severity of peritoneal EM. Thereafter, Tuten et al. (2014) documented that YKL-40 may be utilized as a marker for determining the severity of endometriosis and Ural et al. (2015) detected increased serum YKL-40 levels in patients with EM compared to controls and proposed that circulating YKL-40 levels could be a novel biomarker for diagnosis and follow-up of endometriosis.

As regards uterine myoma, high HOMA-IR index, serum leptin and L/A were significant predictors of UM, but high HOMA-IR which is a measure for insulin resistance associated with obesity and diabetes was the persistently significant predictor for presence of UM. Similarly, Takeda et al., (2008) found BMI, blood pressure, triglyceride level and FPG were significantly higher in fibroid patients than controls and concluded that uterine leiomyomas may share pathogenic features with the development of metabolic syndrome. Dandolu et al., (2010) reported a significant correlation between BMI and both of uterine weight and presence of fibroids with an increase of 4.56 g in uterine weight for every 1-point increase in BMI.

The relation between fibroid and adipocytokines is controversial, where
Dingiloglu et al., (2007) reported that although leptin level was higher in the myomatic women than in the normal women, there was no statistically significant difference and Wakabayashi et al., (2011) found the repression of adiponectin on leiomyoma cell proliferation in the rat may explain a crucial role of adiponectin in the association of metabolic syndrome with uterine leiomyoma. However, the results of these both studies assured a role played by disturbed L/A ratio in pathogenesis of UM; a finding which is coincident with the outcome of the current study.

CONCLUSION

Disturbed serum adipocytokines showed significant relationship with the development of gynecological pathologies. Elevated serum YKL-40 could be used for discrimination of EM out of other pathologies. Lower serum APN levels and high L/A ratio could be used as a significant predictor for presence of EC. High HOMA-IR index can predict the presence of UM. A diagnostic panel of estimation of serum YKL-40 and APN could define and differentiate between EC and EM patients, but its use as a screening test for high-risk women needs further wide-scale study for confirming such applicability and defining a suitable cutoff point for this purpose.

REFERENCES


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مستويات أديبوسيتوكينيتات المضطربة بمصل الدم، مقاومة الأنسولين والأمراض النسجية الأورام الليفية تمثل مثل الخطر

هاني الكلاف - عادل فرج الخولي

أقسام أمراض النساء والتوليد، الكيمياء الحيوية الطبية - كلية الطب - جامعة بنها

هدف العمل: تقييم العلاقة بين مستويات الأديبوسيتوكينيتات بالمصل ومقاومة الأنسولين في النساء المصابات باضطرابات أمراض النساء.

المريضي وطرق الدراسة: شملت الدراسة 25 مريضة، 21 مريضة مصابة بسرطان بطانة الرحم (مجموعة A)، 20 مريضة مصابة بالتهاب بطانة الرحم (مجموعة B) تم تشخيصها باستخدام الفحص بالموجات فوق الصوتية، وفحص عينة من بطانة الرحم. أعطت جميع النساء اثنين من عينات الدم لتشخيص مستويات الجلوكوز بالدم، الأنسولين، أديبوسيتوكينيتات، الليبتين والواي-كيه-إلى-إل-4، تم حساب مؤشر تمكين نموج الكوازن والنسبة بين مستويات البيكين/أديبوسيتوكينيتات بالدم (نسبة L/A).

النتائج: وجد ارتفاع ذو دلالة إحصائية في مستويات الجلوكوز بالدم، ومستويات الأنسولين والليبتين والواي-كيه-إلى-إل-4 بمصل الدم، ومؤشر تمكين نموج الكوازن بين المرضى والمجموعة الضابطة، بينما وجد انخفاض ذو دلالة إحصائية في مستويات الأديبوسيتوكينيتات بمصل الدم بين المرضى والمجموعة الضابطة. وجد ارتفاع ذو دلالة إحصائية في مستويات الليبتين والواي-كيه-إلى-إل-4 في مرضى مجموعة ج مقارنة بباقي المرضى، وفي مرضى مجموعة ب مقارنة بمرضي مجموعة A، وجد انخفاض ذو دلالة إحصائية في مستويات الأديبوسيتوكينيتات في مرضى مجموعة B مقارنة بباقي المرضى، وفي مرضى مجموعة ج مقارنة بمرضي مجموعة A، وكانت نسبة L/A أقل بفارق ذو دلالة إحصائية عند مرضى مجموعة ب مقارنة بباقي المرضى ووجد لها توقعية عالية ذات دلالة إحصائية في تشخيص سرطان بطانة الرحم، وتوقيف عالية ذات دلالة إحصائية.
البكتيريا والدم في تشخيص التهاب بطانة الرحم، ومؤشر تشخيص نموذج التوازن في تشخيص الأورام الليفية بالرحم.

الاستنتاج: ارتفاع مستوي الـL/A-كي-إيه-إلى-4 بمصل الدم يمكن من تمييز بين مرضى التهاب بطانة الرحم، وانخفاض مستوي الأدينوكيناتين مع ارتفاع نسبة L/A يمكن من تمييز مرضى سرطان بطانة الرحم، بينما ارتفاع مؤشر تشخيص نموذج التوازن يمكن من تمييز مرضى الأورام الليفية بالرحم

31. المجلة المصرية لعلوم الظبيبة 6(4) ديسمبر 2015: 25-54.