Award Papers

The Effect of Phenytoin Monotherapy on Serum 25-hydroxyvitamin D and Bone Health Markers: A Prospective Study

[1]Aastha Bansal, [2]Kiran Dahiya, [3]Surekha Dabla, [4]Veena S Ghalaut, [5]Richa Goel

[1,2,4,5]Department of Biochemistry, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India
[3]Department of Medicine, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

OBJECTIVE

Phenytoin monotherapy in patients with epilepsy affects calcium metabolism and bone turnover markers leading to hypovitaminosis D, hypocalcemia, reduced bone mineral density (BMD), and its imminent consequences. This study was planned to assess how early these changes may arise and to find out their correlation with phenytoin levels.

MATERIALS AND METHODS

In this prospective study, BMD, 25-hydroxy vitamin D, urinary hydroxyproline were estimated at baseline, 2 and 6 months after phenytoin monotherapy and serum phenytoin levels were measured at 2 and 6 months of therapy.

RESULTS

At 6 months, BMD showed a decrease (T-score -1.22 ± 1.049 to -1.412 ± 1.055, p-value < 0.001) while vitamin D levels started decreasing as early as 2 months after therapy and decreased further after 6 months (32.93 ± 6.38 to 31.46 ± 5.99 ng/mL at 2 months and then to 29.96 ± 5.94 ng/mL, p-value < 0.05 and < 0.001 respectively). Urine hydroxyproline levels were 16.65 ± 2.22 mg/day at diagnosis and increased to 16.97 ± 2.25 mg/day after 2 months and to 18.544 ± 2.83 mg/day after 6 months (p-value < 0.001 at 6 months). Mean serum phenytoin levels at 2 months were 15.74 ± 9.49 mg/L and while at 6 months these were observed to be 15.92 ± 5.54 mg/L. Urine hydroxyproline levels correlated positively with phenytoin levels (r = 0.447, p-value < 0.05).

CONCLUSION

Bone health derangement starts at around 2 months while at 6 months of phenytoin therapy, there is significant decline in bone health as indicated by status of markers like BMD and urine hydroxyproline.

Decreased TET1-mediated Hydroxymethylation of Promoter CpG Islands Causes Downregulation of Tight Junction Protein, Claudin-6 in Breast Adenocarcinoma Cells

[1]Ashikh Seethy, [2]Sameer A Guru, [3]Vijay Singh, [4]Radhika Agarwal, [5]Nita Khurana, [6]PN Agarwal, [7]Alpana Saxena, [8]PC Ray

[1-3,7,8]Department of Biochemistry, Maulana Azad Medical College, New Delhi, India
[4,5]Department of Pathology, Maulana Azad Medical College, New Delhi, India
[6]Department of Surgery, Maulana Azad Medical College, New Delhi, India

BACKGROUND AND OBJECTIVES

Claudin-6 (CLDN6), an integral tight junction protein, regulates intercellular adhesion and paracellular permeability. Claudins prevent epithelial to mesenchymal transition and breast cancer stem cell generation from differentiated cells. Hypermethylation and hydroxymethylation of promoter CpG islands [mediated by Ten Eleven Translocase 1 (TET1)] are putative mechanisms for regulation of Claudin-6. We studied Claudin-6 expression in breast cancer and breast epithelial cells and its regulation in the context of promoter hypermethylation, TET1 expression, and promoter CpG island hydroxymethylation.

MATERIALS AND METHODS

Claudin-6 expression in breast adenocarcinoma cell line, T-47D, and breast epithelial cell line, HBL-100 was assessed using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and indirect immunofluorescence. Promoter hypermethylation of CLDN6 was studied by methylation-specific PCR. Expression of TET1 was studied using qRT-PCR. The effect of hydroxymethylation on CLDN6 expression was assessed by treatment of the cells with a hydroxymethylating agent, dimethyl sulfoxide (DMSO), followed by qRT-PCR and/or indirect immunofluorescence.

RESULTS

CLDN6 was downregulated in T-47D cells compared with HBL-100 cells (median fold change: 0.125, p: <0.001). There was no difference in the hypermethylation of promoter CpG islands in T-47D and HBL-100 cells. TET1 was downregulated in T-47D cells compared with HBL-100 cells (median fold change: 0.396, p: 0.002). The DMSO treatment caused increased expression of Claudin-6 protein in T-47D cells to a higher extent than in HBL-100 cells, suggesting the already hydroxymethylated status of promoter CpG islands in HBL-100 cells.

CONCLUSION

CLDN6 is downregulated in breast cancer. This could be due to reduced hydroxymethylation of promoter CpG islands, which is mediated by downregulation of TET1 in breast cancer cells.

A Novel Method for Keratin Dissolution and Testing

[1]Asha Kumari, [2]Rambhaj Sharma, [3]Veena S Ghalaut

[1-3]Department of Biochemistry, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

OBJECTIVE

To find a suitable method for dissolution of keratin and performing protein color reactions.

MATERIALS AND METHODS

1 gm Keratin powder obtained from animal horn was dissolved in 100 mL, 10% freshly made KOH. Heat was applied for 1 to 2 minutes to warm the solution. Solutions were then cooled and 10% acetic acid was added in equal amount. The solution was gently mixed and homogenous solution was prepared. Qualitative protein color was performed. Tests were compared with previously described method of preparing keratin solution in 40% KOH and heat. Solutions in 5, 10, 15, 20, and 40% KOH alone and 5, 10, 15, and 20% acetic acid were also tested. In addition, 5 and 20% acetic acid was also used with 10% KOH to see the effect of change in acetic acid concentration.

RESULTS AND OBSERVATIONS

Solution of keratin prepared by 10% KOH and 10% acetic acid made a homogenous solution of keratin and gave positive results with all protein color reactions. Ninhydrin test, Hopkin-Cole test, Xanthoproteic test, and Millon test were negative with keratin in 40% KOH solution.

CONCLUSION

10% KOH and 10% acetic acid should be used for keratin dissolution and its protein color reactions.

Role of Follicle Stimulating Hormone Receptor Gene (Ser680Asn) in Patients with Polycystic Ovarian Syndrome

[1]Shilpa Kumar, [2]Anju Jain, [3]Binita Goswami, [4]Abha Singh

[1-4]Department of Biochemistry, Lady Hardinge Medical College and Hospital, New Delhi, India

OBJECTIVE

To study the follicle stimulating hormone receptor (FSHR) gene (Ser680Asn) polymorphism in polycystic ovarian syndrome (PCOS). To conduct hormonal analysis of the study group [follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, dehydroepiandrosterone, testosterone] and identify any relationship between the polymorphism and FSH levels.

MATERIALS AND METHODS

The FSHR gene (Ser680Asn) polymorphism and FSH levels were assessed and compared between 30 diagnosed cases of PCOS, as per Rotterdam Criteria (2003), and 30 age-matched healthy controls, of age groups from 15 to 40. Hormone and genotype analysis was done using electrochemiluminescence/enzyme-linked immunosorbent assay and polymerase chain reaction-restriction fragment length polymorphism respectively.

RESULTS

The LH, LH:FSH ratio, and testosterone levels were found to be significantly higher in women with PCOS compared to their healthy counterparts (p-value <0.001). No significant difference was seen in genotypic distribution of the FSHR gene (Ser680Asn)

polymorphism between cases and controls. Intergenotypic variation of FSH levels was observed in cases, with the GG genotype (9.21 ± 1.07 IU/L) showing significantly higher levels compared to AG genotype (5.78 ± 0.46 IU/L) with a p-value of 0.018.

CONCLUSION

The FSHR gene (Ser680Asn) polymorphism does not show any differential distribution between PCOS and normal women, but since FSH levels are influenced by the FSHR gene (Ser680Asn), the genotype could contribute to the flawed folliculogenesis in PCOS.

Study of Serum Osteocalcin and Alkaline Phosphatase Levels in Rheumatoid Arthritis

[1]Abhishek Dubey, [2]Chubalemla Longkumer, [3]C Lalrindiki, [4]Victoria Laishram, [5]Santa Naorem, [6]Th Ibetombi Devi

[1-4,6]Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur, India
[5]Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India

INTRODUCTION

Rheumatoid arthritis (RA) is characterized by articular erosions, periarticular bone loss, and chronic inflammation leading to increased risk of osteoporosis. The clinical presentation usually exists of a symmetric polyarthritis of the small joints of hand and feet, but any joint can be involved. Rheumatoid arthritis affects between 0.5 and 1% of the adult population worldwide.

AIMS AND OBJECTIVES

To estimate the values of serum osteocalcin and alkaline phosphatase among control and study groups of RA and to compare and find out any changes in the levels of serum osteocalcin and alkaline phosphatase between study and control groups.

MATERIALS AND METHODS

A case/control study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India between Oct 2013 and Sep 2015 over 76 cases of RA and 76 age, sex-matched healthy controls. Serum osteocalcin was measured by enzyme-linked immunosorbent assay (ELISA) and serum alkaline phosphatase was measured using colorimetric methods.

RESULTS

Values of both serum osteocalcin (cases – 18.50 ± 8.72 ng/mL, controls – 9.98 ± 7.68 ng/mL) and alkaline phosphatase (cases – 216.22 ± 59.96 IU/L, controls – 164.17 ± 50.67 IU/L) were found to be higher among cases as compared with controls. This difference was statistically significant. Also, there was a positive correlation between serum osteocalcin and alkaline phosphatase levels.

CONCLUSION

The results of this study showed a significant increase in the values of serum osteocalcin and serum alkaline phosphatase among cases and controls. This study demonstrates that increased bone formation is associated with RA together with bone resorption. It affects the bone mineral density. Increased bone turnover is a common feature of RA. The results of this study confirm the association of the serum osteocalcin and serum alkaline phosphatase with RA.

HE4: Are We Looking at a Better Marker? A Prospective Study in Comparison with CA125

[1]Jyotsna Volturi, [2]K Suseela, [3]Sudha Murthy, [4]Sujatha, [5]A Santa, [6]Lavanya

[1]Department of Biochemistry, Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India
[2,3]Department of Pathology, Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India
[4]Department of Gynecology, Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India
[5]Department of Medical Oncology, Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India
[6]Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India

OBJECTIVE

The aim of this study is to evaluate a new tumor marker, HE4 and the combination of HE4 with CA125 for the diagnosis of ovarian cancer.

MATERIALS AND METHODS

CA125 and HE4 serum levels were determined in 36 patients presenting with pelvic mass or suspected to have ovarian mass at our institute. 10 women including 5 premenopausal and 5 postmenopausal were taken as healthy controls. CA125 and HE4 were analyzed using Elecsys kits on Roche Cobas e411, which is based on electrochemiluminescence technology. CA125 and HE4 cut-offs were 35 U/mL and 70 or 140 pmol/L respectively, depending on menopausal status.

RESULTS

Serum HE4 and CA125 concentrations were significantly higher in ovarian cancer patients compared with benign disease and nonovarian malignancies ($p < 0.001$ for both). The receiver operating characteristic (ROC)-area under the curve (AUC) value for HE4 in differentiating ovarian cancer from benign and nonovarian malignancies was 0.84 [95% confidence interval (CI), 0.71–0.98] and that of CA125 was 0.71 (95% CI, 0.54–0.89). Compared to CA125, HE4 had higher specificity (64.29% *vs* 50%) and positive predictive value (76.19% *vs* 75.86%). CA125 is 100% sensitive, has 100% negative predictive value (NPV) compared to HE4 (72.73 and 60%). By combining HE4 + CA125, the sensitivity and specificity reached 100 and 58.38% respectively.

CONCLUSION

The finding of ROC-AUC values for HE4 (0.84), being significantly higher when compared to CA125 (0.71), suggests a better performance of HE4 in differentiating ovarian cancer from benign and other nonovarian malignancies. Hence, HE4 appears to be a good single marker than CA125. Further studies are needed to explore in more detail.

Overexpression of Carcinoembryonic Antigen in Diabetes Mellitus Type II can be Related to Tyrosine Kinase Signaling Pathway of Insulin

[1]Yuthika Agrawal, [2]Kiran Chugh, [3]Vipin Goyal

[1]Department of Biochemistry, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Haryana, India
[2]Department of Biochemistry, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India
[3]Department of Chest and TB, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Haryana, India

BACKGROUND

Carcinoembryonic antigen (CEA) is a glycoprotein shown to be overexpressed in adenocarcinomas, especially of colorectal cancer. Recent research indicates that CEA-cell adhesion molecules (CEACAMs) play an important role in signal transduction and CEACAM1 is an important candidate molecule that may cause insulin resistance. It has also been reported that CEACAM1 interacts with other CEACAM protein family members like CEA (CEACAM5) and even both of them share the interacting genes also.

OBJECTIVE

To study serum CEA levels in diabetics and determine its significance in type II diabetes mellitus (DM), which has never been done before in India.

MATERIALS AND METHODS

One hundred fifty nonsmoker diabetic individuals divided equally into obese and nonobese groups were taken along with 50 controls. Serum CEA, serum insulin, HbA1c, fasting, postprandial sugar levels, and lipid profile were also estimated in all diabetics.

RESULTS

There was a significant difference between the mean CEA values in control and cases ($p < 0.001$), while there was not much difference between the CEA values in obese and nonobese diabetics. The CEA values correlated with insulin ($r^2 = 0.756$, $p \leq 0.001$), HbA1c ($r^2 = 0.029$, $p = 0.022$), HOMA-IR ($r^2 = 0.348$, $p < 0.001$), fasting blood glucose ($r^2 = 0.053$, $p = 0.002$), postprandial sugar ($r^2 = 0.197$, $p < 0.001$), triglyceride (TGL) ($r^2 = 0.103$, $p = 0.001$), serum cholesterol ($r^2 = 0.149$, $p < 0.001$), high-density lipoprotein ($r^2 = 0.077$, $p < 0.001$), and low-density lipoprotein ($r^2 = 0.099$, $p < 0.001$).

CONCLUSION

This data supports that increased CEA levels (though not several folds as in cancers) is related to diabetes irrespective of obesity suggesting that CEA functions in the pathophysiology of diabetes, possibly by heterotypically binding to hepatic CEACAM1 that are involved in the regulation of systemic insulin concentration.

Levels of Serum Anti-Müllerian Hormone and Other Circulating Hormones in Breast Cancer Patients and Influence of Chemotherapy to these Hormonal Levels

[1]Jyoti Bala, [2]Simmi Kharb, [3]Veena S Ghalaut, [4]Shashi Seth, [5]Rakesh Dhankhar

[1-5]Department of Biochemistry, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

OBJECTIVE

Despite the advances made in early diagnosis and treatment, the incidence of breast cancer has increased owing to the changes in reproductive pattern and increased screening. The present study was designed to measure the circulating hormone [serum estrogen (E2), progesterone, testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and anti-Mullerian hormone AMH] levels and elucidate the influence of chemotherapy on these hormones in breast cancer patients by comparing them with cancer survivors to age-matched control women.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Biochemistry in collaboration with Department of Radiotherapy in PGIMS, Rohtak. Thirty confirmed female breast cancer patients were selected as cases and 30 age-matched healthy female volunteers served as controls. Females on oral contraceptive pills/hormone therapy or drugs affecting hormone levels were excluded. Serum estrogen (E2), progesterone, testosterone, FSH, LH, and AMH levels were estimated in these patients before and after chemotherapy and in the controls.

RESULTS

In the present study, E2 showed a significant reduction before and after chemotherapy when compared with the healthy subjects ($p < 0.001$). Progesterone and testosterone were significantly increased in prechemotherapy and postchemotherapy group when compared with the controls ($p < 0.001$). There was no significant change in FSH and LH levels between healthy subjects and breast cancer patients, both before and after chemotherapy ($p > 0.05$). The AMH levels were significantly decreased before and after chemotherapy when compared to the control group ($p < 0.001$).

CONCLUSION

Knowledge of the precise time point by which the ovarian reserve is depleted is of great importance for the decision regarding the optimal adjuvant hormonal treatment. The AMH could be a valuable marker in these patients. Further research is required to explore the exact predictive value of AMH levels in breast cancer patients.

Carboxymethyllysine and Pentosidine: The Advanced Glycation End Products could be Early Predictors of Diabetic Neuropathy

[1]K Madhavi, [2]Priscilla A Chandran, [3]AK Meena, [4]M Noorjahan

[1,2,4]Department of Biochemistry, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India
[3]Department of Neurology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India

AIMS AND OBJECTIVES

Hyperglycemia-mediated accelerated formation of advanced glycation end products (AGEs) cause vascular damage leading to micro and macroangiopathy in diabetics, manifesting as peripheral neuropathy, which is the most common cause of neuropathy in India with a prevalence of 30%. Early detection of the ongoing vascular damage is pertinent to reduce morbidity. Our study aimed to estimate serum levels of AGEs, carboxymethyllysine (CML), and pentosidine in diabetic patients with and without neuropathy.

MATERIALS AND METHODS

A total of 40 diabetic subjects were included in the study. Complete history and body mass index (BMI) was obtained. Clinical evaluation for neuropathy and electromyogram were recorded. Based on the ENMG findings, subjects were classified into diabetics with and without neuropathy. Fasting blood sugar (FBS), HbA1c, folate, B12, and homocysteine were estimated. The AGEs – CML and pentosidine – were estimated by enzyme-linked immunosorbent assay.

RESULTS

Significant difference between the two groups was seen in the mean levels of FBS (184 ± 19.12 *vs* 138 ± 12.3 *vs* mg/dL; $p = 0.04$), B12 (1039 ± 129.8 *vs* 511.2 ± 87, $p = 0.002$). CML ($1,352 \pm 215$ ng/mL *vs* 634.8 ± 73.04) and pentosidine (10.6 ± 1.2 *vs* 7.3 ± 1.0 ng/mL)

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were significantly higher in cases p = 0.003 and 0.04 respectively, when compared with controls. Mean ± standard deviation (SD) of age (50 ± 6.7 *vs* 54 ± 6.1 years; p = 0.10), BMI (27.9 ± 1.2 *vs* 27.5 ± 1.2 kg/m^2; p = 0.84), and HbA1c (8.7 ± 0.5 *vs* 7.7 ± 0.4%; p = 0.13), folate (16.04 ± 2.4 *vs* 17.25 ± 1.9 ng/mL; p = 0.69), homocysteine (10.85 ± 1.8 *vs* 12.78 ± 0.9 μmol/L; p = 0.34) are not statistically significant between cases and controls. Logistic regression for HbA1c, CML, and pentosidine showed significance for CML (p = 0.01), which can correctly classify 70% of patients. Receiver operating characteristic (ROC) analysis showed higher sensitivity and negative predictive value (NPV) for CML (85.4%) and pentosidine (84%) than HbA1c (77.8%). Area under the curve (AUC) for CML > pentosidine > HbA1c (0.758 *vs* 0.728 *vs* 0.625).

CONCLUSION

Significantly increased serum levels of AGEs are seen in diabetic neuropathy cases when compared with diabetic controls. Carboxymethyllysine and pentosidine can be used as early screening markers to predict microangiopathy like neuropathy and for further management.

Vitamin D Levels in Patients with Hypothyroidism and its Association with Gender, Age, and Body Mass Index

[1]Pallavi Jain, [2]R Devi

[1,2]Department of Biochemistry, Gauhati Medical College and Hospital, Guwahati, Assam, India

INTRODUCTION

Hypothyroidism affects approximately one in 10 adults in India. The reported prevalence of vitamin D insufficiency in India is around 50 to 90%. Vitamin D affects more than 36 cell types, which possess vitamin D receptor, one of which is the thyroid gland. Both vitamin D and thyroid hormones act through steroid receptors and may affect each other's action as they have similar response elements on genes. Some of the symptoms of vitamin D deficiency overlap with that of hypothyroidism. So, a lower level of vitamin D is likely to aggravate the systemic abnormalities, such as hypertension associated with hypothyroidism as well.

AIMS AND OBJECTIVES

The aim of the present study was to find the levels of 25(OH)D in patients of hypothyroidism and to find the correlation between the levels of 25(OH)D and thyroid stimulating hormone (TSH), free T4 (FT4), and body mass index in the case group. Also, attempt was made to find the association between the levels of 25(OH)D with the gender and age of patients in the case group.

MATERIALS AND METHODS

A case/control study was conducted with 45 cases of hypothyroidism in the case group and 45 apparently healthy individual.

RESULTS AND CONCLUSION

25(OH)D levels were found to be lower in hypothyroid patients than in the control group (p < 0.0001). The levels were significantly decreased in females than in males. Moreover, a negative correlation was seen between 25(OH)D and TSH in the case group (p < 0.0001). The mean values of 25(OH)D were also decreased in the elderly age group. The mean values of TSH showed a decreasing trend with increasing vitamin D tertiles. Thus patients of hypothyroidism suffered from hypovitaminosis D which encourages the advisability of vitamin D supplements and screening of vitamin D levels.