International Journal of Medicine and Medical Sciences

Volume 4 Number 4 April 2012

ISSN 2006-9723

Academic Journals

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Nishimura (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 2001), (Chege, 1998; Stein, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. Parasitol. 116: 85-93.0.

Fransiscus RG, Long JC, (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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International Journal of Medicine and Medical Sciences

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 78-86, April 2012
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS11.126
ISSN 2006-9723 ©2012 Academic Journals

Review

Role of retinoid mediated microphthalmia-associated transcription factor (MITF) in melanoma

T. K. Basak[1,4], S. Chaudhuri[2] and Shobha Gupta[3]*

[1]Director Research, Krishna Engineering College, Ghaziabad, UP, India.
[2]Stem Cell Research, School of Tropical Medicine, Kolkata, W. B., India.
[3]Department of EE, College of Engineering and Technology, Kolaghat, W. B., India.
[4]JDU, Kolkata, W. B., India.

Accepted 22 March, 2012

Retinoic acid is the most effective therapeutic drug for the treatment of many types of cancer, including melanoma. All-*trans* retinoic acid (ATRA) increases the protein level of MITF in melanoma cells within 2 to 4 days (Chan, 2007), indicating that RA induced MITF plays a vital role in its anti-proliferative action. On the contrary, MITF silencing can induce senescence in melanoma cells. These two different roles of the same protein are dependent on a band of threshold level. The expression of MITF below threshold band level results in senescence and above it causes cell differentiation and consequently cell cycle arrest. The anti-proliferative effect of ATRA, mediated by MITF, increases its expression above threshold level and targets other cell cycle regulators to suppress proliferation. This role of RA mediated MITF is elucidated in the present paper showing the internal looping of MITF with other target genes of retinoids to cause differentiation in melanoma cells. The paper presents a novel model of internal looping of genes in double ring resonator form fabricated in MATLAB2008. It is interesting to note that a strong coupling factor of MITF with RA dependent genes reduces proliferation and a weak coupling between these genes increases proliferation, thus reflecting the dual role of the same protein.

Key words: Microphthalmia-associated transcription factor, retinoic acid, homeostasis, ring resonator, Simulink.

INTRODUCTION

Melanoma is the most dangerous type of skin cancer and is the major cause of death in skin disease. It involves cells called melanocytes, which produce skin pigment called melanin, which in turn, is responsible for skin and hair color. Although it is less common than other type of skin cancers, it can spread very quickly, and is leading cause of death among the victims. Retinoids, a group of chemically related molecules derived from vitamin A, have been very effective in treatment of many types of cancer, including melanoma and regulate a large number of biological processes in development, cell growth, differentiation and homeostasis (Watabe et al., 2002).. Retinoic acid (RA) has capability of inhibiting cell growth in both normal and malignant cells by inducing growth arrest in G0/G1 phase of cell cycle and inducing apoptosis. RA induces differentiation and growth arrest by activating RA receptors and incorporating many target genes. One of the most important genes induced by retinoic acid and involved in cell cycle progression and melanin synthesis is microphthalmia-associated transcription factor (MITF). The impact of RA dependent genes and MITF play a crucial role in anti-proliferative role of retinoids in melanoma (Krauss, 2004). Other genes involved in this anti-proliferative role of RA mediated MITF are retinoblastoma protein (pRb), cyclin dependent kinase inhibitor p21, p16, BRAF, ATF-2 and many more. Microphthalmia-associated transcription factor (MITF) mRNA level was induced by retinoic acid in murine cells within 2 to 4 days causing inhibitory effect on proliferation (Chan, 2007; Watabe et al., 2002). Treatment of mouse melanoma cells by ATRA resulted in 20 and 40% reduction in c-Myc protein expression after 2 and 4 days, respectively (Chan, 2007). Mitf silencing-

*Corresponding author. E-mail: mrssgupta1@rediffmail.com, sobhagupta@cemk.ac.in.

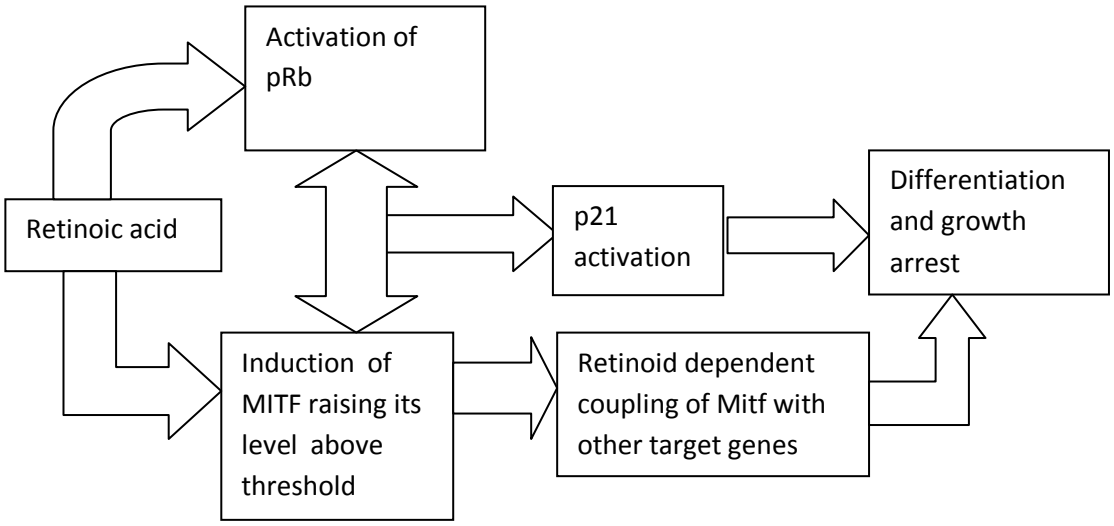

Figure 1. Block diagram representation of the proposed model.

induced senescence is not mediated by c-Myc down regulation because Mitf silencing increases p53 and promotes G1 arrest without affecting level of c-Myc (Giuliano et al., 2010). This indicated that reduction in c-Myc expression after RA treatment may be mediated by Mitf and other genes associated with it. Cyclin D1 protein expression was reduced by 40% after 4 days of ATRA treatment in melanoma cells (Chan, 2007). The decrease in cyclin D1 is associated with accumulation of hypophosphorylated forms of retinoblastoma protein. In hypophosphorylated state, retinoblastoma protein is active and carries out its tumor suppressive activity by suppressing genes needed for progression, thus inhibiting cell cycle progression (Spinella et al., 1999). Mitf can act as an anti-proliferative agent by activation of $p21^{Cip1}$, CDKN1A cyclin dependent kinase inhibitor gene (Carreira et al., 2005). Mitf interacts with pRb and this cooperation increases the ability of Mitf to activate p21 (Carreira et al., 2005; Kuzel and Chien, 2011). p21 is a universal inhibitor of cyclin kinases which controls the cell cycle progression in G1 and S phase. Significantly, $p16^{INK4}$ expression in melanocytes is associated with increased level of MITF. p16, along with p14, functions as a tumor suppressor (Kuzel and Chien, 2011). Mitf expression is suppressed by B-RAF in immortalized mouse and human melanocytes. MITF re-expression in BRAF transformed melanocytes inhibits their proliferation (Wellbrock and Marais, 2005). Moreover, the mutations of genes like BRAF could affect either the stability of MITF or its cooperation with pRb (Carreira et al., 2005). ATRA has been shown to suppress BRAF/ERK signaling in mouse skin cancer model (Cheepala et al., 2009). Transcriptionally inactive ATF2 inhibits melanoma deveopment and progression (Shah et al., 2010). B16 melanoma cells have much higher levels of phosphorylated (active) ATF-2 than immortalized melanocytes. It has been shown that RA decreases ATF-2 phosphorylation in B16 melanoma cells. This decrease in activity of ATF-2 by RA is mediated in a dose and time dependent manner (Huang et al., 2008).

MODEL DOMAIN

The proposed model

Retinoids have been effective in treatment of melanoma cell lines having low as well as high metastatic ability (Chan, 2007). The effect of retinoids and some of their associated genes has been described previously. This paper presents a novel model of microphthalmia-associated transcription factor based differentiation of melanoma cell lines. The role of MITF in proliferation and differentiation is dependent on the input signals associated in the biofeedback system (Hoek and Goding, 2010). The four layered model proposed by Suzanne (Carreira et al., 2005; Giuliano et al., 2010) reveals that higher MITF level is associated with differentiation of cells in melanoma. In the present model, retinoid is the main input stimulus which initiates the signals responsible for anti-proliferative activity of network of genes (proteins). The complete process of retinoid dependent induction and hence increased level of MITF is dependent on its coupling with other retinoid dependent genes and their consequent effect on other genes. Hence, retinoids function on the basis of single to multiple internal loops. This is relevant because the effect of retinoids is both, time dependent and dose dependent (Chan, 2007; Bejar et al., 2003; Watabe et al., 2002; Huang et al., 2008).

The block diagram of the proposed model is shown in the Figure 1. This model has been fabricated by digital signal processing technique looking into the fact that cell-

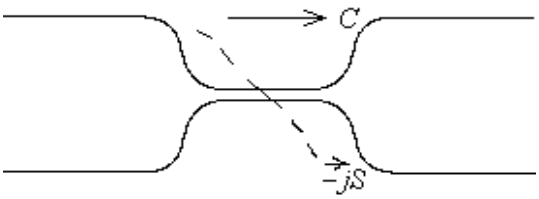

Figure 2. Schematic diagram of a directional optical coupler.

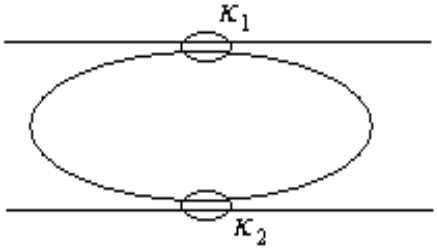

Figure 3. Directional optical couplers forming a simple ring resonator.

to-cell communication in epithelial layers could be modeled in discrete form via autocrine and paracrine signaling and that the discrete model takes into consideration each cell individually (Gupta et al., 2010; Pribyl et al., 2003).

Description of a ring resonator

A ring resonator is a fiber optical coupler whose main characteristic is its frequency response, which describes the variation of the magnitude and phase angles with frequency. It works as an optical filter consisting of a waveguide in a closed loop to one or more input and output waveguides. It works on the principle of interference where the signal of the coupled waveguide could build up in intensity and resonate with the specific signal. The transfer function of this ring resonator is taken in Z domain and is determined with the help of delay line digital signal processing technique and Mason's rule.

Figure 2 is the schematic diagram of an optical coupler, which is formed when two waveguides are brought close to each other for overlapping their evanescent fields. A power coupling ratio, k, is associated with each directional coupler. For an input on one port, the power coupled to the cross port is k times the input power. Two directional optical couplers form a simple ring resonator as shown in Figure 3 (Madsen and Zhao, 1999).

In the directional optical coupler, the optical signals are split and are recombined after a time delay. The frequency response of ring resonators is usually periodic in nature and this one period is known as free spectral

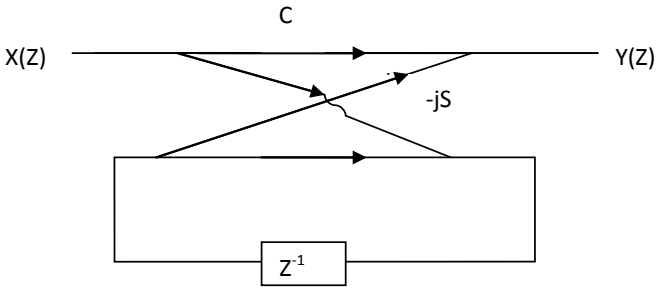

Figure 4. The Z-transform schematics of a single ring resonator.

range (FSR). The output of a directional coupler could be represented in terms of input as:

$Eo^1=q(CEi^1-jS\ Ei^2)$
$Eo^2=q(-jSEi^1+CEi^2)$

where Ei^1 and Ei^2 represent coupler input, 'q' is amplitude transmission coefficient of the coupler, C and –jS are through port and cross port transmission respectively, Eo^1 and Eo^2 are coupler outputs. The through port transmission is given by:

$C=\cos\theta=\sqrt{(1-k)}$

and cross transmission is given by

$-jS=-j\sin\theta = -j\sqrt{k}$

where, 'k' is the power coupling ratio of the coupler which is assumed to be independent of wavelength and 'θ' is equal to the coupling strength integrated over the coupling length (Madsen and Zhao,1999). The directional optical coupler forming a ring resonator is shown in Figure 2. The Z-transform schematics of a single ring resonator are shown in Figure 4.

For digital signal processing technique, a continuous signal is sampled at equal interval of time, $t = aT$, where a is the sample number and T is the unit delay which is the smallest optical path length

The transfer function, in Z domain, of an optical fiber single ring resonator is given as:

$$\frac{Y(Z)}{X(Z)} = \frac{C-\gamma Z^{-1}}{1-\gamma C Z^{-1}}$$

where γ = exp(-αL), is the round trip loss of the ring, α is the average ring loss per unit length and L is the ring perimeter. Z^{-1} is known as unit delay in Z-domain and the whole ring perimeter represents unit delay length for single ring resonator (Gupta et al., 2010; Mandal et al., 2007).

Same rule is applicable for determining overall transmittance (transfer function) of a double ring resonator.

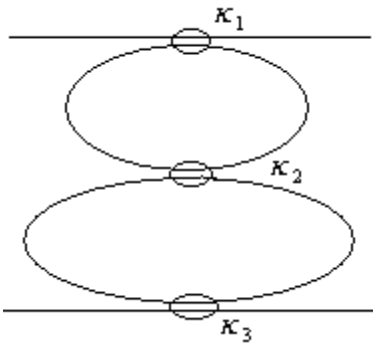

Figure 5. Directional optical couplers forming double ring resonator**.**

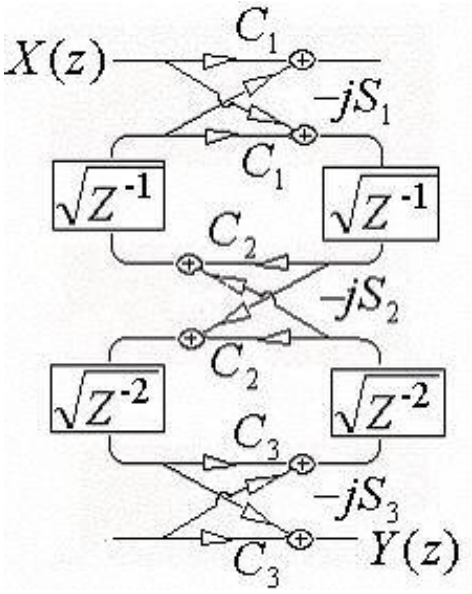

Figure 6. The Z-transform schematics of a double ring resonator.

Schematic of a double ring resonator and its Z-transform representation is shown in Figures 5 and 6, respectively. The transfer function, in Z domain, of an optical fiber double ring resonator is given as:

$$T_f = \frac{j\sqrt{(\gamma\ 1\ \gamma 2 k1 k2 k3)}\ \sqrt{z^{-3}}}{(1 - C1C2\gamma 1 z^{-1} - C2C3\ \gamma 2 z^{-2} + C1C3\ \gamma 1\ \gamma 2 z^{-3})}$$

where γ1 and γ2 are the two ring losses.

The denominator part of this function could be represented in the form of

$$Den = 1 - a1z^{-1} - a2z^{-2} + a3z^{-3}$$

where a1=c1c2γ1, a2=c2c3γ2, and a3=c1c3γ1γ2.

MODELLING AND SIMULATION

A homeostatic biofeedback model with retinoid as input stimulus comprising of brain and endocrine regulator and their consequent transduction phases are shown in Figure 7 which has been realized by MATLAB tool.

The input stimulus (retinoid in this case) is taken as a step function because any other form of input could be simplified in the form of step function (Gupta et al., 2010; Basak et al., 2005). The transfer function of homeostat1 (MITF homeostat) and 2(p21) are taken as first order systems for the sake of simplicity, although it could be a higher order term. The per unit scale values signify normalization of the curve to correlate a particular physiological phenomenon. The output has been simulated in MATLAB (7.6, R2008a) SIMULINK. Different time constants for rising and decaying phases are considered for simulation within a time interval of 4 days. Per unit values are taken as maximum expression level of genes. The exponentially varying curves are obtained after implementing the second and first order transfer functions and the double ring resonator effect to the input signal. The transfer function of MITF homeostat is taken as:

$$G1(s) = 5/(s+2)$$

and transfer function of corresponding transduction phase is taken as

$$H1(s) = 0.2/(s^2 + s + 1)$$

such that the overall transfer function becomes

$$T1(s) = G1(s)/(1+G1(s)H1(s)) = 5(s^2+s+1)/(s^3+3s^2+3s+2)$$

The output of modified MITF homeostat is shown in the Figures 7a and 8a. This output is associated with activity of ATF2 as shown in Figures 7b and 8b. The input is governed by brain and endocrine regulator, which are responsible for directing the signaling pathways. RA also activates pRb and consequently other dependent genes. The homeostat2 (p21) has transfer function of G1(s) = 5/(s+5) and transfer function of corresponding transduction phase is taken as

$$H1(s) = 0.1/(s^2 + s + 1),$$

such that the overall transfer function becomes

$$T1(s) = G1(s)/(1+G1(s)H1(s)) = 5(s^2+s+1)/(s^3+6s^2+6s+5.5)$$

The output of modified p21 homeostat is given in Figures 7c and 8c. Homeostat3 acts in a ring resonator form, where the transduction phase is accommodated in the loop itself. The transfer function of this resonator had

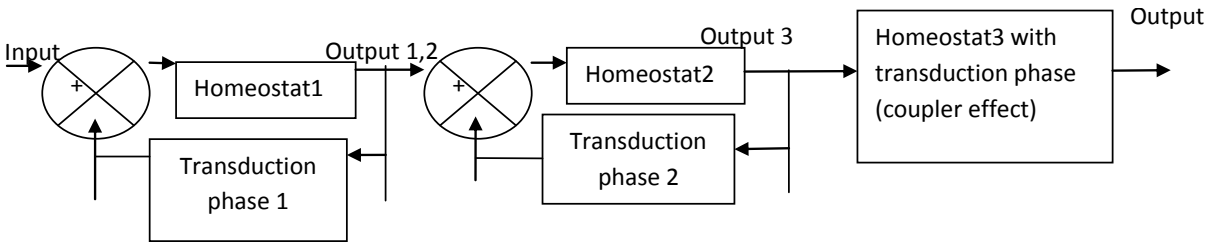

Figure 7. Homeostatic model of retinoid mediated dependent growth arrest.

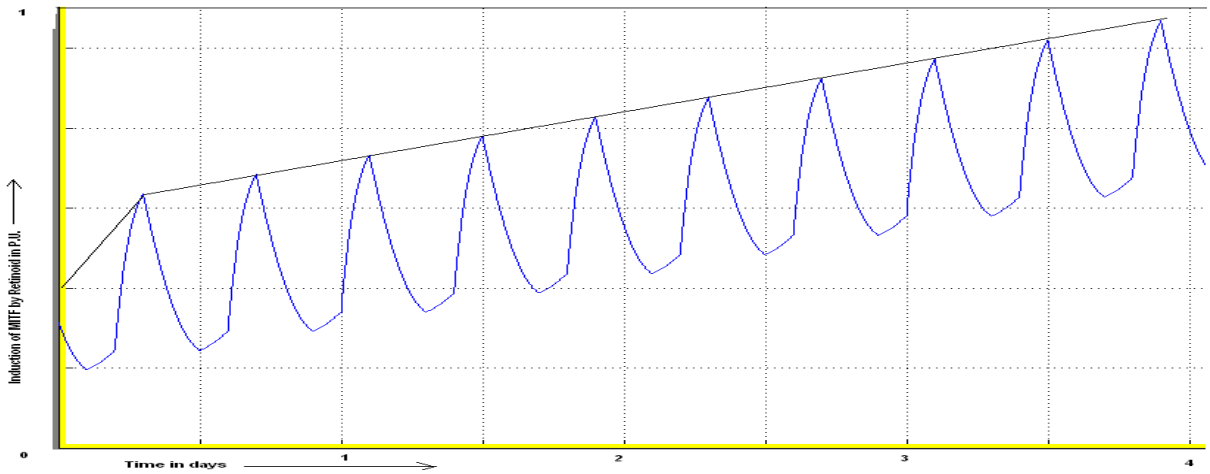

Figure 7a. Induction of MITF by retinoid with k1=k2=k3=0.9 and γ1=γ2=0.01.

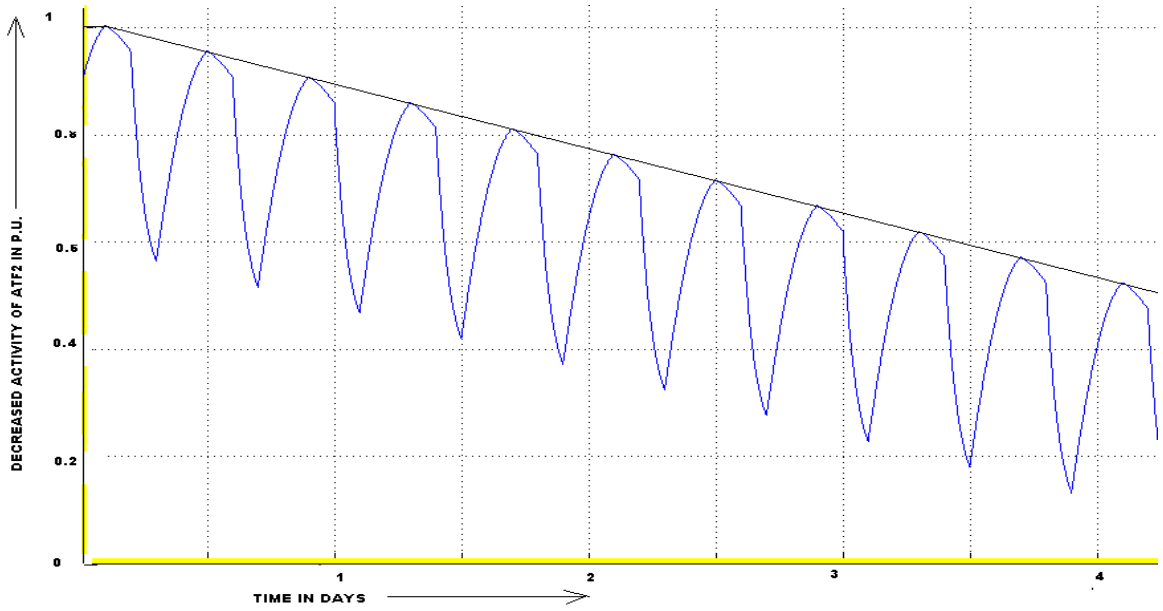

Figure 7b. Reduced activity of ATF2 due to retinoid with k1=k2=k3=0.9 and γ1=γ2=0.01.

been given previously. The through port transmission is given by C=cosθ = √(1-k), where 'k' is the coupling ratio. The decrease or increase in melanoma progression with respect to the coupling factor is shown in Figures 7d and

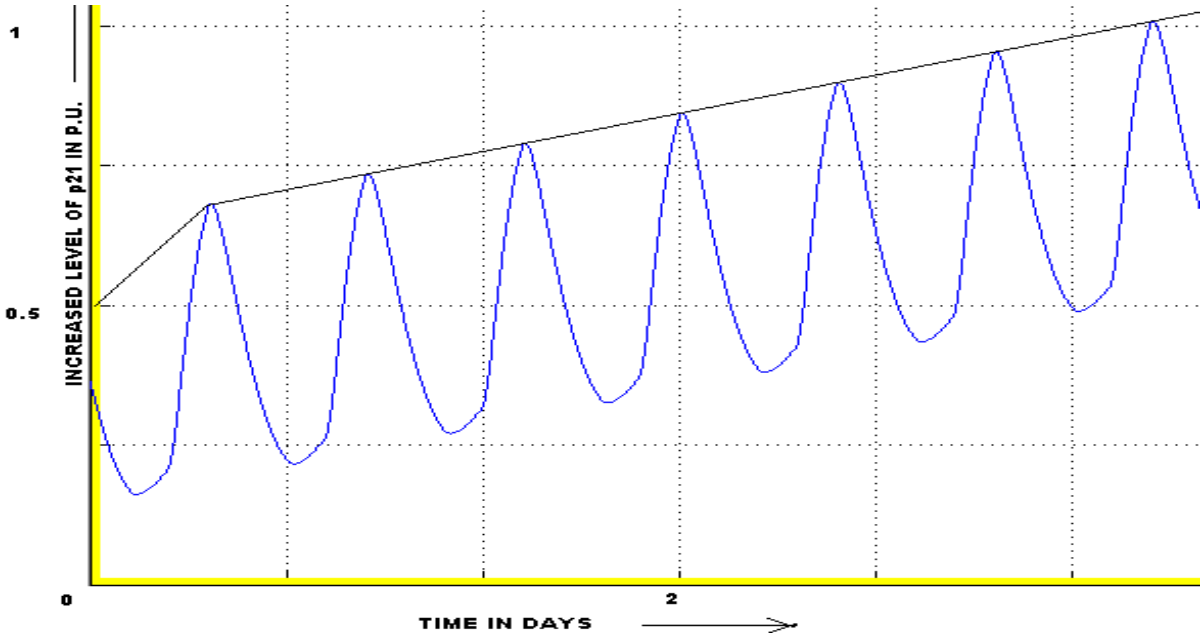

Figure 7c. Increased activity of p21 due to combined effect of MITF and retinoid.

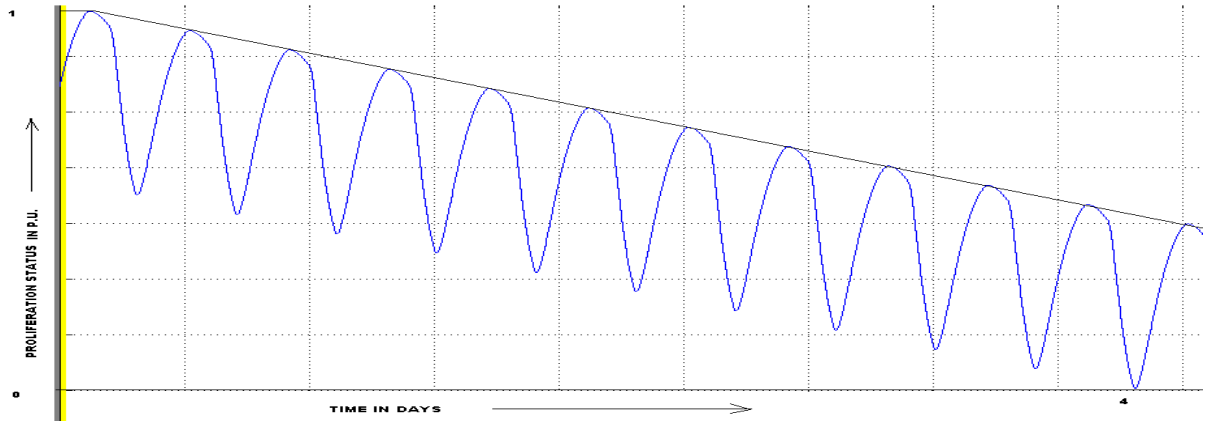

Figure 7d. Decreased proliferation due to coupling of MITF with retinoid dependent genes with k1=k2=k3=0.9 and γ1=γ2=0.01.

8d, respectively. The coupling ratio between retinoid or retinoid dependent gene/s and MITF is taken as k1, between retinoid induced MITF and its direct target gene/s is taken as k2 and k3, which is responsible for its altered signaling pathway. It is note worthy that the values of this coupling ratio alter the output significantly. The induction of MITF with coupling ratio k1=k2=k3=0.9 and loss component of γ1=γ2=0.01, raising its level above threshold by retinoid is shown in 7a. The corresponding reduced activity of ATF2 due to RA is shown in 7b. The consequently increased level of p21 is revealed in 7c (which increases further due to combined effort of MITF with pRb). The double ring resonator effect is shown in Figure 7d, where it causes anti-proliferative effect in melanoma cells.

The level of MITF below threshold with coupling ratio k1=k3=0.4 and k2=0.1 and loss component of γ1=0.4 and γ2=0.9 is shown in Figure 8a. The corresponding increased activity of ATF2 is shown in Figure 8b. The consequently decreased level of p21 is revealed in Figure 8c (which may be due to lack of cooperation between MITF and pRb) The double ring resonator effect is shown in Figure 8d, where it causes proliferative effect in melanoma cells. These two different roles of MITF has concurrence with the rheostat model of Carreira et al. (Suzanne et al., 2005; Sandy et al., 2010; Keith, 2010). It It could be shown from the same model that MITF silencing also results in decreased proliferation rate and

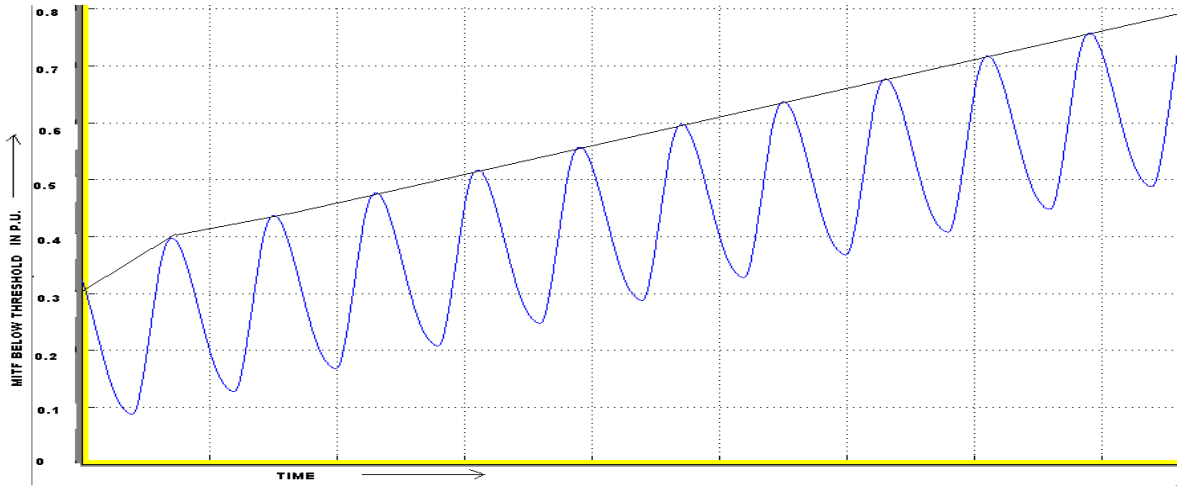

Figure 8a. Increased activity of MITF below threshold with k1=k3=0.4 and k2=0.1.

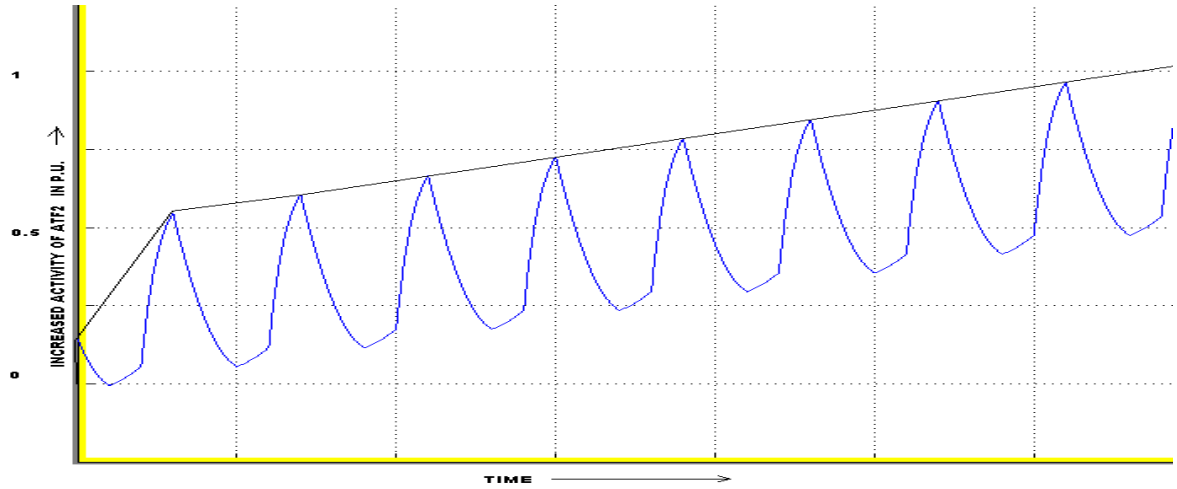

Figure 8b. Increased activity of ATF2 with k1=k3=0.4 and k2=0.1.

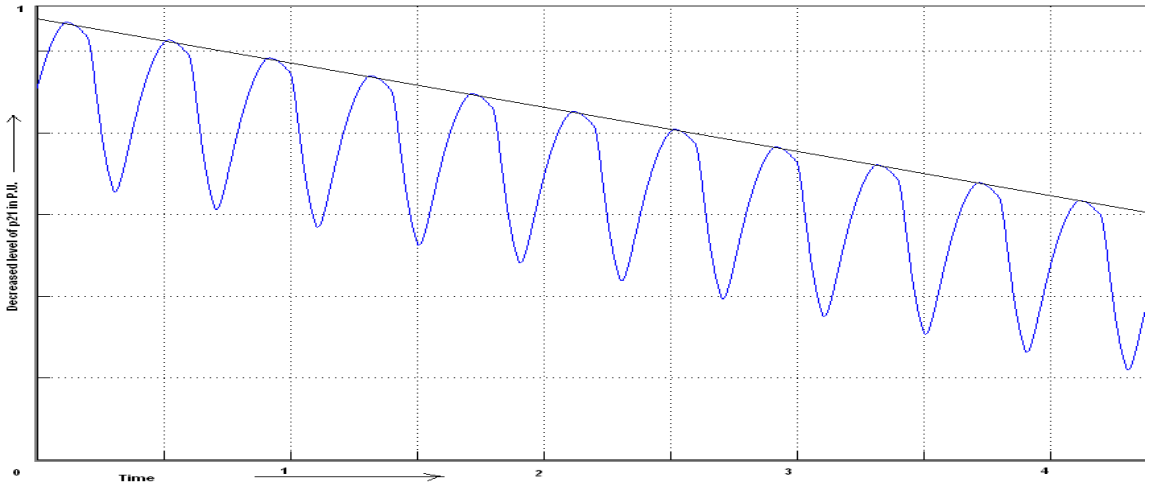

Figure 8c. Decreased activity of p21 with k1=k3=0.4 and k2=0.1.

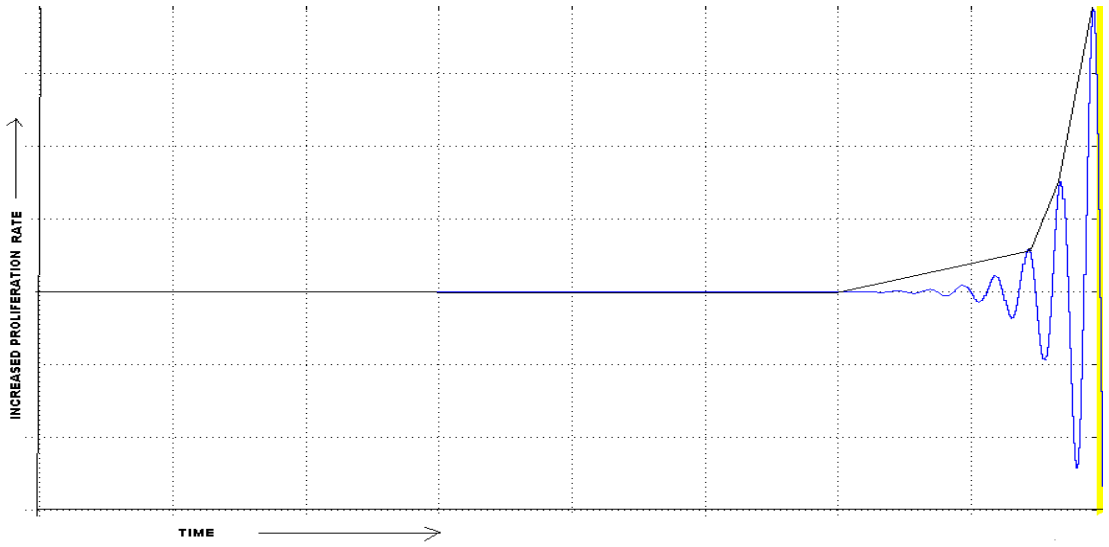

Figure 8d. Increased proliferation with k1=k3=0.4 and k2=0.1.

growth arrest.

RESULTS

From the output obtained by this model, it has been shown that the levels and activity of MITF are key regulators of melanoma cell differentiation and metastatic proliferation. It is note worthy that the values of these coupling ratios alter the output significantly. It is interesting to note that coupling ratio of 0.9 each and loss component of 0.01 each, results in a decreased progression of melanoma. This is shown in Figure 7d. The induction of MITF raising its level above threshold by retinoid is shown in Figure 7a. The activity of pRb has a simultaneous effect. The reduced activity of ATF2 due to RA is shown in in Figure 7b. The increased level of p21 is revealed in 7c. The double ring resonator effect is shown in Figure 7d, where it causes anti-proliferative effect in melanoma cells. Similarly coupling ratio of k1=k3=0.4 and k2=0.1 and loss component of 0.4 and 0.9 results in increased melanoma progression as shown in Figure 8a to d.

DISCUSSION

The present model has focused on the two contradictory roles of MITF concomitant with retinoid therapy on melanoma cell lines. It has also shown the possible pathway of cell differentiation and growth arrest by retinoid in melanoma and other types of cancer. A complex biological effect of retinoid on various tumor responsive genes and MITF has been simplified as double ring resonator effect, where the internal loops are formed, resulting in altered target genes. The induction of MITF and other tumor responsive genes concomitant with retinoid are responsible for activating or deactivating specific genes which take important part in tumor genesis and are responsible for proliferative and anti-proliferative effect. The combined effort of retinoid dependent genes and MITF is in a complex coupler form, wherein the coupling ratios of these genes are closely associated. Though, MITF acts as a key regulator in decreasing the progression of melanoma, its target are dependent on retinoids and retinoid activated genes in RA therapy. Here two factors are of great importance. Firstly, it is important that the level of MITF has to be above the threshold level. And secondly, the coupling of MITF with retinoid dependent genes should be strong. The coupling ratios of k1=k2=k3=0.9 and a negligible loss component of $\gamma1=\gamma2=0.01$ results in a decreased proliferation. It could be shown that the weak coupling ratios between retinoid dependent genes and MITF, alters the signaling pathway of MITF target genes and consequently increase growth rate. Taking the coupling ratio k1=k3=0.4 and k2=0.1 and more loss component of 0.5 and 0.9, the proliferation rate increases. It is shown in Figures 8a to d that increased level of MITF below threshold and consequent weak coupling between retinoid dependent genes result in increased proliferation. This has been earlier proposed by Suzanne et al. (2005) as a rheostat model of MITF.

CONCLUSION AND FUTURE ENHANCEMENT

The role of retinoid mediated MITF on melanoma cell lines is target gene specific and depends largely on the increased level of MITF expression. The two extreme MITF

MITF levels cause differentiation or apoptosis. Both, very high level of MITF with retinoid coupled genes and MITF silencing, need specific signaling pathways. The four layered model of MITF activity could be refined and hypothesized by a five layered model, where the middle three layers have proliferative action or invasiveness. The upper middle layer and the lower middle layer are highly resistant to the changes and hence hold the prior level of this gene. Hence, the upper middle layer need some other genes also to cross the threshold level and reach the safe level to induce differentiation and growth arrest. These genes are mostly retinoid dependent in a subject. The resistance to retinoid therapy is largely based on the effective structure and activity of MITF with other oncogenes in the upper middle band. The role of retinoid mediated MITF could be explicitly understood by developing antigens to overcome the resistance for retinoid in melanoma cell lines. This approach could give a new direction to the treatment of melanoma and possibly other types of cancer.

ACKNOWLEDGEMENT

The authors are grateful to the Almighty and their respective working places.

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 87-89, April 2012
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS12.019
ISSN 2006-9723 ©2012 Academic Journals

Case Report

Erythromelalgia in a patient treated with penicillin

Lora-Cabral A. and Pardos-Gea J.

Internal Medicine Department, Vall Hebrón Hospital, Barcelona, España.

Accepted 25 March, 2012

Erythromelalgia is a rare neurovascular peripheral disorder of unknown cause that affects hands or feet causing painful skin redness in affected area. The attacks are periodic and are commonly triggered by heat, pressure, mild activity, exertion, insomnia or stress. Erythromelalgia can occur either as a primary or idiopathic disorder, or as secondary forms associated with hematologic diseases. Drugs have been implicated in several skin disorders but only punctual single cases of erythromelalgia have been related to drug therapies. Here we describe two cases of erythromelalgia associated with penicillin treatment, a new non-described association.

Key words: Erythromelalgia, penicillin, drug.

INTRODUCTION

Erythromelalgia is a rare disease, with 1 case per 100,000 diagnosed per year, a median age of onset of 40 to 50 years and a female predominance (Davis et al., 2000). It presents itself as burning pain, paresthesia and redness of distal portion of extremities and less frequently neck, face, ears, nose and genitals (Prevost and English, 2007). Diagnosis is based on the clinical aspect. Provoking an attack by placing affected area in hot water for 10 to 20 min, or the dramatic relief of symptoms with the administration of aspirin can help. There is no specific treatment for erythromelalgia. It is recommended that the patient avoid those factors which can aggravate the symptoms, especially heat. Aspirin, gabapentin (Ceyhan et al., 2010), beta-blockers have been used, with positive benefits towards symptoms. Studies have shown that the use of oxcarbazepine (Skali et al., 2009) has had good results in some of the patients resistant to other treatments.

The etiology of erythromelalgia is not well understood and consequently it is often a diagnosis of exclusion. Erythromelalgia is clinically classified into primary and secondary subtypes. Primary form is idiopathic and thought to be due to a genetic susceptibility on chromosome 2 that results in a mutation of the gene that codes for voltage gated sodium channels (Drenth et al., 2001; Yang et al., 2004; Cheng et al., 2011). A 10 to 15% of cases of erythromelagia occur as a result of mutations of this gene that leads to a dysfunction of vasomotor regulation and results in a shunting process (Dib-Hajj et al., 2007).

Symptoms of primary erythromelalgia usually present early in life, are symmetric, and alleviated by cold. Secondary erythromelalgia can develop associated with underlying medical condition, most commonly myeloproliferative disorders (polycythaemia vera, essential thrombocythaemia), neurological and autoimmune diseases. Drugs such as antibiotics have been implicated in several skin diseases, mainly in form of allergic reactions, with scanty cases associated with erythromelalgia. Here we describe two cases of erythromelalgia associated with penicillin therapy.

CASE PRESENTATION

First case was a 54-year-old bar owner whose symptoms started 3 years ago during treatment with penicillin benzathine (2.4 million units) on a weekly dosage for latent syphilis. In week three, coinciding with the last dosage, patient began with a burning and painful sensation in distal extremities. Upon examination redness erythema in his feet, neck and hands were present (Figure 1). Exacerbations of symptoms occurred without set timeframes and/or schedule. The rest of the physical examination was normal. Blood tests did not show any

*Correspondingauthor. E-mail: jpardosgea@yahoo.es. Tel: 34 93 2746100. Fax: 34 93 2746882.

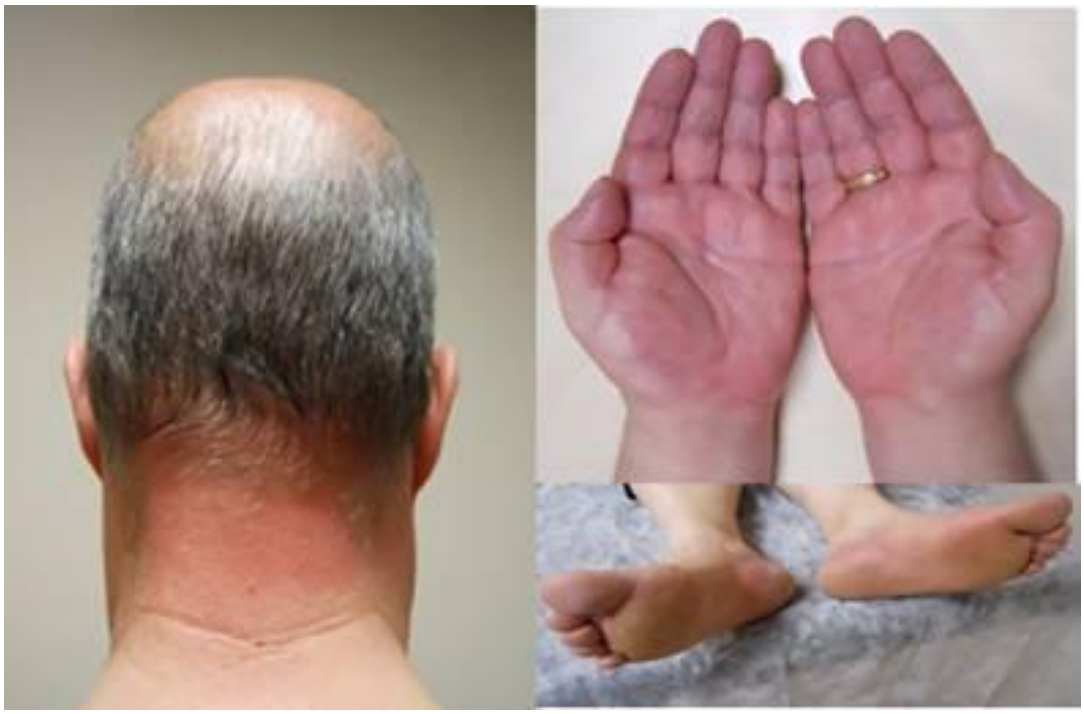

Figure 1. Erythromelalgia affecting neck, hands and feet.

alteration and electromyography was normal. After 6 months of penicillin treatment, non-treponemal tests (RPR, VDRL) were negative considering syphilis cured.

Based on the signs and symptoms, the patient was diagnosed of erythromelalgia. Treatment started with an aspirin intake of 500 mg every 8 h as well as gabapentin 300 mg on daily basis. Patient was revisited in consult where he explained partial improvement, so gabapentin treatment was incremented beginning with capsaicin cream 2 to 3 times a day and amytriptyline 50 mg a day. After three months of treatment, patient noted the disappearance of all clinical features of erytromelalgia, which is actually asymptomatic under therapy.

The second patient was a 38-year-old truck driver, a smoker with moderate alcohol intake for more than twenty years. Symptoms started two years ago while patient was receiving treatment with penicillin, 4 million units every 4 h, for cerebral abscesses secondary to oral cavity infection. Painful redness in lower extremities and hands started after 8 weeks of antibiotic. Patient was diagnosed with erythromelalgia and studied for underlining causes, with negative results. Penicillin regimen was changed conveniently to another antibiotic in order to finish treatment for brain abscesses. Patient was then started with aspirin 1 g every 8 h, propranolol 20 mg every 12 h, sertraline 80 mg a day and gabapentine 600 mg every 12 h. Relief of symptoms was achieved in one month. Patient is controlled through consult on a yearly basis; he is currently asymptomatic without treatment.

Conclusion

Erythromelalgia associated with pharmacological treatments has been reported in few publications. We found single cases of patients under several drugs as nifedipine, verapamil (Drenth et al., 2001), bromocriptin, clonazepam, rosuvastatine that developed erythromelalgia (Sunahara et al., 1996; Nanayakkara et al., 2007; Kraus, 1990; Cimolai and Cimolai, 2009). The appearance of the disease in the aforementioned cases was temporarily associated with the introduction of drugs. That aspect is essential in order to relate disease onset with pharmacological treatments, mainly because of the lack of objective test to confirm those associations. The patients we describe were under penicillin treatment and not taking any other medications. When penicillin was discontinued, symptoms of erythromelalgia still continued for several weeks till disappearance unless symptomatic treatment. We then suggest a possible triggering role of penicillin for erythromelalgia, a circumstance not previously described. The underlying pathophysiologic mechanism would be unknown. Regardless, the putative relationship between penicillin treatment and the development of erythromelalgia is inferential, not clearly proved, so our description should be taken cautiously.

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 90-96, April 2012
Available online at http://www.academicjournals.org/IJMMS
ISSN 2006-9723 ©2012 Academic Journals

Full Length Research Paper

Chronic care model for diabetics by pharmacist home health in Bangkok Metropolitan: A community based study

Sirirat Tunpichart[1]*, Rungpetch Sakulbumrungsil[2], Ratana Somrongthong[1] and Duangtip Hongsamoot[3]

[1]College of Public Health Sciences, Chulalongkorn University, Thailand.
[2]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.
[3]Nation Health Security Offices, Bangkok, Thailand.

Accepted 2 April, 2012

Diabetes was increased in Thailand with increasing burden of morbidity and mortality. There were 42.8% of diabetes patients in Bangkok who had been treated, but the disease conditions were uncontrolled. Diabetes with drug related problems (DRPs) frequently occurred, leading to problems of uncontrolled disease conditions. The objective of this study was to apply chronic care model (CCM) which has been introduced using medication therapy management (MTM) services by community pharmacist home health care and monitor patients' drug utilization in diabetic patients at home. An action research was conducted in the community in Bangkok Metropolitan. The uncontrolled diabetes conditions were purposively selected and identified by nurse home care team. The community pharmacists provided the MTM service 3 times as the delivery service design template that was planned over the 6-month period. The study implemented on CCM with MTM services as the main delivery system. The outcomes were evaluated on three aspect of ECHO model. Data were gathered for 288 uncontrolled diabetic patients with high prevalence of drug related problems. The number of drug were taking mean standard deviation (SD) 7.1 (3.1) per patient at enrollment. The 2.98 number problems per patient and 95.8% non-adherence were identified by community pharmacist. After 3 interventions, non-adherent patients' state was changed to adherent medication level and partially medication adherent level by 18.2 and 26.0%, respectively. The pharmacists identified problems and improved in safety issues (adverse drug reactions, drug interactions), adherence issue and effectiveness issue (sub-therapeutic dosage). The clinical outcome found the average systolic and diastolic blood pressures to improve significantly in 48.6% patients with hypertension including those in pre-hypertension, stage I and stage II. The data was limited and results showed that the fasting plasma glucose (FPG) was not significantly reduced from baseline due to lack of linkage among hospital and community settings. The non-compliance issue had an effect on excessive medications per patient on the average of $543.24 per year. This study concluded that implementation MTM service through CCM by community pharmacist home health care could alleviate patients' medication utilization problems and would thus improve overall quality of patient care.

Key words: Chronic care model (CCM), drug related problems (DRPs), medication adherence, home health care, medication therapy management (MTM).

INTRODUCTION

In Thailand, diabetes is a common chronic disease with increasing burdens as the prevalence had risen to 6.9% in 2009. It was found out that 42.8% of patients in Bangkok were unable to control the disease condition (Aekplakorn, 2009). The co-morbidities and diabetes-related complications were associated with an increase in health care costs and hospitalization. The fundamental

*Corresponding author. E-mail: s_tunpichart@yahoo.com. Tel: 662 538-4906.

role of the diabetes management team by multi-disciplinary professionals is the development of a model for continuity of care and services for diabetes (McGill and Felton, 2007). The chronic care model (CCM) was a guide to higher-quality chronic illness management that brought new conceptual frameworks and innovations for redesigning the service platform and structure of the healthcare setting (Bodenheimer and Grumbach, 2007). The CCM strives to foster more productive interactions between prepared, proactive practice teams and well-informed, motivated patient by delivery system design involves diabetes care visit (Wagner et al., 2001). A good illustration of this new service concept was pharmacist home health care service for elderly taking polypharmacy and those with poor cognition had improved their medication adherence within a week after being monitored (Stewart et al., 1988; Osterberg and Blaschke, 2005).

Drug related problems (DRPs) were frequently found among patients discharged from hospitals and could potentially interfere desired health outcomes (Hepler and Strand, 1990). Typically, the control of hyperglycemia required multidrug regimens, associated with an increase risk of adverse drug events (Hanlon et al., 1996; Grant et al., 2003; Chrischilles et al., 1992). The medication therapy management (MTM) service was driven by the philosophy of pharmaceutical care, which was viewed as a comprehensive framework for patient care service focusing on drug use monitoring (American Pharmacists Association and the National Association of Chain Drug Stores Foundation, 2008). Pharmacists had used MTM as a strategy to reduce drug related problems from polypharmacy (Viktil et al., 2006; Christensen et al., 2007). The MTM program could improve medical adherence and lead to a reduction in the overall health care expenditures by optimizing therapeutic outcomes, especially in elderly patients. In Thailand, the CCM for diabetes was mainly delivered in secondary and tertiary hospital settings. The role of primary health care settings, especially community pharmacies, in continually monitoring and managing patients' chronic medications was limited. The pharmacist home health care service was initiated as a mechanism to ensure the continuity of care for patients. Improvement of community and home-based diabetes care programs was needed to strengthen the service of home health care (Katekaew, 2005; Debavalya and Moolasarn, 2008). Therefore, this study integrated the MTM service into pharmacist home health care as the delivery care element for CCM. The proven effectiveness of this model would not only reduce drug related problems and improve diabetes patients' outcomes, but also reinforce the drug cost containment through the decrease of medication utilization and the optimization of therapeutic outcomes.

STUDY DESIGN

The study period was during May 2009 to July 2010. It was an action research with one group before-and-after design. A total of 34 communities in 5 community health centers were purposively selected as the study areas. The sample of 288 chronic patients identified by nurses from the community health centers as having uncontrolled diabetic conditions were referred to community pharmacies for home health care visits to periodically monitor patients' drug utilization. The pharmacist providing home health care intervention followed 5 components of MTM services, including the medication therapy reviews, a personal medication record, a medication action plan, intervention and referral, documentation and follow-up for problems solving. The framework of MTM services by home health care pharmacists is as shown in Figure 1. Pharmacist provided each MTM services for patient every 2 to 4 weeks for 3 visits and 2 more follow-ups for an outcome assessment during the next 2 months. The time spent in each home health care visit was 20 min for interview as well as medication review among patient and/or caregiver and 40 min on intervention, patient medication record, documentation and referral if needed. This study was designed with the emphasis on the practice level of the CCM with the MTM service as the main delivery system as in practice elements. The implement of CCM was outlined as shown in Table 1.

The outcomes were evaluated on three aspects of ECHO model: economic, clinical and humanistic outcomes (Kozma et al., 1993). During each home health care visit, DRPs were identified and intervened. The drug related problems were classified into categories such as adverse drug reactions, drug interactions observed by the symptoms occurs, over-dosage or under-dosage identified from medication labels, untreated indication, improper drug used and non-adherence evaluated by modified brief medication questionnaire (Svarstad et al., 1999). The economic aspect was assessment in excessive drug cost per patient. The average cost of excessive drug was calculated from actual drug list for prescriber only by review of records and pills count between interval visits.

RESULTS

The baseline demographic characteristics of all patients were collected during the first visit. Out of 288 patients, 81.7% had hypertension as the main co-morbidity and 90.0% had two or more chronic diseases. They were taken on the average (±SD) of 7.1 (±3.0) medications, and 89.3% of patients had 4 or more medications. Some patients dropped out from the project and some relocated during the studied period remaining 236 patients or 81.9% with completed 3 pharmacist visits/interventions. Patients were classified into 3 groups according to adherence levels using the pill count method. Adherent were those with the average of ≥ 80% medication compliance, partially adherent were those between ≥ 60 to <80% and non-adherent were those taking medication less than 60% (Asher-Svanum et al., 2009). Table 2 revealed that the number of patients in non-adherence level was improved to 18.2% and became adherent and 26.0% improved to partially adherent after completion of pharmacist home health care visits. The partially adherent level also improved to adherent in 32.8% of patients.

The pharmacists identified that a total of 858 DRPs issues were detected during the first visit with the mean number of 2.98 DRPs per patient. Majority of the

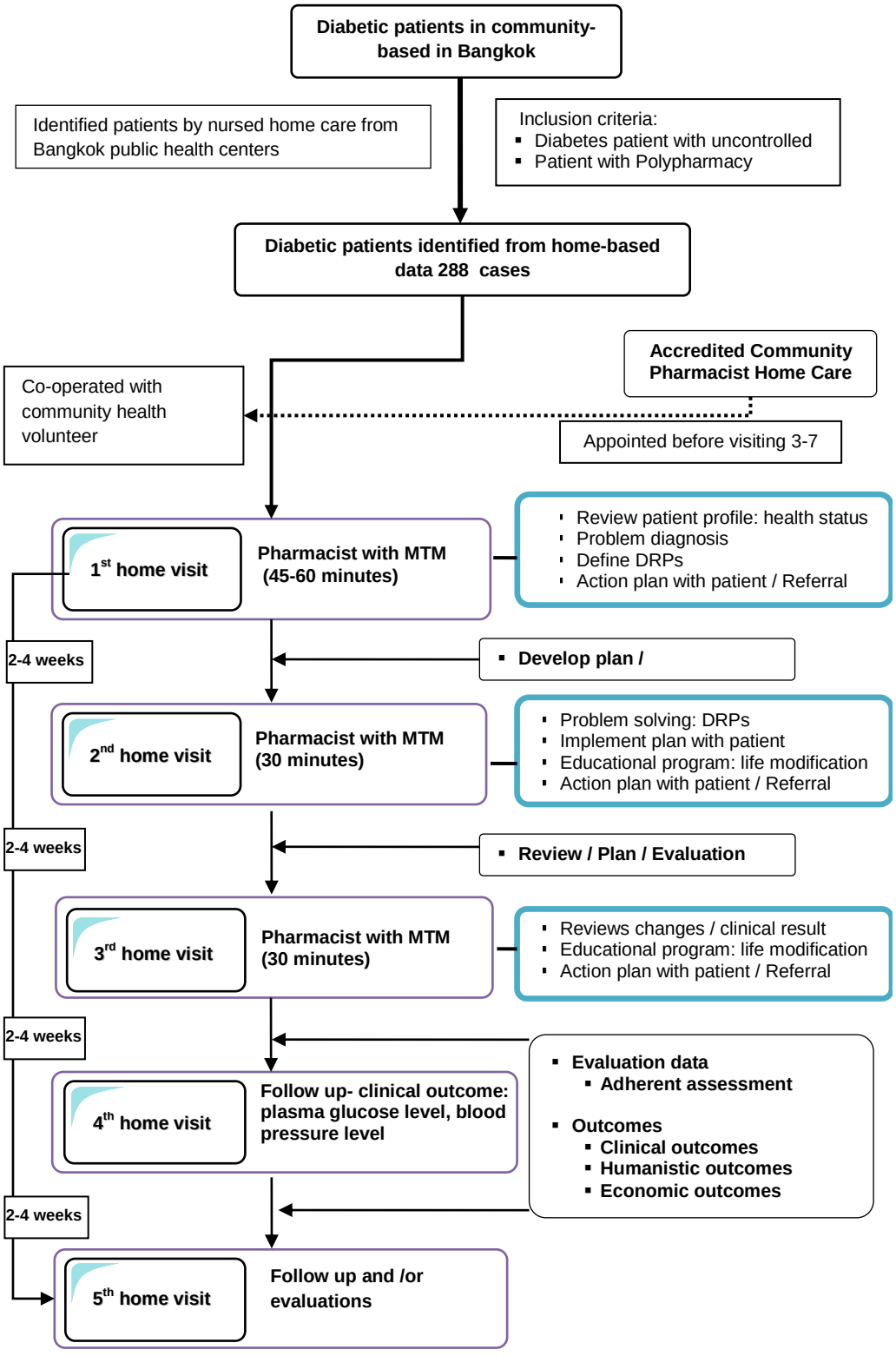

Figure 1. Framework of MTM services by pharmacist home health care.

problems 822 (95.8%) were non-adherence and 21 adverse drug reactions problems. After the third pharmacist home visit, the change of drug related problems were improved in safety issues (adverse drug

Table 1. Implement of CCM for diabetes care.

CCM component	Management and activities
Policy level	
Health System Health care organization	Financial incentives supported from National Health Security Office (NHSO)
Community resources and policies	To encourage patients to participate in effective community program, the following should be done: – Community Pharmacy Association organized and supported
Practice level	
Self-management support	Pharmacist provided the materials and services. – Emphasis on patient empowerment and acquisition of self-management skill. – A personal medication record handbook for patient – Education family/or care giver
Delivery system design	MTM services for each visiting. Pharmacist home health care by 3 times of medication therapy management services:
Decision support	– Specialist expertise team for support about the clinical management – Develop drug related problem guideline – Provide the case/problem based learning program
Clinical information systems	– An application on handheld devices. – An application for registering patients, care givers and pharmacies

Table 2. The change stage of adherent level by pharmacist home health care services.

Adherence level baseline (N[a]=236)	The change stage of adherence level after MTM service at home		
	Adherent (%) (n=66)	Partially adherent (%) (n=70)	Non-adherent (%) (n=100)
Adherent (n=65)	38.5	32.3	29.2
Partially adherent (n=67)	32.8	32.8	34.4
Non-adherent (n=104)	18.2	26.0	55.8

[a]Number of patients who had completely pill counts in 3 visits and exclude error data.

reactions, drug interaction), adherence issue and effectiveness issue (sub-therapeutic dosage). The untreated indication issues found out that there were no changes, whereas pharmacists could detect more problems, as shown in Table 3. The pharmacists provided all patients education or counseling that did not require a physician response. Thirty-four patients (11.8%) were referred to their physicians for immediate actions due to safety issue. The physicians acted 55% of response rates from referral patients. The changes in drug therapy were recommended as stopping, switching medication or dose changes. The clinical outcome found out that the average systolic and diastolic blood pressures showed significant improvement in 48.6% of patients with hypertension including those in pre-hypertension, stage I and stage II. Stage II patients showed decrease in significantly both systolic and diastolic blood pressure levels after intervention. This study had the constraint in acquiring patients' HbA1c test results due to the difficulty in linkage among hospital and community settings, only fasting plasma glucose (FPG) levels before the first visit and after the third visit. Data was limited and results showed that the FPG was not significantly reduced from baseline.

The humanistic outcomes were measured by patient satisfaction using a diabetes specific instrument, modified diabetes quality of life (DCCT Research Group, 1998) during the follow-up visit. The results showed that patients

Table 3. The change of number of DRPs issues after pharmacist home services.

DRPs issues	Number of problems 1st visit	Number of problems 3rd visit	Change of drug-related issue
Number of DRPs per patient	2.98	2.69	-
Adherence			
Non-compliance	822	684	Decreased problems
Indication			
Untreated indication	4	15	Increased problems
Improper drug selection	2	2	Not changed
Invalid indication	1	0	Decreased problems
Safety			
Adverse drug reaction	21	8	Decreased problems
Drug interaction	5	0	Decreased problems
Effectiveness			
Sub-therapeutic dosage	3	0	Decreased problems
Over-dosage	0	1	Not changed
Total of number of problems	858	710	Decreased problems

CCM for diabetics by pharmacist home health in Bongkok Metropolitan: A Community Based Study

during the follow-up visit. The results showed that patients were satisfied with all 3 dimensions in 33 items with Cronbach's Alpha *0.780 (r)* on five-point Likert scale, including life and daily activity (4.485 ± 0.537), 0.870 *(r)*, diabetic disease impact (3.875 ± 1.028), 0.877*(r)* and worries about diabetes (4.019 ± 1.122), 0.933*(r)*. On the economic aspect, the outcomes showed that most patients carried more medications than necessary. The average excessive drug expenditures were $45.27 per patients per month or $543.24 per year. It was noticeable that patients under the Health Universal Coverage has the lowest excessive cost at $12.84 and those paying out-of-pocket had the highest excessive drug cost at $205.90. These excessive drug expenditures were calculated from current drug items by interval visiting prescribed by physicians only.

DISCUSSION

This study found out that the pharmacist home health care provided the MTM services through the chronic care model that improved patient outcomes on clinical, humanistic and economic outcomes. The results show that the pharmacist improve diabetes care by addressing the important issue of adherence to medication, although, this was not explicitly measured in fasting plasma glucose level. The levels of blood plasma glucose and glycated hemoglobin were not recorded and perceived by diabetes patients. The patient data profiles were limited due to lack of linkage between hospital and pharmacy. The medication adherence stages improved by MTM service which identified the problems, planning, medication dose interventions and co-operation with health care professionals. Adherence is complex and is bound up with the need of integration with social life as well as health beliefs. However, pharmacist home care services operated reminder system, consulted the medication management, self-medication record, supplied patient education and facilitated communication between patients and physicians for medication adherence. This continuity of care model between hospitals and community pharmacies initiated in this research was in its early stage; the cooperation between them was for patient clinical outcomes. Thus, only 77 patients had FPG data, showed no significant change. If more data could be obtained, the result could have been more informative. However, patients' blood pressure levels showed significant improvement with stage II. These results correspond to the improvement of patients' adherence. Researcher found out that better patients' adherence was partly due to the impact from personal medication record (PMR), which was used as self-management support for patients. Not only did it serve as a memory recall for patients, but it was also an effective tool for pharmacist to continuously monitor patients' drug utilization. The data linkage between hospitals and community pharmacies will allow the program to render patient medication monitoring to be more effective and efficient. DRPs found a great number of non-compliance

that caused the misunderstanding in medication used, the stop taking drug, health beliefs in herbals, many of drug items and several drug regimen too complex with daily life. Pharmacists helped patient adherent to develop the level of trust in each other to support the cooperation needed for effective drug therapy management. The safety issues were addressed and solved in adverse drug reactions and drug interactions that were acceptant recommended from physicians. The CCM by pharmacist home health care using MTM services as delivery system design in this study enhanced the effectiveness of pharmacists in providing patient care leading to achievement of the therapeutic goals by improving overall health, at the same time it decreased the overall health care system costs. The economic efficiency was increased through reducing excessive and improper medication use, preventing adverse drug events, and other undesirable outcomes. The role of the community pharmacist in primary health care team had proven to be a good linkage between tertiary, secondary and primary care. This research confirmed that community pharmacists could effectively provide diabetic care, reduce drug related problems and improve medication adherence.

Conclusions

This study concluded that redesigning care using implemented CCM through the MTM services by pharmacist home health care was an effective cooperative model for diabetic care management. The findings of this study led to the recommendation that health care providers should integrate MTM services by pharmacists to help improve the quality of patient medication utilization in chronic conditions. The continuity of the institution and home through community pharmacist home health care would benefit diabetic patients therapeutically and economically, leading to improved patient care and better health outcomes.

POLICY RECOMMENDATIONS

The MTM service by community pharmacists should then be valued and recommended as a part of benefit package for patients. The financial incentives supported by the National Health Security Office (NHSO) would strengthen the sustainability of pharmacist home health care services. Patient registration at their selected community pharmacy would allow better continuity of care for all patients and preventive care for their families. This suggested system would endorse the “family pharmacist” concept by community pharmacists to manage family medication and health.

LIMITATIONS

The limitations of this study should be considered. Lack of data linkage between service units impeded the completion on some clinical information. Some of the clinical outcomes as HbA1c or FPG could not be analyzed for all patients.

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 97-102, April 2012
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS12.026
ISSN 2006-9723 ©2012 Academic Journals

Full Length Research Paper

The effect of preconceptional gamma irradiation on the morphometric assessment of adult female mice and the embryo

Innocent A. Edagha[1]*, Matthew T. Shokunbi[2], Adeniyi A. Adenipekun[3], Rosemary B. Bassey[1] and Moses B. Ekong[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Nigeria.
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ibadan, Nigeria.
[3]Department of Radiotherapy, University College Hospital, Ibadan, Nigeria.

Accepted 2 April, 2012

The aim of this work is to study the effect of preconception gamma irradiation on the gross morphometry of the adult female mice and its embryo. 27 mice (18 females and 9 males) were subdivided into 3 groups (control, non-irradiation and radiation) containing 6 females and 3 males mice each in 2:1 ratio. They were anaesthetized with ketamine intraperitoneally for mild unconsciousness. A gamma irradiation dose of 1 Gy/min was delivered to each batch of mice exposed by a cobalt 60, Theratron 780c model, by Atomic Energy of Canada Limited (AECL) at the Radiotherapy Department of the University College Hospital, Ibadan. All the animals were mated 1 week post irradiation. Vaginal plugs were confirmed and the pregnant females were sacrificed on day 14 of gestation by chloroform inhalation. The gross morphology of the female mice and their harvested litters were assessed and statistically analysed. A total of 113 embryos were harvested in all the groups (54 for control, 50 for non-irradiated and 9 for the irradiation group). The gross morphologic assessments of the foetuses were statistically significant ($p < 0.05$) for all the 3 groups compared. These findings suggest that a preconception irradiation affects the morphology of the female mice and its progeny.

Key words: Preconception, irradiation, gamma ray, gross morphology, embryo.

INTRODUCTION

Radiation is an example of a physical mutagenic agent. There are also many chemical agents as well as biological agents (such as viruses) that cause mutations (ECRR, 2010). The effect on the foetus of preconception x-irradiation is less well described. Some human studies showed an increased incidence of chromosomal abnormalities in offsprings of mothers who received preconception diagnostic x-rays (Wald, 1993). Moreover, other studies suggested an association between preconception paternal exposure to diagnostic x-rays and infant leukaemia (Shu et al., 1994). Research of the past decade has increasingly suggested the potential importance of preconception paternal exposures to foetal growth and development. Mechanisms by which father may contribute to the birth outcomes include genetic and epigenetic phenomena (Olshan and Faustman, 1993). A wide range of animal studies of internal irradiation have revealed profound developmental and offspring mortality effects which have not been addressed by International Commission on Radiological Protection (ICRP) or other risk agencies (European Committee on Radiation Risk (ECRR), 2010).

Various biological endpoints of transgenerational effects have been analyzed in this connection, and congenital malformations are one class of these endpoints (Nomura, 1982). Malformations can be induced by ionizing radiation in mammals when the exposure takes place during organogenesis. It has been postulated that exposures during other phases of prenatal

*Corresponding author. E-mail: iedagha@yahoo.com. Tel: +2348032113340.

development do not cause such malformations (ICRP, 2003). However, it has been found that in certain mouse strains, malformations can be induced by ionizing radiations as well as genotoxic substances when the exposures take place during pre-implantation period (Streffer, 2006).

Apart from studies on mutation rates in somatic cells, considerable progress has also been made in the analysis of radiation-induced instability in the mammalian germ-line, where the effects of radiation exposure were investigated among offsprings of irradiated parents. Transgenerational studies have been designed to test the hypothesis that radiation-induced instability in the germ line of irradiated parents could be transmitted to the offsprings and may, in turn, affect their mutation (Dubrova, 2003). It is now recognized that transgenerational genetic effects are mainly due to transmissible induced genetic instability (Natarajan, 2006).

MATERIALS AND METHODS

Experimental animals

The animals were acclimatized for 3 weeks in the animal holding room of the Department of Anatomy, College of Medicine, Ibadan. They were daily fed with mouse pellets and tap water was provided *ad libitum.* All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

Experimental design

27 mice (18 females and 9 males) were used in this study and divided in three groups as follows: Group A consists of 3 male and 6 female mice that served as control. They were placed in two separate cages labelled A1 and A2, respectively. Group B consists of 3 male mice exposed to gamma irradiation and 6 female mice not exposed to gamma irradiation. They were place in two separate cages labelled B1 and B2, respectively. Group C consists of 3 male mice and 6 female mice that were both exposed to gamma irradiation. They were placed in two separate cages labelled C1 and C2, respectively. Identification marks were placed on the mice using a red permanent marker for the purpose of distinguishing them in their individual cages.

Choice of irradiation dose

The choice of irradiation of 1 Gy/min was arrived at, after review of similar experimental protocol (Malomo et al., 2005).

Irradiation procedure

This procedure was carried out at the Radiotherapy Department of the University College Hospital, Ibadan. The mice exposed to gamma irradiation were carefully anaesthetized with ketamine intraperitoneally for mild unconsciousness at a dose of 0.04 mg/kg. Mice in a prone position were then gently strapped to a flat board and buckled. Four of these small boards were then arranged neatly together for exposure to gamma irradiation. The gonads of the mice were then irradiated with cobalt 60, Theratron 780C model (Canada), with an area of exposure being from the waist down (hemi-body). The source surface distance (SSD) used was 80 cm (distance between the source of the irradiation beam and the object irradiated), with the length of 16 cm (width 4 cm and depth 2 cm).

Mating procedures

This began one week post irradiation. Each group consisting of 6 female mice and 3 males were mated at a ratio of 2:1. The cages of the male and female mice were placed side by side in a dark room for 1 h, after which the male mice were transferred into the female cages. The cages were left in a dark room overnight. Vaginal plugs were subsequently examined early in the next morning. Female mice with vaginal plug were removed from their cages and transferred to another cage and were recorded as day ‘0’ of pregnancy.

Measurement of gross and microscopic parameters

The weight of the embryos was measured using a Metler analytical balance; crown rump length (CRL) of the embryos was measured using a manual sliding calliper; bi-parietal index (BPI) of the embryos was measured using a manual sliding calliper and fronto-occipital index (FOI) of the embryos was measured using manual sliding calliper.

Statistical analysis

Data obtained was analyzed using one-way analysis of variance (ANOVA) through the Statistical Package for Social Sciences (SPSS) 14 software package. Confidence interval was calculated at 95% level. The level of significance was fixed at less than 5%.

RESULTS

During the course of the experiment, results and observations were categorized into two major headings; the gross observation and the measurement of gross morphologic parameters.

Gross observation within 24 h after irradiation

Reduced motor function and activity, breathing difficulties, excessive urination, clustering of animals together in a corner of their cage, reduced feed and water intake were observed. They laid down in a stretched prone position, looked drowsy and sleepy, pupil of the eye were dilated and less pinkish in colour, consciousness and agile activities resumed towards a 24 h period, one death was recorded after 12 h and a large tumor in one of the irradiated female mice 1 week post irradiation (Figure 1).

The body weights of all the female mice serving as control in the experiment were recorded and statistically

Figure 1. A tumour beneath left hind limb of irradiated female mice.

Table 1. Weight of adult female mice (in grams) on day 7 post irradiation.

Group (n)	Mean (SD)	P value
Control (n=10) Non-irradiated (n=10)	23.9 (0.8) 23.9 (0.8)	1
Control (n=10) Irradiated (n=9)	23.9 (0.8) 22.7 (0.8)	0.01*
Non-irradiated (n=10) Irradiated (n=9)	23.9 (0.8) 22.7 (0.8)	0.01*

*Statistically significant at $p < 0.05$.

compared with those exposed to irradiation on day seven, that is, one week post-irradiation. The result obtained was statistically significant ($p < 0.05$) for the control and irradiated groups, as well as among the non-irradiated and irradiated groups as shown in Table 1.

The body weights of all the female mice in the experiment were recorded for each group on day 14 post vaginal plug. The result showed that there was no statistical significance on their weight variation. This is presented in Table 2. A total of 113 pups were obtained at the end of the experiment. These consist of 54 for the

Table 2. Weights of adult pregnant mice on day 14 post vaginal plug.

Group (n)	Mean (SD)	P value
Control (n=6) Non-irradiated (n=6)	29.1 (0.9) 28.6 (0.8)	0.36
Control (n=6) Irradiated (n=1)	29.1 (0.9) 27.0 (0.0)	0.1
Non-irradiated (n=6) Irradiated (n=1)	28.6 (0.8) 27.0 (0.0)	0.12

*, Statistically significant at $p < 0.05$.

Table 3. Litter size.

Group (n)	Mean (SD)	P value
Control (n=6) Non-irradiated (n=6)	9.0 (1.2) 8.3 (0.0)	0.51
Control (n=6) Irradiated (n=1)	9.0 (1.2) 9.0 (0.8)	1
Non-irradiated (n=6) Irradiated (n=1)	8.3 (2.0) 9.0 (0.0)	0.77

*Statistically significant at $p < 0.05$.

control, 50 for the irradiated and non-irradiated female group and 9 for the irradiated male and irradiated female group, as only one female was confirmed pregnant in this group. This is presented in Table 3. The mean of the litter size of all the three groups, namely, the control, irradiated male with non-irradiated female and the irradiated male with irradiated female was not statistically significant at p value ≤ 0.05. This is presented in Table 4. Some morphological measurements of the litters were recorded in all the three groups and their statistical analysis showed that they were all significant. This is presented in Tables 4 to 6.

DISCUSSION

According to Upton et al. (1992), the effects of exposure to low radiation doses may damage health after several years of exposure. Ionizing radiation is known to have detrimental effects on the reproductive systems of both males and females (Ladner et al., 1991). There are evidences of association between occupational exposure, cytogenetic alterations and the increase in cancer rates (Mitelmann. et al., 1997).

Parental exposure of mice to radiation and chemicals causes a variety of adverse effects (e.g. tumours,

Table 4. Gross morphologic assessment of foetuses in group 1 (day 14).

Gross parameter	Groups (n)	Mean (SD)	P value
Weight of litters (g)	Control (n=10) Non-irradiated (n=10)	0.41 (0.03) 0.23 (0.02)	0.001*
CRL (cm)	Control (n=10) Non-irradiated (n=10)	1.23 (0.04) 1.02 (0.07)	0.001*
BPI (cm)	Control (n=10) Non-irradiated (n=10)	0.44 (0.05) 0.30 (0.01)	0.001*
FOI (cm)	Control (n=10) Non-irradiated (n=10)	0.68 (0.03) 0.59 (0.02)	0.001*

*, Statistically significant at $p < 0.05$.

Table 5. Gross morphologic assessment of foetuses in group 2 (day 14).

Gross parameter	Groups (n)	Mean (SD)	P value
Weight of litters (g)	Control (n=10) Irradiated (n=9)	0.41 (0.03) 0.21 (0.02)	0.001*
CRL (cm)	Control (n=10) Irradiated (n=9)	1.20 (0.04) 0.90 (0.11)	0.001*
BPI (cm)	Control (n=10) Irradiated (n=9)	0.44 (0.05) 0.24 (0.15)	0.001*
FOI (cm)	Control (n=10) Irradiated (n=9)	0.68 (0.03) 0.50 (0.01)	0.001*

*Statistically significant at $p < 0.05$.

congenital malformations and embryonic deaths) in the progeny, and tumor-susceptibility phenotype is transmissible beyond the first post-radiation generation (Nomura, 2006). Since ionizing radiation is capable of inducing a wide spectrum of cellular, molecular, biochemical and hormonal abnormalities. The aim of this experiment was to determine the morphological development and the growth, in mice progeny mediated through the father.

In this study, we investigated the association between mice paternal gamma-ray exposure prior to coitus and conception, and the subsequent effect on the morphology of the mice progeny. The reported data from parental gonadal gamma irradiation at 1 Gy/min and subsequent mating at 1 week post-irradiation presented evidence of a significant effect on the fertility of the female mice with respect to their ability to conceive after mating, and even with the confirmation of a vaginal plug when compared with the control and intermediate groups. This is also similar to the results obtained by Steffer (2006).

Although, a statistical significant value was not obtained for the litter size of the foetuses, this may be due to the fact that there was only one conception from a single female mouse in the irradiation group when compared with the control and non-irradiation groups which yielded foetuses from all the female mice. A gross observation indicates that the acute dose generated radiation sickness which led to a significant weight loss and mortality. This is consistent with several other findings (Shea and Little, 1997; Vorobsova, 2000; Malomo et al., 2005; Nomura, 2006).

Gross results in this study revealed that the body weight of the control group as compared to non-irradiated group was not statistically significant. The body weight of the control group as compared to the irradiation group at 7 days post irradiation was statistically significant. The body weight of the non-irradiated group when compared with the irradiation group was statistically significant.

The reason for this weight loss remains unknown but could be due to the fact that the animals ate less and

Table 6. Gross morphologic assessment of fetuses in group 3 (day 14).

Gross parameter	Groups (n)	Mean (SD)	P value
Weight of litters (g)	Non-irradiated (n=10) Irradiated (n=9)	0.23 (0.02) 0.21 (0.01)	0.016*
CRL (cm)	Non-irradiated (n=10) Irradiated (n=9)	1.02 (0.07) 0.90 (0.11)	0.014*
BPI (cm)	Non-irradiated (n=10) Irradiated (n=9)	0.30 (0.01) 0.24 (0.05)	0.004*
FOI (cm)	Non-irradiated (n=10) Irradiated (n=9)	0.59 (0.02) 0.50 (0.01)	0.001*

*Statistically significant at $p < 0.05$.

were therefore less active. Increased catabolism may have resulted in weight loss. This could also be due to their response to trauma. The development of copious diarrhoea by all animals that were given irradiation is likely due to radiation sickness, inflammatory responses and decreased absorption of water and nutrients of the continuously cycling cells of the gastrointestinal epithelium from scattered effect of irradiation. This leads to loss of water absorptive capacity of the simple columnar epithelial cells. The water loss may be responsible for the weight loss (Malomo et al., 2005).

Experimental studies on plants and animals suggest that such effects will occur, and that such effects will range from the undetectably trivial, through gross malformations and loss of function, to premature death. Since this statement was made, applications of the minisatellite DNA testing procedure have shown unambiguous evidence of such effect including mutation in the offspring of the Chernobyl 'liquidators' (ECRR, 2010).

Conclusion

Irradiation of hemi-body of locally bred adult mice (male and female) with 1 Gy/min of gamma rays obtained from cobalt 60 elicited systemic problems leading to radiation sickness which includes excessive urination, reduced motor function and activity, breathing difficulties and change in coloration of the eye. The study also revealed:

(1) Statistically significant weight loss in the irradiated mice at $P < 0.05$
(2) Statistically significant effect on gross parameters from litters of experimental groups when compared with the control group mice which include; weight, CRL, BPI and FOI.

Thus, the reported data shows that a transgenerational transmission occurs for radiation induced instability in progeny of preconceptional gonadal gamma irradiated mice, especially at their gross structure and were statistically significant for weight, CRL, BPI and FOI of the litters.

ACKNOWLEDGEMENT

We acknowledge Mrs Ekishola of Radiotherapy Department, University College Hospital, Ibadan.

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 103-109, April 2012
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS12.028
ISSN 2006-9723 ©2012 Academic Journals

Full Length Research Paper

Biological evolution of tryptophan and phenylalanine in the occurrence of breast cancer in Senegalese women

Fatimata Mbaye[1,3]*, Malick Fall[1], Ahmadou Dem[2] and Mbacké Sembène[1,3]

[1]Faculty of Science and Technology, University Cheikh Anta Diop, P. O. Box 5005 Dakar, Senegal.
[2]Faculty of Medicine, Pharmacy and Odonto-Stomatology, University Cheikh Anta Diop, Institute of cancer, Dakar, Senegal.
[3]Laboratory of Biology Animals Populations Sahelo-sudanian, IRD, CBGP, Bel Air BP.1386 Dakar.

Accepted 3 April, 2012

The reports provided by OMS in 2011 revealed that cancer is a major cause of death worldwide, causing 7.6 million deaths in 2008. Breast cancer represents in the world, the most common malignancy of women, and it seems that the low penetrance genes, frequently mutated in the general population would play an important role in the development of this cancer. The purpose of this study is to evaluate the involvement of the protein diversity of cytochrome B (mitochondrial gene) in the occurrence of breast cancer in Senegalese women. We analyzed by PCR-sequencing cytochrome B variability in thirty Senegalese patients suffering from breast cancer. The nucleotide sequences obtained were transformed into amino acid sequences with BioEdit software version 7.0.8. Changes of one or more tryptophan to other amino acids, ranging normal tissue to cancerous tissue, are noted in some individuals with a penetrance of 72.41%. Our results also show a significant increase (79.3%) in the rate of phenylalanine in cancerous tissues with very different proportions between individuals. Any increase in the rate of tryptophan and phenylalanine in cancerous tissues could be correlated with an increased risk of developing breast cancer.

Key words: Cytochrome B, tryptophan, phenylalanine, breast, cancer, Senegal.

INTRODUCTION

The reports provided by OMS in 2011 showed that cancer is a major cause of death worldwide, causing 7.6 million deaths in 2008, about 13% of global mortality. It therefore represents a major public health problem. Cancer is the emergence of a cell clone that proliferates, invades, and metastasizes, despite the different levels of control of the body. It is a dogma that the last 30 years of research have continued to check (Stoppa-Lyonnet et al., 2010). Once considered a disease of the rich, it is now found among the poor in Senegal. Most cancer patients die from lack of means to support this costly disease in terms of drug costs, resulting from the level of living. Recent advances in molecular biology have allowed passing mathematical models proposed in the fifties to a biological reality. We now know that cancer is a disease of DNA resulting from the accumulation of successive mutational events: The acquired or germline mutations alter the normal function of some genes (Sobol and Eisinger, 2004). The discovery of oncogenes and their return, the tumor suppressor genes, established the pattern of cancer forming and progressing, following the onset of spontaneous somatic mutations. We now know that the genomes of tumors undergo many changes that disrupt profoundly affect the structure and functioning (Theillet, 2010). Most mutations are acquired (somatic) in tumor transformation. However, some are present from conception (the constitutional changes, or germ) and explain the genetic predisposition to cancer (Stoppa-Lyonnet et al., 2010). In nearly 25 years, more than 70 genes predisposing to cancer have been identified (Futreal et al., 2004).

Breast cancer is in the world, the most common malignancy of women; causes approximately 30% of

*Corresponding author. E-mail: mbaye_fatimasy@yahoo.fr. Tel: 00 (221) 773665998.

cancers and about 16% of cancer deaths in women (D'hondt et al., 2008). It is the second most common cancer in women in Sub-Saharan Africa after that of the cervix (Ly et al., 2011), particularly in Senegal (Dem et al., 2008).

It occurs in young women with a mean age at diagnosis between 42 and 53 years depending on the region, and is most often diagnosed at a late stage. These are mainly invasive ductal carcinoma with features of aggressive tumors (high SBR grade [grade III], low expression of hormone receptors and HER2). Under the new classification of breast cancers, some studies show that 16 to 55% of these tumors belong to the sub-group known as the triple negative (Ly et al., 2011). One of the problems the Curie Institute in Dakar faces is the resurgence of the disease in young women. In the literature, breast cancer in young women occurs at either the age of 35, sometimes less than 40 years, or sometimes less than 50 years (Espié and Cottu, 2003). Here, we will stick to the third option.

The objective of this work is to study the diversity of protein cytochrome B (Cyt. B), between healthy tissue and cancer, to determine the penetrance of the mitochondrial gene encoding breast cancer in Senegalese women, especially women who are received at the Institute Joliot Curie in Hospital Aristide Le Dantec.

PATIENTS AND METHODS

One of the first stages of this study is to obtain and collect samples necessary for its implementation. Sampling was done following a number of parameters, given in Table 1. From each patient, a perfectly healthy tissue sample and a sample of cancerous tissue are removed. Samples are taken in their interventions, by Prof. Ahmadou DEM Institute Joliot Curie in Hospital Aristide Le Dantec and colleagues. They are immediately forwarded to the Joint Laboratory IRD-ISRA-UCAD Molecular Biology of the Center for Biology and Population Management (CBGP) IRD Bel-Air, where the various stages of analysis are carried out. The samples are preserved in alcohol 96°C.

Currently, biological samples representing different stages of tumor progression: normal breast tissue, benign tumors, malignant tumors, and samples of special interest (tumors of the young woman) are available in the laboratory. The analysis focuses on 30 patients, all black. A portion of these biopsies was extracted nucleic acid (DNA).

Total genomic DNA was extracted from 25 mg to healthy tissue and cancerous (approximately 2 mm × 2 mm) using standard Qiagen method (Qiagen Dneasy Tissue Kit). Once the DNA was extracted, a portion of Cyt.B of great interest was amplified and sequenced. The Cyt.B has an area of over a thousand base pairs of the mitochondrial genome, located at positions 14201 and 15341 in the human sequence (Anderson et al., 1981), has a low recombination rate (related to the maternal inheritance only) and has a relatively high variability although it is a coding sequence, which justifies the choice of this marker. Primers used to amplify Cyt.B were as described previously (Montgelard et al., 2002). The 50 µl PCR reaction mixture contained 28.9 µl water, 5 µl enzyme buffer supplied by the manufacturer, 2 µl dNTP, 5.10^{-6} pmol of each primer H6 (5'TCT-CCA-TTT-CTG-GTT-TAC-AAG-AC 3') and L7 (5' ACC-AAT-GAC-ATG-AAT-AAA-CAT-GGT-T 3'), 0.1 µl of Taq polymerase and 4 µl of DNA extract. PCR conditions were 94°C for 3 min, followed by 40 cycles (92°C for 45 s, 50°C for 1 min, 72°C for 1 min 30 s), and a final elongation at 72°C for 10 min.

Table 1. Parameters sampling.

Patient	Age (years)	Tumor seat
1	50	Right breast
2	21	Left breast
3	46	Left breast
4	32	Right breast
5	40	Left breast
6	34	Right breast
7	35	Right breast
8	19	Right breast
9	50	Left breast
10	52	Left breast
11	60	Right breast
12	70	Right breast
13	42	Undefined
14	46	Right breast
15	57	Right breast
16	38	Left breast
17	73	Right breast
18	35	Right breast
19	44	Left breast
20	28	Right breast
21	49	Left breast
22	45	Right breast
23	52	Left breast
24	54	Right breast
25	40	Right breast
26	73	Right breast
27	35	Undefined
28	38	Undefined
29	48	Left breast
30	73	Left breast

Sequencing was performed by Macrogen in South Korea. A portion of the mitochondrial Cytochrome B gene was sequenced. The sequences of Cyt.B, healthy and cancerous tissue are carefully checked, adjusted and aligned with BioEdit software version 7.0.8 (www.mbio.nscu.edu/BioEdit/bioedit.html). Each healthy tissue is aligned next to the cancerous tissue to visualize and locate the mutations. The standard indices of genetic variation: genetic distances intra-healthy tissue, intra-cancerous tissues and between tissues, as well as genetic distances correlated on the one hand, at the age of the patients and secondly, the location of tumors (right breast or left breast) are explained with the software MEGA 4 (Evolutionary Molecular Genetics Analysis 4) (Tamura et al., 2007). The mtDNA coding is used. This allows you to convert nucleotide sequences into protein sequences using different reading frames possible. The reading frame is the sequence of triplets along a portion of mRNA. For a ribonucleotide sequence data, there are three different reading frames. The transformation into amino acid sequences was performed with the BioEdit editor. At this level, firstly, we try to establish a correlation between the rate of mutated

amino acids and age of the patients according to the Pearson coefficient correlation note (r), and secondly to look for mutations of interests within a single individual between cancerous and healthy tissue:

$$r = \frac{\sum (X - \overline{X}).(Y - \overline{Y})}{\sqrt{\sum (X - \overline{X})^2} \times \sqrt{\sum (Y - \overline{Y})^2}}$$

RESULTS

Alignment and genetic distance of cytochrome B

A portion of Cyt.B cells cancerous was sequenced in 30 individuals. The results are compared with those obtained from matched non-cancerous breast tissue, derived from the same patients. The sequences obtained are in number 60. Following a careful correction, we are left with 58 sequences, with a maximum length of 806 base pairs. The individual No 13 was eliminated because of a high genetic diversity. Analyses of genetic diversity and protein therefore focused on 29 individuals.

A comparison of nucleotide sequences at the intra and inter-individual variability shows strong Cyt.B. Strong disruption of normal tissue is observed in cancer tissue. The value of genetic distance (0.400) in healthy tissue is higher than cancerous tissue (0.281). Between the two groups, the genetic distance is 0.340. Young women represented 63.3% of our study population; the genetic distance within the tissue (0.461) is higher than that observed in healthy tissue of older women (0.310). By against, the genetic distance within the cancerous tissue of younger women (0.275) is lower than the intra-cancerous tissue of older women (0.291). The genetic distance between cancerous and normal tissues of young women (0.364) is higher than that observed between healthy and cancerous tissues of older women (0.297). In our study population, 53.3% of patients had a tumor that is located in the right breast, 36.7% in the left breast and the rest is not defined in the cards collection. The genetic distance intra-healthy tissue located at the right sound (0.461) is much higher than intra-healthy tissue located in the healthy left (0.249). The opposite effect is observed at the intra-cancerous tissue. The genetic distance between cancerous and healthy tissue located in the right breast (0.366) is higher than that observed between healthy and cancerous tissue of the left breast (0.255). In general, the results show that the genetic distance intra-healthy tissue is higher than intra-cancerous tissues regardless of the hierarchical level compared (the age of the patients and the right breast). For cons, the opposite is found in the left breast.

Protein diversity of cytochrome B

The protein sequences were obtained following transformation of the nucleotide sequences into amino acid sequences. After a test phase on three individuals, it was agreed that the second reading frame was by far the most appropriate, because it had the least stop codon. The total percentage of mutated amino acids in our patients was 36.47%. The correlation index R amino acid levels mutated versus age of patients was 0.0385. The correlation is shown in Figure 1. Changes of one more Trp to other amino acids from healthy tissues to cancerous tissues are noted in some individuals with a penetration of 72.41%. Among these individuals, mutations lead to 47.62% in truncated proteins. Meanwhile, an increasing number of Trp in 68.97% of cases is noted in the cancerous tissue. Our results also show a significant increase (79.3%) in the number of phenylalanine in cancerous tissues, with very different proportions. The results are given in Tables 2 and 3.

DISCUSSION

The objective of this work is to study the Cyt.B diversity of protein between healthy and cancer tissue, and to determine the penetrance of the mitochondrial gene encoding the breast cancer in Senegalese women. The choice to study mitochondrial DNA, fell on the Cyt.B because of the work done in laboratories; suggesting that the Cyt.B had a relatively high variability, although it is a gene. We analyzed by PCR-sequencing variability Cyt.B in 30 Senegalese patients suffering from breast cancer, a total of 60 sequences. The results are compared not in terms of epidemiology but in terms of mutational changes. The risk factors of breast cancer, which included previous exposure to hormone therapy, family history, obesity, ethnicity, were not taken in to account in our data processing. Only the patient age and tumor localization (left breast or right breast) were considered.

Genetic diversity of cytochrome B

The Americans, in their project "Cancer Genome Atlas'' found 189 genes whose mutations are involved in the onset or development of tumors. Only two of these genes are common to breast and colon cancer, all others differ. The researchers posited that each type of cancer is a very specific disease, requiring a specific treatment. Going further, they explain that each patient is different from the other. Our results show a high variability of nucleotide at both intra and inter-individual. This could be explained on the one hand, by the peculiarities of the mitochondrial genome: heteroplasmy and mitotic segregation. The heteroplasmy is the coexistence in the same cell of two species of mitochondrial DNA. During cell division, mitochondria of a cell are not distributed homogeneously in the daughter cells. Thus, from a cell with two types of mitochondrial populations, the daughter

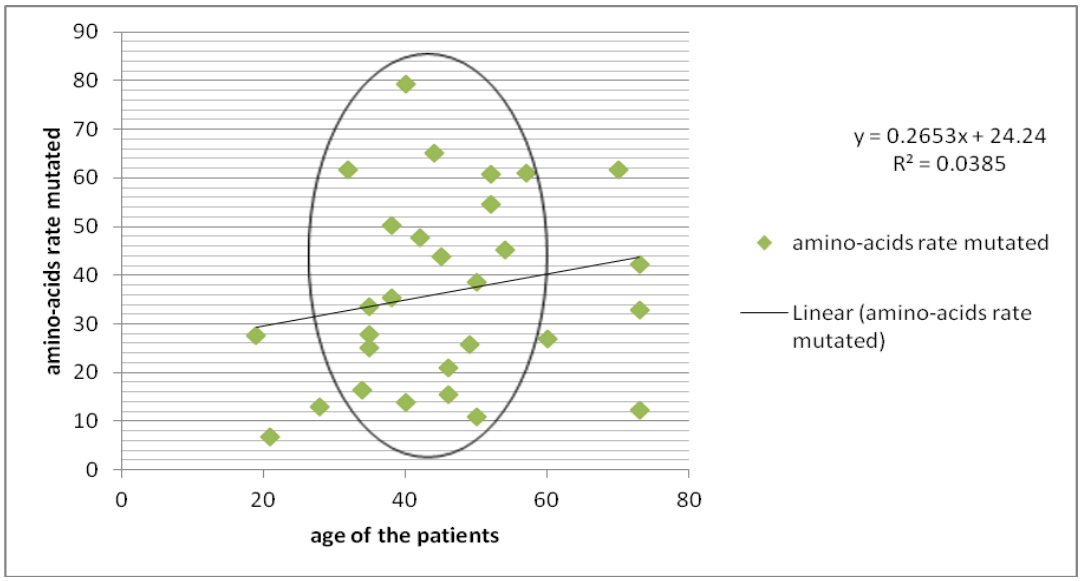

Figure 1. Curve of correlation of the rate of mutated amino acids at the age of the patients.

Table 2. Summary of changes in individuals with tryptophan.

Patient	Substitutions of Trp to TS-TC	Amino acid substitution of Trp to TS- TC
1	Cys	Gly ; His ; Cys
2	_ _ _ _	Gly
3	End ; Met	2 (Arg) ; End ; Cys
4	Met ; End ; Phe	Pro ; End
5	_ _ _ _	End
6	Arg	End ; His
7	_ _ _ _	Phe ; Gly
8	Gln ; End	Phe ; Cys ; Pro
9	Gly	Leu
10	End ; Lys ; Ile ; Lys	End
11	Cys	_ _ _ _
12	Val ; Arg ; End	His ; End ; Arg ; Phe
15	Thr ; Cys ; Arg	2 (End)
16	Glu	End
17	Gly ; Val ; Arg	2 (Gly) ; Arg ; Phe
18	Cys	_ _ _ _
19	End	End ; Gly
20	_ _ _ _	Gly
21	Cys	_ _ _ _
22	End ; Gly	_ _ _ _
23	End ; Gly	_ _ _ _
24	Arg ; Phe ; Gly	_ _ _ _
25	Lys ; leu	End ; 2 (Phe) ; Gly
26	End	End
27	_ _ _ _	Phe
28	_ _ _ _	2(Phe)
30	End ; Cys	_ _ _ _

- - - -, No modification.

Table 3. Summary table of individuals with a greater number of Phe in cancerous tissues.

Patient	Mutated amino-acids to Phe TS, TC	Nature of mutation
1	End, Glu	
2	Val	
3	2 (Leu), 3 (Tyr)	
4	Ser, 2 (Gly), Asp, His, Tyr, 2 (Leu), Trp	
5	Leu	
6	Glu, Ile	
7	His, Cys, Tyr	
8	2 (Ser), Cys, Leu	
10	End, Arg, Ser, Cys, Glu, Val, Ala, Leu	
11	End, 2 (Ile), 3 (Ser), Pro	
12	2(Leu), His	
14	Ile, Leu	Missense mutation
15	End, Ile, 2 (Cys), Glu, Ser	
16	Ser, 2 (Leu), Cys	
17	Leu, His	
19	Pro	
20	Leu	
22	End, Ser, Val, Gly	
23	End, Gln, 3 (Gly), Val, Leu, Ile	
24	End, 2 (Leu), Glu, Trp, Ile, Val, Ser, Gly	
27	Gly, Leu, Ile, Ser	
28	Pro, Ile	
30	End, 2 (Val)	

cells with variable rates of each of the two populations can be obtained. The phenomenon called mitotic segregation explains that from an egg containing a given proportion of normal and mutated mitochondrial DNA, an individual can have highly variable sex normal DNA / DNA mutated in its various tissues and organs. Associated with the threshold effect, this phenomenon explains the heterogeneity of clinical expression of diseases associated with mitochondrial DNA (DiMauro and Schon, 2001). On the other hand, this inter-individual variability observed could be due to mutations in precancerous especially as the samples are from the same breast. Indeed, Palacios et al. (2008) have shown that loss of heterozygosity observed in 90% of tumors BRCA1/2, are also present in preneoplastic lesions: carcinoma *in situ* of these patients, but also in non-tumor tissue. These results suggest that non-tumor tissues have a certain degree of genetic alterations that are predispose to neoplastic transformation.

The genetic distance within the normal tissue compared with intra-cancerous tissue, reveals a genetic differentiation. The proliferation of normal cells appears to be much faster. This shows that the main characteristic of the cancer cell is that its proliferation is no longer under control of the regulatory mechanisms of the body, and instead it evolves at a pace of its own. Thus, it does not necessarily divide faster than normal cell from which it derives, but its proliferation is no longer understood as meeting the unique needs of the organization, it escapes the different levels of its control. The high genetic distance observed between healthy and cancerous tissues could be explained by the fact that the mammary gland is constantly changing during the life of a woman.

The genetic distance correlated with patient age, which reveals that the genetic distance observed at the intra-healthy tissue of younger women is higher than the genetic distance of intra-healthy tissue of older women. This shows that genetic differentiation is related to the age of the patients. The proliferation of normal cells is faster in younger women. For cons, the genetic distance within the cancerous tissue is higher in older women; thus, we are told that the rate of proliferation of the cancer cell increases with age. The activity of repair genes in cell division decreases. This would explain the fact that cancer is a disease of aging. The genetic distance inter-healthy tissue and cancerous higher among young, could explain the large tumors observed in them during sampling. Would vascularization of the tumor not be more developed among young people? Taking into account the location of the tumor, the results show that the genetic distance intra-healthy tissue, localized in the right breast is higher than that observed in healthy

tissue located in the left breast. The proliferation of normal cells would be much faster in the right breast. The opposite effect is observed at the intra-cancerous tissue. The proliferation of the cancer cell is faster in the left breast, suggesting that the location of the tumor in it would be in favor of a faster evolution of the different process of carcinogenesis. In general, the proliferation of normal cells is faster than the cancer cell. This is valid not only in younger women than older women, but also for tumors that are localized in the right breast. For tumors localized in the left breast, the proliferation of the cancer cell is faster than the normal cell from which it is derived.

Protein diversity of cytochrome B

The total percentage of mutated protein levels on all patients is not significant enough. However, the Cyt.B being a gene, these substitutions may change the nature of the amino acid encoded, depending mainly on the position of substitution in codon but also according to the nature of the substitution. Through the right correlation between the rate of mutated amino acid and age of patients, our results confirm the work of Dem et al. (2008) namely that in Senegal, breast cancer occurs from 20 years, increase in frequency from 30 years to reach a peak between 44 and 50. As such, it is important to identify specific alterations, reflecting the occurrence of these tumors to less than 50 years without any hereditary background.

Mutations leading to a deficit of tryptophan (Trp) in normal tissues, as well as increased levels of Trp and phenylalanine (Phe) in cancerous tissues were identified. The Trp plays an important role in T cell proliferation, and Phe is among the eight essential amino acids that cannot be synthesized by the body so it is our food that should bring them. T cells are key players of immune rejection reactions that can lead to the elimination of cancer cells, which are based on various approaches to immunotherapy currently tested. Indeed, the methods used are designed to stimulate the immune system to recognize and destroy tumor cells. However, *in vivo*, cancer cells are able to develop mechanisms that allow tumors to resist and evade the immune system. Among these mechanisms, two enzymes are the key players: tryptophan 2, 3-dioxygenase (TDO) and indoleamine 2, 3-dioxygenase (IDO) (Moineaux et al., 2010). TDO is present in the liver, and IDO is expressed by the vast majority of tissues. By inhibiting the proliferation of T cells via the reduction of local rates of Trp, IDO is involved in the survival of tumor cells (Andre, 2008). Always in the same vein, Eyndeb et al. (2003), studying a new mechanism of tumor resistance to the immune system, based on the degradation of Trp by indoleamine, firstly, observed that the majority of human tumors expres this enzyme, and secondly, that expression of this enzyme by tumor cells of mice enabled them to escape immune rejection. Consistent with these results, such a change could be a risk factor for the occurrence of breast cancer. Immunotherapy could be suggested as a treatment by administration of an inhibitor of IDO obviously with other additional studies. These changes were observed in 21 individuals, or 72.41% of the study population.

The opposite effect occurs, always healthy tissue to cancerous tissue, leading to the appearance of a greater number of tryptophan in cancerous tissues. The amino acids that change in Trp healthy tissue to cancerous tissue are most often: Phe, Gly, Arg, Cys, the Pro, His, Leu, Glu sometimes in the same position, sometimes very different positions. It is as if there were repair systems that are mobilized to eliminate damage in the cancerous tissue. This is undoubtedly responsible for the transformation of the stop codon or other amino acids Trp, to allow T cells to proliferate and to play their advocacy role, recognizing cancer cells. Indeed, in normal cells, there are mechanisms of DNA repair involved to correct mutations that could for example be the cause of the cancer process. There are many eukaryotic repair systems, each suited to one or more types of lesions: MMR (mismatch repair): mismatch repair; NER (Nucleotide Excision Repair): nucleotide excision repair; BER (Base Excision Repair): base excision repair; the TS (Translesion Synthesis): direct repair; repair of DNA carrying agent and DSBR (Double Strand Break Repair): double-strand break repair; which includes the HR (Homologous Repair) and NHEJ (Non Homologous End Joining) (de Feraudy, 2007). The balance between the occurrence of DNA damage and repair is critical to the risk of developing cancer (Moisan, 2009). Mutations that are causing the change in Trp to stop codon, resulting in the appearance of a truncated protein (nonsense mutation) or other amino acids (missense mutations) are inactivating. Therefore, the gene Cyt.B could be considered a new susceptibility gene for breast cancer.

Similarly, an increase of Phe was observed on almost all (79.3%) cancerous tissues of individuals sampled, with very different proportions. This could be explained by the progress of the disease. Recall that we used biological samples representing different stages of tumor progression. Sometimes it is a transition, a transversion sometimes but in most cases it is a transversion. Adenine, guanine or cytosine is replaced with a thymine or cytosine. Phe UUU and UUC encoded by the formula UUU was found in 86.75% of cases. In other words, situations where the mutation is the thymine was found to be much more. Penetrance is high, the quantification of the rate of Phe in the body could be considered as a screening.

Conclusion

Faced with this real public health problem posed by

breast cancer in women, the establishment of a genetic test will allow sensitive and rapid management of patients. The results revealed one hand, nucleotide variability at intra-and inter-individual as well as genetic differentiation between healthy tissue and cancerous tissue; and secondly, that this genetic differentiation is linked both to the patient age and tumor localization (left breast or right breast). Any modification of Trp leading to a deficiency of this amino acid in normal tissues, as well as increased levels of Trp and Phe in cancerous tissues, could be correlated with an increased risk of developing breast cancer. The search rate of tryptophan and phenylalanine in the blood could be proposed as a screening test, and immunotherapy as a treatment. But these assumptions need to be confirmed by genetic analysis of a larger number of samples and by sequencing a larger number of coding genes.

ACKNOWLEDGEMENT

The authors warmly thank all members of the CBGP, especially Tofféne Diome for being very helpful.

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International Journal of Medicine and Medical Sciences Vol. 4(4), pp. 110-114, April 2012
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS12.027
ISSN 2006-9723 ©2012 Academic Journals

Full Length Research Paper

Nutritional assessment of the diets in rickets prevalent communities in Kaduna State of Nigeria

Moji Taibat Bakare-Odunola[1]*, Turaki Kalik-Zik[1], Magaji Garba[1], Rasheed Adekeye Odunola[2] and Kudi Bello-Mustapha[3]

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Kaduna state, Nigeria.
[2]University Health Services, University of Ilorin, Kwara State, Nigeria.
[3]National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

Accepted 10 April, 2012

Absorption (SHIMADZU MODEL 650) was used to determine the levels of calcium and phosphorus in the food samples. Wet digestion method was adopted for the food samples preparation. The results obtained showed that calcium levels in all foods were low with mean values of 0.056±0.02, 0.069±0.04 and 0.127±0.06 mg\kg (S.E.M) in Gonin Gora, Jankasa and Kaso respectively. Phosphorous levels were high with mean values of 0.538±0.09 and 0.431±0.01 mg\kg (S.E.M) in Gonin Gora and Kaso respectively; compared with the allowable limit of 0.412 mg/kg. However, phosphorus concentration was low (0.261 mg/kg) in Jankasa. The low levels of calcium in foods or the low calcium intake with high phosphorus intake could be a contributing factor to the cause of the disorder in these settlements especially during the period of the children growth.

Key words: Nigeria, Kaduna state, rickets, food, calcium, phosphorus.

INTRODUCTION

Rickets is a bone disease of children, resulting in progressive softening and weakening of the bone structure as a result of loss of calcium and phosphate from the bone, which eventually causes destruction of the supportive gland matrix (Blok et al., 1998; Rowe, 2001; Rajakumar, 2003). Nutritional rickets can be caused by inadequate intake of nutrients (vitamin D in particular), calcium, or phosphorus, or inadequate sunlight exposure. It is common in dark-skinned children who have limited sun exposure and in infants who are breastfed exclusively. Vitamin D-dependent rickets - type I, results from abnormalities in the gene coding for 25(OH) D_3-1-alpha-hydroxylase, and type II results from defective vitamin D receptors. The vitamin D-resistant types are familial hypophosphatemic rickets and hereditary hypophosphatemic rickets with hypercalciuria. Other causes of rickets include renal disease, medications, and malabsorption syndromes. Rickets prevalence is reported to be on the increase in several countries (Finberg, 1979; Pettifor et al., 1981; Igbal et al., 1994; Muhe et al., 1997; Dux et al., 2001; Rowe, 2001; Delucia et al., 2003). There have also been increasing reports of nutritional rickets in healthy children without vitamin D deficiency (Muhe et al., 1997; Delucia et al., 2003).

In developing countries where calcium intakes are characteristically low and the population relies heavily on cereal-based staples, with few or no dairy products, dietary calcium deficiency appears to be the major cause of rickets among children outside the infant age group (Thacher et al., 2006).

Gonin Gora, Jankasa, and Kaso, are communities with high prevalence rate of rickets; there is hardly a family without a child afflicted with the disease. This work assessed the nutritional status of these communities in order to determine the adequacy of their diet.

MATERIALS AND METHODS

Measurements were made with a Buck model 210 variant giant pulse correction (UGP) system, and Atomic Absorption Spectrophotometer (AAS) equipped with calcium or phosphate

*Corresponding author. E-mail: mojitaibat@yahoo.com. Tel: +2348035896043.

hollow cathode lamp. Lamp current of 10 mA, wavelength 420.0 nm, band pass 0.5 nm with air/acetylene flame and stiochiometric fuel flow at 0.9 to 1.21 min^{-1} was used.

Description of sampling areas

The study areas/sampling points are areas most affected by the rickets disorder. These areas include: Jankasa, Kaso, and Gonin Gora, all in the southern part of Kaduna Metropolis. Jankasa and Kaso are rural land locked villages approximately 25 km along Kaduna- Kachia road while Gonin- Gora is a few kilometers from the metropolis along Kaduna-Abuja road. The inhabitants of these villages are 'Gbaygi, Kadara, Hausa and Fulani by tribe.

Sample collection and preservation

The water sample collection and preservation method as described by Department of Water Affairs and Forestry (DWAF), Hydrological Research Institute, Pretoria, South Africa (Analytical Methods Manual) and the vegetable/foods samples collection and preservation methods as described by Bakare-Odunola (2006) were adopted in this study. The treatment of both water and vegetables/foods samples, which were kindly provided by the occupants of the communities were done following the procedures described by Laboratory Procedure for Fertilizer and Water Analysis, Ahmadu Bello University, Nigeria, Training Workshop (Bakare-Odunola, 2006). The address and place of collection, date of collection, name of sample and time of collection were recorded at the collection sites. Samples were all collected in the Months of October and November 2006.

Official methods were used for the identification of calcium and phosphorus (British Pharmacopoeia, 2002)

Preparation of calibration curves for calcium and phosphorus

Calcium and phosphorus sample solutions were prepared by dissolving 5 g each of dried ground calcium/phosphorus in a digestion tube using 20 ml of digestion acid mixture (nitric acid, sulphuric acid and perchloric acid). The resulting solution was then heated at low temperature to avoid fuming and loss of volatile minerals. When the initial reaction subsided, the temperature of the digestion block was slowly increased to 200°C. This digestion was continued at this temperature with occasional shaking until there were no visible particles and the colour of the digestion acid was cleared. The temperature was allowed to rise from heating source to 240°C and evaporation of the digestion acid ensued. This was confirmed by the formation of white fumes within the digestion tube. The tube was removed from the heating source and the content was filtered through acid washed filter paper in a 100 ml volumetric flask using deionized water.

Working standard solutions of concentrations 0.001, 0.002, 0.005, 1.000, 2.000, 3.000, 4.000, and 5.000 mg/l each were prepared by dilution using deionized water. 5 ml of standard was drawn into a 25 ml volumetric flask. 10 ml of lanthanum was added to calcium standards while molybdate vandate was used as a complexing agent for phosphorus standards. The resulting solution was shaken and made up to the volume with deionized water. The solution of the mixture was aspirated into the flame and the absorbance was recorded.

Sample preparation for calcium and phosphorus

Each of the samples was weighed in duplicate (5 g) and added into series of weighed beakers labeled accordingly and treated as described under calibration curve.

5 ml of the digested sample was also treated as described for calibration curve of calcium and phosphorus. The concentration of calcium and phosphorus was determined from the respective calibration curve. The stability of the instrument was checked at intervals by introducing the lowest or the highest working standard solution and blank.

RESULTS

The results obtained for calcium concentrations are shown in Tables 1, 2 and 3, for Gonin Gora, Jankasa and Kaso communities respectively. Calcium concentration in all foods in the study areas were found to range from 0.00 to 1.30 mg\kg. The highest concentration of 1.300 mg\kg was determined in soya beans for Kaso area and calcium was not detected in kunu sample from Gonin Gora (Tables 1 and 3) respectively. Despite the different nature of the samples, the calcium concentrations have similar patterns. In Gonin Gora, the concentration was found to be high, compared to that of other study areas.

The result obtained for phosphorus concentrations are shown in Tables 4, 5 and 6 for Gonin Gora, Jankasa and Kaso respectively. The concentrations were found to range from 0.007 to 5.43 mg\kg. The lowest concentration (0.007 mg\kg), found in orange and the highest concentration (5.42 mg\kg), found in fish, were recorded in Gonin Gora. Jankasa and Kaso samples showed high concentration of phosphorus (Tables 5 and 6, respectively). Phosphorus levels were higher in all the study areas compared with calcium level. It was significantly low ($p < 0.05$) for Kaso.

DISCUSSION

The prevalence of rickets disease is lower in Gonin Gora compared with Jankasa and Kaso. Gonin Gora has a higher level of calcium in most of their food due to closeness of the village to Kaduna town which improved the standard of the diet in the community

The mean value of calcium level in Kaso was 0.127±0.066 mg\kg (S. E M.) which is higher than that of Gonin Gora and Jankasa since soya beans with high calcium concentration (1.30 mg/kg) formed part of their stable food which affecs the mean value of calcium levels. Despite the high mean value of calcium levels, the area was mostly affected with the disease than other communities. Nearly all families had a victim of rickets. It is possible that they do not take much of the soya bean meal or they have metabolic defect. The low calcium intake among infants and children has been attributed to the development of rickets (Kooh et al., 1977; Legius et al., 1989). Rickets among rural children has been reported to be attributed to low dietary calcium intake (Pettifor et al., 1978)

The health condition of Jankasa with the mean calcium value of 0.069± 0.045 mg\kg is better than Kaso but most

Table 1. Calcium levels (mg/kg) for different food samples from Gonin Gora settlement of Kaduna State.

S/N	Sample	Concentration of calcium
1	Tuwo	0.069
2	Gari	0.034
3	Kuka	0.156
4	Okro	0.044
5	Karkashi	0.045
6	Honey	0.001
7	Fish	0.223
8	Beef meat	0.000
9	Yoghurt	0.003
10	Nono	0.348
11	Orange	0.001
12	Yakuwa	0.048
13	Water	0.006
14	Yam	0.005
15	Kunu	0.000
16	Fura	0.032
17	Spinach	0.024
18	Cabbage	0.015
19	Potatoes	0.012

Mean = 0.056 ± 0.021 mg\kg.

Table 2. Calcium levels (mg/kg) for the different food samples from Jankasa settlement of Kaduna State.

S/N	Sample	Concentration of calcium
1	White kaura	0.018
2	Yellow kaura	0.008
3	Red kaura	0.012
4	Millet	0.021
5	Maize meal	0.042
6	Acha	0.008
7	Water	0.012
8	Beans	0.051
9	Patte	0.045
10	Nono	0.871
11	Groundnut	0.030
12	Fura	0.053
13	Maize	0.007
14	Yam	0.004
15	Sweet potatoes	0.021
16	Kuli kuli	0.016
17	Bread	0.002
18	Onion	0.032
19	Lettuce	0.054

Mean = 0.069± 0.045 mg\kg.

Table 3. Calcium levels (mg/kg) for different food samples from Kaso settlement in Kaduna State.

S/N	Sample	Concentration of calcium
1	White kaura	0.003
2	Red kaura	0.002
3	White beans	0.058
4	Black beans	0.047
5	Yam	0.013
6	Water	0.013
7	Gurjiya	0.021
8	Sweet potatoes	0.009
9	Maize meal	0.068
10	Tomatoes	0.006
11	Dadawa	0.022
12	Patte	0.189
13	Rice	0.010
14	Soya beans	1.300
15	Kindirmo	0.623
16	Gauta	0.015
17	Sesame seed	0.010
18	Millet	0.002
19	Bread	0.002
20	Eggs	0.014

Mean = 0.127± 0.066 mg\kg.

Table 4. Phosphorus levels (mg/kg) for different food samples from Gonin-Gora settlement of Kaduna State.

S/N	Sample	Concentration of phosphorus
1	Maize meal	0.310
2	Gari	0.034
3	Kuka	0.184
4	Okro	1.064
5	Karkashi	0.451
6	Honey	0.036
7	Fish	5.426
8	Beef meat	1.097
9	Yoghurt	0.036
10	Orange	0.007
11	Nono	0.068
12	Orange	0.080
13	Yakuwa	0.067
14	Water	0.053
15	Yam	0.080
16	Kunu	0.098
17	Fura	0.881
18	Spinach	0.056
19	Cabbage	0.193

Mean = 0.538± 0.283 mg/kg.

Table 5. Phosphorus levels (mg/kg) for different food samples from Jankasa Settlement of Kaduna State.

S/N	Sample	Concentration of phosphorus
1	White kaura	0.172
2	Yellow kaura	0.550
3	Red kaura	0.398
4	Millet	0.423
5	Tuwo	0.300
6	Acha	0.400
7	Water	0.016
8	Beans	0.333
9	Patte	0.037
10	Nono	0.026
11	Groundnut	0.262
12	Fura	0.110
13	Maize	0.360
14	Yam	0.000
15	Sweet potatoes	0.062
16	Kuli kuli	0.348
17	Bread	0.077
18	Onion	0.489
19	Lettuce	0.873

Mean = 0.262± 0.054 mg\kg.

Table 6. Phosphorus levels (mg/kg) for different food samples in Kaso settlement Kaduna state.

S/N	Sample	Concentration of phosphorus
1	White kaura	0.360
2	Red kaura	0.470
3	White beans	0.260
4	Black beans	0.228
5	Yam	0.073
6	Water	0.009
7	Gurjiya	0.234
8	Sweet potatoes	0.065
9	Tuwo	0.320
10	Tomatoes	0.765
11	Dadawa	1.264
12	Patte	0.390
13	Rice	0.173
14	Soya beans	0.338
15	Kindirmo	0.982
16	Gauta	0.376
17	Sesame seed	0.338
18	Millet	0.430
19	Bread	0.088
20	Eggs	0.400
21	Eguisi	1.484

Mean = 0.431± 0.084 mg\kg.

of the houses also have at least a victim of the rickets disease.

The levels of phosphorus in the foods were found to be generally higher than the levels of calcium for the communities. The functional consequences of this high intake of phosphorus in the presence of low calcium remain a topic of controversy (Sax, 2001). Wyshak (2000) reported that high phosphorus intake contributed to hypocalcaemia and fractures in children. The high phosphorus intake in the presence of low calcium could also be responsible for the problem (Thacher et al., 2000).

Rickets therefore remained common in many parts of the world and calcium deficiency, not vitamin D deficiency, was the important cause of the disease (Thacher et al., 2006; Graff et al., 2004).

Conclusion

In conclusion, most staple food in the three communities (Gonin Gora, Kaso and Jankasa) are low in calcium but high in phosphorus. The low levels of calcium in the foods and/or the low calcium intake with high phosphorus intake could be the major cause of the disease in these communities, especially during the period of child growth.

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