LEVELS OF 5-HYDROXYINDOLEACETIC ACID, GAMMA-AMINOBUTYRIC ACID AND NITRIC OXIDE METABOLITES IN CEREBROSPINAL FLUID OF EGYPTIAN WOMEN SUFFERING OF FIBROMYALGIA

SAHAR AHMAD SAAD, MANAL A. M. MANDOUR * AND HASSAN KOTB **

Rheumatology & Rehabilitation, Biochemistry* Departments and Pain Unit** Assiut University Faculty of Medicine and Assiut University Hospitals**

KEY WORDS: CEREBROSPINAL FLUID IN FIBROMYALGIA, 5-HYDROXYINDOLEACETIC ACID, GAMMA AMINOBUTYRIC ACID, NITRIC OXIDE METABOLISM.

ABSTRACT

Hypothesis: Fibromyalgia (FM) is a complex chronic disorder predominately affects women. The lack of objective analytical image for diagnosis and prognosis of FM attract many investigators to evaluate the neuroendocrine changes in body fluids that may be related to the pathophysiology of FM.

Objectives & Methodology: To compare the levels of 5-hydroxyindoleacetic acid (5-HIAA, serotonin metabolite), gamma-aminobutyric acid (GABA) and nitric oxide (NO) metabolites in cerebrospinal fluid (CSF) of normoglycemic women suffering of FM with those of control group. In addition, levels of insulin, insulin like growth factor-1 (IGF-1), cortisol, 5-hydroxytryptamine (5-HT, serotonin), GABA, and NO metabolites were determined in serum of FM patients compared to control group. Moreover, the correlations between all the studied parameters were performed.

Results: The present study showed altered serum levels of insulin and cortisol in normoglycemic women suffering of FM (n=15) compared to those of control group (n=15). However, the data did not find significant differences between serum levels of IGF-1 in women suffering of FM and those in healthy women. Cortisol levels in FM sera were inversely correlated with the number of tender points (r=-0.76, p<0.001). Also, negative
correlation was observed between serum IGF-1 levels and age of FM patients \( (r=-0.63, p<0.05) \). Low levels of serum 5-HT and CSF 5-HIAA were also noticed among women suffering of FM. A decrement of both serum and CSF levels of GABA in those patients compared to controls was detected. Furthermore, there was a positive correlation between serum GABA levels and serum cortisol levels \( (r=0.61, p<0.05) \), and between CSF GABA and 5-HT \( (r=0.56, p<0.05) \). However, CSF GABA levels were negatively correlated with age of women suffering of FM. These findings may clarify the significant role of GABA in the pathogenesis of FM. A higher serum and CSF levels of NO metabolites were detected in FM patients than in control group. In addition, serum levels of NO metabolites were correlated with those levels in CSF \( (r=0.63, p<0.05) \). The involvement of NO in pain process was revealed by a strong correlation between serum and CSF levels of NO metabolites with the number of tender points \( (r=0.91, p<0.001; r=0.73, p<0.01 \) respectively). Also, by a negative correlation between serum levels of NO metabolites and serum cortisol levels \( (r=0.70, p<0.01) \) in FM patients.

**Conclusions:** The changes in the levels of studied hormones and neurotransmitters in women suffering of FM support the hypothesis of neuroendocrine involvement in FM pathogenesis. The current study suggested that GABA and NO may play a role in modulating FM. Alterations of their levels in serum and CSF of women suffering of FM and their correlations with age, number of tender points and/or cortisol may confirm this suggestion. Accordingly, this investigation recommended the examination of GABAergic agents, NO synthase (NOS) inhibitors, or antioxidants for therapy of various symptoms in women suffering of FM. Furthermore, our study may find utility of these neurotransmitters as possible markers of FM, but this study warrant more investigations to confirm these findings.

**INTRODUCTION**

Fibromyalgia (FM) is a complex chronic disorder characterized by symptoms of diffuse pain, unrefreshing sleep, fatigue, emotional distress, and irritable bowel syndrome \( (\text{Wolfe et al., 1990}) \). The disorder
predominately affects middle-aged women. FM is now recognized and diagnosed frequently among females with somatic pain (Wolfe et al., 1995).

The etiology of FM is unknown, although several lines of evidence support the involvement of disturbed serotonin (5-HT) metabolism along with disturbances in several other chemical pain modulators (Russell et al., 1992 and Alnigenis & Barland, 2001). Moreover, there is alteration of neuroendocrine functioning include altered hypothalamic-pituitary-adrenal axis (HPA) activity with hypocortisolism (Riedel et al., 1998, Torpy et al., 2000 and Fries et al., 2005). Other neuroendocrine axes, such as the growth and thyroid axes are perturbed in FM, as evidenced by low levels of insulin-like growth factor 1 (IGF-1) (Bennett et al., 1997). Neeck (2000) revealed that neuroendocrine and hormonal perturbations may be related to the serotonergic system in FM.

Determination of gamma-aminobutyric acid (GABA) concentration in human cerebrospinal fluid (CSF) can be used to assess GABAergic activity in the central nervous system (CNS) (Schechter & Sjoerdsma, 1990). Most of the data from direct and indirect studies are consistent with GABA involvement in mood disorders and depressive illness and showed low CSF GABA levels in these states (Petty 1995, Tunnicliff & Malatynska, 2003 and Kalia, 2005). Recently, GABA analogue and GABA agonist were examined for treatment of CNS disorders including FM, and has been found to be effective in raising the pain threshold and improving other related symptoms (Dixit & Bhargava 2002 and Grofford et al., 2005).

Nitric oxide (NO) metabolites in CSF could be useful prognostic markers in neurological diseases and may facilitate understanding the involvement of NO in these diseases (Bratasz et al., 2004 and Wang et al., 2005). NO may be pro- or antinociceptive molecule depending on its concentration and sites of action (Durate et al., 1992, Meller et al., 1992 and Christopherson & Bredt, 1997). The involvement of NO in pain process in FM was previously documented (Larson et al., 2000, Pall 2001 and Sackner et al., 2004). FM patients show abnormalities in allodynia (heightened pain sensitivity) that could result from aberrant CNS perception of normal stimuli (Central sensitization) (Woolf, 1983, Codere et al., 1993 and Bradely et al., 2000). Central sensitization appears to depend on activation of N-methyl D-aspartate (NMDA) receptors (Woolf & Thompson, 1991). Activation of excitatory receptors as NMDA receptors (Codere & Melzack, 1992) and substance P (Radhakrishnan et al., 1995) increases intracellular calcium and activates NO synthase (NOS) (Garthwaite et al., 1988 and Garthwaite & Boulton, 1995).
The diagnosis of FM is based exclusively on subject data because of the lack of objective analytical image or pathological data (Andreu & Sanz, 2005). On the other hand, understanding specific etiological factors and mechanisms involved in individual patients will allow clinicians to determine treatments that are most effective for a given patients. Therefore, the current study aimed to estimate the levels of insulin, IGF-1, cortisol, 5-HT, GABA and NO metabolites in serum of women suffering of FM. In addition, levels of 5-hydroxyindoleacetic acid (5-HIAA; metabolite of 5-HT), GABA, and NO metabolites were determined in CSF of those patients in a trial to elucidate their roles and relations with clinical signs of FM.

**SUBJECTS AND METHODS**

**Subjects:**

Fifteen normoglycemic patients all of them females their ages range between 23 and 55 years old (mean ± SD, 37.4 ± 8.5) diagnosed as primary fibromyalgia according to the American College of Rheumatology (ACR) criteria (Wolfe et al., 1990). Laboratory investigations were done in form of level of blood sugar, ESR, CRP, complete blood picture. Rheumatoid factor and antinuclear antibodies to exclude any other diseases interfere with the study. Fifteen control subjects all of them females without fibromyalgia they were matched in age with the patients’ group. All subjects agreed to donate CSF obtained by lumbar puncture and blood sample. None of the patients or healthy control subjects was on psychotropic drugs. After giving informed consent using a document approved by the local ethics committee, all subjects agreed to donate CSF obtained by lumbar puncture. Primary fibromyalgia patients who were on analgesics were discontinued 48 hours prior to lumbar puncture.

Serum and CSF collection: The samples were collected between 09:00 and 11:00 h after overnight fast. None of the CSF samples were blood stained. The serum and CSF samples were divided into aliquots, frozen at –70 °C until thawed for analysis.

**Determination of 5-HT and 5-HIAA:**

Because of the low levels of 5-HT in normal CSF samples, determination of its metabolite 5-HIAA levels were performed. 5-HT and 5-HIAA levels were determined fluorimetrically according to Curzon & Green (1970) with minor modification (Jonsson & Lewander, 1970). Briefly, 2ml of sample were treated with 3ml acidified butanol. After centrifugation for 5 min. at 3,000 rev/min., 2.5ml of the supernatant were
pipette into 25 mL glass stoppered tube and shaken for 5 min. with 5 ml n-heptane and 0.4 ml 0.1N HCL containing 0.1% cysteine. The aqueous phase was used for determination of 5-HT and the organic phase retained for the 5-HIAA determination. For 5-HT determination 0.004% O-phthalaldehyde (in 10N HCL) was used. For 5-HIAA determination, organic phase was added to 0.5M phosphate buffer (pH 7), centrifuged for 3 min. at 3.000 rev/min. and divided into two portions (one for test and other for blank). 1% cysteine, 0.1% O-phthalaldehyde (in methanol), conc. HCL, and 0.02% sodium periodate was used. Activation and fluorescent wavelengths were 360 nm and 470 nm respectively.

**Determination of GABA levels:**

GABA levels were estimated in serum and CSF samples by fluorimetric method with minor modification (Uchida & Brien, 1964). An equal volume of 10% trichloracetic acid was added to sample, centrifuged, and the supernatant was pipette in 10 ml test tube, 0.05M glutamic acid in 0.2M phosphate buffer (pH 6.4) and ninhydrin solution were added. The tubes were kept at 60° for 30 min and then cooled and copper tart rate reagent was added. Activation and fluorescent wavelengths were 380 nm and 450 nm respectively.

**Determination of NO metabolites:**

This assessed by using Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine diHCL, and 2.3% orthophosphoric acid) according to method of Ding et al. (1988).

**Determination of insulin, IGF-1, and cortisol:**

Insulin levels were estimated in FM sera using a solid phase enzyme amplified sensitivity immunoassay (MEDGENIX-INS-EASI) kit (Frier et al., 1981). IGF-1 levels were determined using enzyme linked immunosorbent assay (ELISA) kit (Blum et al., 1993). Also, cortisol levels were estimated using ELISA kit (Tijssen, 1985).

**Statistical analysis:**

Prism software program, graph Pad version 3.0 was used for all analyses. Clinical data was expressed as mean ±SE or numbers (percentages). Data of hormones and neurotransmitters was expressed as mean + SE and quartiles [median (25th, 75th percentile)] because of the wide range of their levels. Hormone and neurotransmitter concentrations for women suffering of FM were compared with those in the control group by unpaired 2-tailed t-test. For nonparametric comparisons, Mann-Whitney test
was performed because of non-normal distribution of hormones and neurotransmitters. Spearman’s rank correlation coefficient was used for evaluating the correlation between age, number of tender points, hormones, and neurotransmitters. P<0.05 was considered a significant difference.

**RESULTS**

Clinical characteristics of women suffering of FM were demonstrated in table 1. The present data showed altered serum levels of some hormones in normoglycemic women suffering of FM (table 2). This is revealed by reduced levels of cortisol (p<0.05) and elevated levels of insulin (p<0.01) in FM sera. The data did not find significant differences between serum levels of IGF-1 in women suffering of FM and those in healthy women.

Table (1): Clinical characteristics of women suffering of FM.

<table>
<thead>
<tr>
<th>Variables</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.4 ± 2.19</td>
</tr>
<tr>
<td>Number of tender points</td>
<td>13.27 ± 0.35</td>
</tr>
<tr>
<td>Pain</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Morning stiffness</td>
<td>11 (73.3%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14 (93.3%)</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Psychiatric syndrome &amp; depression</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Irritable bowel</td>
<td>4 (26.7%)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SE or number (percentage).

In addition, cortisol levels in FM sera were inversely correlated with number of tender points (r= -0.76, p<0.001). Negative correlation (r= -0.63, p<0.05) was also observed between IGF-1 levels and age of FM (table 4).

Quartiles [median (25th, 75th percentile)] of serum and CSF levels of neurotransmitters were presented in table 2 and 3. Low levels of serum 5-HT (p< 0.01) and CSF 5-HIAA (p<0.001) in women suffering of FM compared to control group was demonstrated in figure 1. In this study (table 4) serum levels of 5-HT were correlated positively with the levels of CSF GABA (r= 0.56, p< 0.05).
Table (2): Serum levels of hormones and neurotransmitters in studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n= 15)</th>
<th>FM (n= 15)</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin (μIU/mL)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>19.37 ± 1.83</td>
<td>27.72 ± 1.61</td>
<td>38</td>
<td>0.002</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>20.6 (13.25, 26.3)</td>
<td>30.3 (23.9, 33.1)</td>
<td></td>
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<tr>
<td><strong>IGF-1 (ng/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>216.2 ± 14.12</td>
<td>246.3 ± 22.45</td>
<td>93.3</td>
<td>0.443</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>202 (175, 275)</td>
<td>238 (178.5, 329.5)</td>
<td></td>
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<tr>
<td><strong>Cortisol (µg/ dl)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SE</td>
<td>10.29 ± 0.85</td>
<td>7.86 ± 0.93</td>
<td>63</td>
<td>0.040</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>10 (7.5, 17.3)</td>
<td>7 (5, 10.25)</td>
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<tr>
<td><strong>5-HT (ng/ ml)</strong></td>
<td></td>
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<tr>
<td>Mean ± SE</td>
<td>128 ± 12.57</td>
<td>73.9 ± 10.32</td>
<td>48.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>135 (83.75,177.5)</td>
<td>82.5 (36.25, 100)</td>
<td></td>
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<tr>
<td><strong>GABA (ng/ ml)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SE</td>
<td>85.35 ± 6.39</td>
<td>55.99 ± 5.91</td>
<td>40</td>
<td>0.003</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>75.5 (62.75, 110)</td>
<td>55 (40.25, 72.5)</td>
<td></td>
<td></td>
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<tr>
<td><strong>NO (nmol/ ml)</strong></td>
<td></td>
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<tr>
<td>Mean ± SE</td>
<td>33.69 ± 2.78</td>
<td>63.07 ± 5.36</td>
<td>35.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>29 (25, 42.25)</td>
<td>69 (59.1, 79.5)</td>
<td></td>
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</tbody>
</table>

*p< 0.01, *p< 0.05, comparing controls vs. FM groups using Mann-Whitney test.

Table (3): CSF levels of neurotransmitters in studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n= 15)</th>
<th>FM (n= 15)</th>
<th>U</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td><strong>CSF 5-HIAA (ng/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean ± SE</td>
<td>29.07 ± 1.35</td>
<td>19.75 ± 0.89</td>
<td>15</td>
<td>... &lt; 0.001</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>27.5 (26.65, 34)</td>
<td>19 (17.5, 24)</td>
<td></td>
<td></td>
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<tr>
<td><strong>CSF GABA (ng/ ml)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>41.58 ± 2.06</td>
<td>29.95 ± 2.36</td>
<td>38</td>
<td>0.002</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>43 (37, 55.3)</td>
<td>25 (22.75, 38.5)</td>
<td></td>
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<tr>
<td><strong>CSF NO (nmol/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>21.13 ± 1.02</td>
<td>28.78 ± 2.48</td>
<td>61</td>
<td>0.034</td>
</tr>
<tr>
<td>Median (25, 75th percentile)</td>
<td>21 (18, 23.3)</td>
<td>27.6 (19.79, 40.25)</td>
<td></td>
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</tr>
</tbody>
</table>

***p< 0.001, **p < 0.01, *p< 0.05 comparing controls vs FM groups using Mann-Whitney test.
Fig. (1): Mean serum levels of 5-HT and mean CSF levels of 5-HIAA in FM and control group.

Fig. (2): Mean serum and CSF levels of GABA in FM and control group.
The decrement of both serum and CSF levels of GABA (p<0.01) were detected in women suffering of FM compared with control group (figure 2). Moreover, CSF GABA levels were positively correlated (r= 0.67, p<0.01) with serum levels of GABA in those patients. In contrast, negative correlation was showed between age and CSF GABA levels (r= -0.74, p<0.01) among women suffering of FM. Our statistical analysis demonstrated a positive correlation (r= 0.61, p<0.05) between serum cortisol and serum GABA levels in women suffering of FM (table 4).

The higher serum and CSF levels of NO metabolites were detected (figure 3) in women suffering of FM than control group (p<0.01, p<0.05 respectively). Table 4 mentioned that levels of serum NO metabolites were correlated with levels of CSF NO metabolites in those patients (r=0.63, p<0.05). Our study also noticed strong correlation between levels of NO metabolites (either in serum or CSF) and number of tender points (r= 0.91, p<0.001; r=0.73, p<0.01 respectively). In contrast, there was inverse correlation between those levels in sera of women suffering of FM and serum levels of cortisol (r= -0.70, p< 0.01).
DISCUSSION

During the last years, FM research directed from psychological and behavioral issues to sleep, nociception, and neuroendocrinology (Lash et al., 2003). Almost all of the hormonal feedback mechanisms controlled by the hypothalamus are altered in this syndrome, mainly observed in females (Neeck, 2000 & Torpy et al., 2000 and Landis et al., 2001).

The present data showed altered serum levels of some hormones in normoglycemic women suffering of FM. This is revealed by reduced levels of cortisol and elevated levels of insulin in FM sera. The data did not find significant differences between serum levels of IGF-1 in women suffering of FM and those in healthy women. These results are in agreement with several studies and provide further evidence for altered neuroendocrine functioning in women suffering of FM (Gur et al., 2004, Denko & Malemud, 2005 and Fries et al., 2005). Hypocortisolism is a neuroendocrine finding that has been described in FM (Neeck & Grofford, 2000 and Gur et al., 2004). There is increasing evidence for a relatively decreased, rather than an increased cortisol secretion in individuals who have been exposed to severe stress or suffer from stress-response related disorders (Heim et al., 2000). Regarding IGF-1 levels there are controversial results in previous studies. Denko & Malemud (2005) found no change in IGF-1 levels and increment in insulin levels, and concluded that fasting serum insulin levels appear to be valuable surrogate markers in normoglycemic FM patients. However, Bennett et al., (1992 and 1997) suggested that the hypothalamic-pituitary-IGF axis was deficient in FM. In the current study, cortisol levels in FM sera were inversely correlated with number of tender points, consistent with a study of Gur et al. (2004). Additionally, Malt et al. (2002) showed a negative association between pain and baseline cortisol. Negative correlation was also observed between IGF-1 levels and age of FM. Increase in age was strongly associated with low activity levels and thus this correlation confirms that the activity levels influence serum IGF-1 (Veldhuis et al., 1997).

The relation between neuroendocrine perturbation and serotonergic system in FM is clarified by Neeck (2000), who suggested that 5-HT precursor (tryptophan) and drugs which release 5-HT or act directly on 5-HT receptors stimulate HPA axis, indicating a stimulatory serotonergic influence on HPA axis function. Most current studies in nociception affirm that patients with FM exhibit low serum 5-HT and CSF 5-HIAA in combination with increased substance P levels in the CSF (Russell et al.,
Substance P levels are normally high in the axons of primary unmyelinated C-fiber sensory neurons (McNeill et al., 1989) and its physiologic functions are mediated by 5-HT (Murphy & Zemla, 1987). On the other hand, Jonsson et al. (2004) stated that concentrations of monoamine metabolites in human CSF have been used extensively as indirect estimates of monoamine turnover in the brain. This study concerning serum 5-HT and CSF 5-HIAA confirm those of the aforementioned researches. Low levels of serum 5-HT and CSF 5-HIAA showed in this study probably result from disturbed 5-HT metabolism. 5-HT is recognized chemical mediator of sleep and of pain perception by both the thalamus and the peripheral nervous system (Harvey et al., 1975). The role of 5-HT is supported by an observation of Juhl (1998) who examined 5-HT substrates as supplements in FM. The author reported that L-tryptophan or 5-hydroxytryptophan has been shown to improve symptoms of depression, anxiety, insomnia and somatic pain in a variety of FM patient cohorts. The effects of 5-HT2 and 5-HT3 receptor antagonists were performed for treatment of FM (Farber et al., 1998 and Stratz & Muller, 2000). The investigators sought a possible reduction in pain based on the premise that binding of 5-HT to its receptor can trigger pain, probably due to the release of potent pain mediators such as substance P and other neurokines. Some medical literature demonstrated the relationship between age and CSF levels of 5-HIAA (Larsson et al., 1988 and Russell et al., 1992). However, the present data showed no correlation between age and CSF 5-HIAA levels in FM. This finding is compatible with other investigations (Kruesi et al., 1988 and Faustman et al., 1990).

The balance between excitatory signaling mediated by glutamate and inhibitory signaling mediated by GABA plays a pivotal role in mechanisms underlying the modulation and maintenance of a variety of neural functions. Therefore, abnormalities in GABAergic signaling molecule would lead to a crisis of severe symptoms relevant to number of neuropsychiatric disorders including depression (Fujimori & Yoneda, 2004, Kalueva & Nutt, 2004 and Kalia 2005). The decrement of both serum and CSF levels of GABA were detected in women suffering of FM compared with control group. Moreover, CSF GABA levels were correlated with serum levels of GABA in those patients. Petty (1995) reported that low GABA levels are found in brain, CSF and plasma of patients with depression. This could be attributed to decreased levels of glutamate decarboxylase (Fatemi et al., 2005). Other study showed no differences in CSF GABA between depressed patients and normal controls (Roy et al., 1991). The current investigations showed
negative correlation between age and CSF GABA levels among women suffering of FM. Later, the CSF GABA concentrations were found to be varied with age, sex, CSF fraction collection and storage (Schechter & Sgoerdsma, 1990 and Takayama et al., 1992). GABA, the principal neurotransmitter in the cerebral cortex is formed within GABAergic axon terminals and released into synapse, where it acts at one of the two types of receptor. GABAA, which control chloride entry into the cell, and GABAB which increases potassium conductance, decreases calcium entry and inhibits the presynaptic release of other neurotransmitters (Treiman, 2001). GABA analogue and GABA agonist have been found to be effective for treatment of CNS disorders (Dixit & Bhargava, 2002 and Grofford et al., 2005). GABA analogue is effective in raising the pain threshold, reducing allodynia increasing slow-wave non rapid eye movement sleep, relieving anxiety, modulating acute pain symptoms, and reducing colon-related pain in FM (Grofford et al., 2005). Our statistical analysis demonstrated a positive correlation between serum cortisol and serum GABA levels in women suffering of FM. There is no explanation for this correlation. However, a recent study revealed a role of GABA in mediating the effects of corticotrophin-releasing factor on the rat dorsal raphe 5-HT system (Waselus et al., 2005). In this study serum levels of 5-HT were correlated positively with the levels of CSF GABA. 5-HT might elicit the inhibitory effect through a Ca (2+)-sensitive release of GABA from intercalated GABAergic local neurons that are excised first by 5-HT (Kang et al., 2002). On the other hand, the pineal hormone, melatonin (5-HT product) seems to be the natural hormone to facilitate sleep in insomnia patients acts via GABA receptor (Rohr & Herold, 2002).

Recent literatures demonstrated that the inhibition of NOS especially inducible NOS (iNOS) produces antinociception in different models of pain suggest that the iNOS-NO system plays in pain processing (Eriksen 2004, Goadsby 2005 and Labuda et al., 2005). NO exhibits a peripheral analgesic effect, whereas it mediates hyperalgesia at spinal or supraspinal level (Durate et al., 1992 & Kawabata et al., 1992 & Meller et al., 1992). Herein the higher serum and CSF levels of NO metabolites were detected in women suffering of FM than control group. Furthermore, levels of serum NO metabolites were correlated with levels of CSF NO metabolites in those patients. Our study also noticed strong correlation between levels of NO metabolites (either in serum or CSF) and number of tender points. In contrast, there was inverse correlation between those levels in sera of women suffering of FM and serum levels of cortisol. These data clarified...
the implication of NO in pain processing in FM (Larson et al., 2000, Pall, 2001 and Sackner et al., 2004). The elevated levels of NO metabolites may be due to increase NOS activity with increased pain (Larson et al., 2000). NOS activity was associated with NMDA receptor-mediated events as well as substance P activity during thermal and mechanical nociceptive transmission in the cat dorsal horn (Radhakrishnans & Henry, 1993 and Mens, 2001) reported that in contrast to excitability, the resting activity of dorsal neurons which is likely to induce spontaneous pain does not appear to depend on the release of substance P and glutamate but on the concentration of NO in the spinal cord.

The ability of NOS inhibitors to prevent the development and expression of many types of hyperalgesia including FM may be by blocking the downstream mediators of NMDA activation (Meller et al., 1992, Rao, 2002, Llabuda et al., 2005 and Pall 2001) suggested that variation in symptoms might be explained by a variation in NO /peroxynitrite tissue distribution. Peroxynitrite is formed from reaction of NO with superoxide radical (Henry et al., 1991). Peroxynitrite concentration may affect dopamine concentration inversely and may act to facilitate glutamate and GABA release (Trabace & Kendrik, 2000).

Because arginine is the first amino acid at the amino terminus of substance P, one might speculate that the source of arginine in patients with FM may reflect, in part, degradation of substance P in CSF (Russell et al., 1993).

Apart from the known role of 5-HT in the pathogenesis of FM, GABA and NO are involved in either mood, depression, anxiety, or pain and fatigue. Therefore, the current study suggests that they may play a role in modulating FM. This is clarified by the alterations of these neurotransmitters either in serum or CSF of women suffering of FM. In addition, levels of the studied neurotransmitters appear to be partly correlated to age, number of tender points and/or cortisol. Accordingly, this study recommended the possibility of using innovative therapy as GABAergic agents, NOS inhibitors, or antioxidants for therapy of various symptoms in women suffering of FM. Moreover, our study may find utility of these neurotransmitters as possible markers of FM, but this warrant more studies to confirm these findings.
REFERENCES


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مسوطنات حمض ال 5-هيدروكسيد الاملأسيتيك وحمض الجاما أمينوبيرتريك ونواتج أكسيد النيترزول في مسار النخاع الشوكي للمصابات ذات الاملأ الفسيولوجي العضلي

ىوتم أحمد محمد سعد ومنى أحمد محمد مدكور و حسن قطب

اقسام الروماتيزم والتهابي والكيمياء الحيويه والسلامه ووحدة الاملا

كلية الطب جامعة أسوان و مستشفى أسوان الجامعي

مقدمة: يعد الاملأ الفسيولوجي العضلي من الاضطرابات المتهمة الممتعة التي غالباً ما تصيب السيدات. و الافترار إلى صورة واضحة تحدي من أجل تشخيص أو متابعة تقدم المرض يديث العديد من الباحثين لقياس تغيرات المواصلات العصبية والهرمونات في سائل الجسم والتي قد تكون لها علاقة فسيولوجيةً للمرض.

الهدف: إذا فإن هذه الدراسة استهدفت مقارنة مستويات حمض ال 5-هيدروكسيد إندول أسيتيك و حمض جاما أمينوبيرتريك و نواتج أكسيد النيترزول في السائل الشوكي لمصابات بالاملأ الفسيولوجي العضلي وذوات مستوى سكر الدم الطبيعي في المد التُلمي الذي تنتج أدوات الضماطة، بالإضافة إلى ذلك فإن مستويات الإنسولين و عامل جسمة الإنسولين-1 والكورتيزول و السيروتينين و حمض ال 5-هيدروكسيد إندول أسيتيك و حمض جاما أمينوبيرتريك و نواتج أكسيد النيترزول قد تم تقديرها في مصل مرضى الاملأ الفسيولوجي العضلي بالمقارنة بالمجموعة الضاملة.

وايضاً فقد أجريت علاقات ارتباط بين كل القياسات المدروسة.

النتائج: قادرت الدراسة الحالية تغييراً في مستويات الإنسولين و الكورتيزول في مصل السيدات المصابات بالاملأ الفسيولوجي العضلي ذات مستوى الاملأ الطبيعي (15 سيده) في المد مقارنة بالمجموعة الضاملة (15 سيدة). ولكن النتائج لم تحق أي فروق جوهري في مستويات عامل جسمة الإنسولين-1 في أمثل السيدات المصابات بالاملأ الفسيولوجي العضلي عنها في السيدات الضاملة. وكانت هناك علاقة إرتباط عكسي بين مستويات الكورتيزول وعدد نقاط المرض. وأيضاً وافقت على علاقة ارتباط ساقي بين مستويات سيدات عامل جسمة الإنسولين-1 في المصل و عمر مرضى الاملأ الفسيولوجي العضلي. وقد ن자를 مستويات السيروتينين في المصل و حمض ال 5-هيدروكسيد إندول أسيتيك في السائل الشوكي برمي مرضى الاملأ الفسيولوجي العضلي. و تبين تقلص في مستويات حمض جاما أمينوبيرتريك في كل من المصل والسائل النخاعي الشوكي في حالات المرضي المقارنة بالمجموعة الضاملة. بالإضافة إلى ذلك كان هناك علاقة إرتباط إيجابية بين مستويات حمض جاما أمينوبيرتريك في المصل و مستويات الكورتيزول في المصل و أيضاً بين مستويات حمض جاما أمينوبيرتريك في السائل النخاعي الشوكي و السيروتينين في المصل. مع ذلك فإن مستويات حمض جاما أمينوبيرتريك في السائل النخاعي الشوكي أدركت سلبياً مع عصر السيدات المصابات بالاملأ الفسيولوجي العضلي. هذه النتائج قد توضح الدور الهام لحمض جاما أمينوبيرتريك في السرب المرضي للاملأ الفسيولوجي العضلي. أنتج ارتفاع في مستويات نواتج أكسيد النيترزول في المصل والسائل النخاعي الشوكي لمرضى الاملأ الفسيولوجي العضلي عن المجموعة الضاملة. بالإضافة إلى ذلك فإن مستويات نواتج أكسيد النيترزول في المصل قد ارتبطت إيجابياً بطريقة الغليظة بين السائل النخاعي الشوكي. إذ اشترك أكسيد النيترزول في عملية الأمان قد اتطورت بعلاقة الارتباط القوية بين

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الناتج: إن التغيرات في مستويات الفيرومونات والمواد الكيميائية التي وردت في هذه الدراسة للسيدات المصابات بالألم الليفي العضلي دعمت نظرية إشراك الفيرومونات والمورثات العصبية في السبب المرعب للألم الليفي العضلي. وقد اقترح أن الدراسة الحالية أن حمض جاما أمينوبيريكيك وأكسيد البيتريك ربما تلعب دورًا في تقليل الألم الليفي العضلي وتغيرات مستوياتهم في المصل والسائل النخاعي الشوكي لمرضي الألم الليفي العضلي. وبذلك، من المحتمل أن يكون برامج التدريبية المخصصة للأكسيد البيتريكيك أو مضادات الإنتيبيوتريكيك للterior العصبية كعلاجات للألم الليفي العضلي، ولكن هذه الدراسة تحتاج المزيد من الأبحاث للتأكد من هذه النتائج.