Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

Rev Electron Biomed / Electron J Biomed 2009;2:1-85 (pdf)

Mayo - Agosto 2009 / May - August 2009

EDITORIAL /EDITORIALS

ORIGINALS / ORIGINALES

CASE REPORTS / CASOS CLÍNICOS

INTERNET REVIEWS / REVISIONES EN INTERNET

LETTERS TO THE EDITOR / CARTAS AL EDITOR

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicina

ISSN: 1697-090X

Inicio
Home

Indice del volumen
Volume index

Comité Editorial
Editorial Board

Comité Científico
Scientific Committee

Normas para los autores
Instruction to Authors

Derechos de autor
Copyright

Contacto/Contact:

Rev Electron Biomed / Electron J Biomed 2009;2: 3-5.

Editorial:

IS STRICT GLYCAEMIC CONTROL BENEFICIAL?

Prof. Francisco Abad Santos PhD.

**Professor of Pharmacology and Therapeutic. Universidad Autónoma de Madrid.
Department of Clinical Pharmacology. Hospital la Princesa. Madrid. España**

fabad.hlpr @ salud.madrid.org

Version española

Cardiovascular disease is one of the principle causes of morbidity and mortality, and diabetes mellitus is becoming one of the main risk factors. In fact, coronary disease is the principle cause of death in diabetic patients. Several studies on patients with type 1 diabetes showed that strict glycaemic control reduces coronary disease by more than 50%; but the data are not so clear in type 2 diabetes, as they usually have other underlying risk factors, such as high blood pressure, hyperlipidaemia and obesity. For these reasons, some authors doubt whether strict blood glucose control is necessary to reduce macrovascular events and total mortality in patients with type 2 diabetes mellitus.

Due to the negative results of several recent clinical trials, some experts suggest that the efforts to achieve strict glycaemic control in patients with type 2 diabetes should be relaxed. However, this could be wrong, as the microvascular benefits of strict blood glucose control are well established. It may be that all

these studies did not have sufficient power to detect a cardiovascular benefit, as the difference between the two comparison groups (standard and strict glycaemic control) was small, or the follow up period was too short. A recently published meta-analysis has shone new light on this subject (Ray et al. Lancet 2009; 373: 1765-72).

This meta-analysis combines the results of 5 recent clinical trials, with a total of 33,040 patients (including patients with stable type 2 diabetes) who were randomly assigned to standard or intensive diabetic treatment. The strict diabetic treatment was different in each study and was based on sulphonylureas, metformin, glitazones, insulin or a combination of several of them, with a mean follow up of 5 years.

The final analysis showed that strict hypoglycaemic treatment significantly reduced the incidence of myocardial infarction by 17% and coronary disease by 15%. However, no significant effect was found with stroke or overall mortality. Intensive treatment was also associated with a higher incidence of hypoglycaemic episodes (38.1% patients vs. 28.6% with standard treatment) and an increase in weight of 2.5 kg, which could limit the benefit gained over other cardiovascular risk factors.

It is important to point out that the results seem to be applied to the majority of the patients, regardless of the baseline glycosylated haemoglobin level. The mean reduction in HbA1c was 0.9% greater with the strict diabetic treatment, which shows us that better glycaemic control is achieved.

However, the cardiovascular benefits associated with glycaemic control are less than those obtained by a reduction in blood pressure or cholesterol. The results of this meta-analysis show us that each 1% reduction in HbA1c prevents around 3 coronary events per 200 patients treated for 5 years, a lower benefit than that obtained for each 1 mmol/l (38 mg/dl) of LDL-cholesterol or 4 mmHg in blood pressure (8.2 and 12.5 cardiovascular events prevented, respectively). Furthermore, the reduction in cholesterol with statins and blood pressure control decrease total mortality, which does not happen with strict glycaemic control.

Therefore, the overall cardiovascular risk of the patient must be assessed, and if there has to be a choice, controlling the hypertension and the dyslipaemia must be priorities, which does not mean that we have to forget to control the glycaemia the best we can. The doctor and the patient must weigh up the expected benefits with the will and ability of each individual patient to manage to control the main risk factors. A practical approach could be to gradually reduce the HbA1c, being careful to prevent severe hypoglycaemic episodes. Blood glucose control must begin as soon as possible.

REFERENCE

Ray KK, Seshasai SR, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D, Erqou S, Sattar N. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet 2009; 373: 1765-1772

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

Rev Electron Biomed / Electron J Biomed 2009;2: 6-8.

Editorial:

¿ES BENEFICIOSO EL CONTROL INTENSIVO DE LA GLUCEMIA?

Prof. Francisco Abad Santos PhD.

**Profesor Titular de Farmacología y Terapéutica. Facultad de Medicina. Universidad Autónoma de Madrid.
Servicio de Farmacología Clínica. Hospital la Princesa. Madrid. España**

fabad.hlpr @ salud.madrid.org

English version

Las enfermedades cardiovasculares son una de las principales causas de morbimortalidad y la *diabetes mellitus* se está convirtiendo en uno de los principales factores de riesgo. De hecho, la principal causa de muerte en pacientes diabéticos es la enfermedad coronaria. Varios estudios realizados en pacientes con diabetes tipo 1 demuestran que el control estricto de la glucemia reduce más de un 50% la enfermedad coronaria, pero los datos no son tan claros en la diabetes tipo 2 porque se suelen superponer otros factores de riesgo cardiovascular como la hipertensión arterial, la hiperlipidemia y la obesidad. Por estos motivos, algunos autores dudan de si es necesario un control estricto de la glucemia para reducir los eventos macrovasculares y la mortalidad total en los pacientes con diabetes mellitus tipo 2.

A raíz de los resultados negativos de varios ensayos clínicos recientes, algunos expertos sugieren que se pueden relajar los esfuerzos para conseguir un control glucémico estricto en los pacientes con diabetes tipo 2. No obstante, esto sería un error porque los beneficios microvasculares del control glucémico estrecho están bien establecidos. Es posible que cada uno de estos estudios no tuviese un poder suficiente para detectar un beneficio cardiovascular porque la diferencia entre los dos grupos de comparación (control glucémico estándar o estricto)

fuese pequeña o la duración del seguimiento fuese demasiado corta. Un metanálisis publicado recientemente aporta nueva luz a esta cuestión[1]

Este metanálisis combina los resultados de 5 ensayos clínicos recientes, con un total de 33.040 pacientes, en los que participaron pacientes con diabetes tipo 2 estables que se asignaron aleatoriamente a un tratamiento antidiabético estándar o intensivo. El tratamiento antidiabético intensivo evaluado era diferente en cada estudio y se basaba en sulfonilureas, metformina, glitazonas, insulina o una combinación de varios de ellos durante una media de 5 años.

El análisis final reveló que el tratamiento hipoglucemiante intensivo reduce significativamente la incidencia de infarto de miocardio un 17% y de enfermedad coronaria un 15%. Sin embargo, no se encontró un efecto significativo sobre el ictus ni la mortalidad total, y el tratamiento intensivo se asoció a una mayor incidencia de episodios hipoglucémicos (38.1% de los pacientes vs 28.6% con el tratamiento estándar) y a un aumento de 2,5 kg de peso, lo que puede limitar el beneficio producido sobre otros factores de riesgo cardiovascular.

Es importante destacar que los resultados parecen aplicarse a la mayoría de los pacientes, independientemente del nivel basal de hemoglobina glicosilada. La reducción media de HbA1c fue 0.9% mayor con el tratamiento antidiabético intensivo, lo que nos indica que se consigue un mayor control glucémico.

Sin embargo, los beneficios cardiovasculares asociados al control glucémico son menores que los que produce la reducción de la presión arterial o el colesterol. Las estimaciones de este meta-análisis nos indican que por cada reducción de 1% en la HbA1c se evitan alrededor de 3 episodios coronarios por cada 200 pacientes tratados durante 5 años, un beneficio inferior al que se consigue por cada reducción de 1 mmol/l (38 mg/dl) de LDL-colesterol o 4 mmHg de la presión arterial (8.2 y 12.5 episodios cardiovasculares prevenidos, respectivamente). Además, la reducción del colesterol con estatinas y el control de la presión arterial disminuyen la mortalidad total, lo que no ocurre con el control intensivo de la glucemia.

Por tanto, el riesgo cardiovascular del paciente debe ser valorado globalmente y si es necesario elegir, controlar la hipertensión y la dislipemia deben ser prioritarios, lo cual no significa que debamos olvidarnos de controlar la glucemia lo mejor posible. El médico y el paciente deben sopesar los beneficios esperados con la voluntad y la capacidad de cada paciente concreto para conseguir controlar los principales factores de riesgo. Una aproximación práctica puede ser reducir la HbA1c continuadamente con cuidado para evitar los episodios de hipoglucemia grave. El control de la glucemia se debe empezar lo más pronto posible.

REFERENCIA

Ray KK, Seshasai SR, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D, Erqou S, Sattar N. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet 2009; 373: 1765-1772

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

INFLUENCE OF ARYLPIPERAZINES AROMATIC STRUCTURE OVER DIFFERENTIAL AFFINITY FOR 5-HT$_{1A}$ AND 5-HT$_{2A}$ RECEPTORS

António Machado, Eduardo Tejera, Irene Rebelo

IBMC, Department of Biochemistry, Faculty of Pharmacy, University of Porto. Porto, Portugal

machadobq @ gmail.com

Rev Electron Biomed / Electron J Biomed 2009;2:9-19

Comment of the reviewer Prof. Pilar Muñiz Rodríguez PhD. Titular del Área de Bioquímica y Biología Molecular de la Facultad de Ciencias de la Universidad de Burgos. España.

Comment of the reviewer Prof. Amalio Garrido Escudero PhD. Head Environmental Engineering and Toxicology Dpt. Universidad Católica S. Antonio. Guadalupe. Murcia. España.

RESUMEN:

INFLUENCIA DE LA ESTRUCTURA AROMÁTICA DE LAS ARILPIPERAZINAS EN LA AFINIDAD DIFERENCIAL CON LOS RECEPTORES 5-HT$_{1A}$ Y 5-HT$_{2A}$

Las piperazinas son una familia de compuestos químicos muy amplia y con una gran capacidad para interactuar con diversos receptores serotonérgicos (5-HT). Debido a estas propiedades, estos compuestos tienen un importante potencial farmacológico, sin embargo muestran también algunos efectos tóxicos asociados. En la actualidad el subtipo 1A del receptor serotonérgico (5-HT$_{1A}$) ha resultado ser un importante blanco para el tratamiento eficaz de la depresión y ansiedad, mientras que el subtipo 2A del receptor serotonérgico (5-HT$_{2A}$) ha sido asociado con numerables efectos adversos.

En este estudio, se utilizan diversos métodos computacionales con el fin de efectuar una caracterización de los fragmentos estructurales y las propiedades químicas asociadas, responsables por la afinidad de las piperazinas para los receptores 5-HT$_{1A}$ Y 5-HT$_{2A}$. En este trabajo, se discuten también, algunas propiedades de las estructuras aromáticas en las arilpiperazinas que son similares para los dos subtipos del receptor serotonérgico. Por otra parte se sugiere, que la substitución con calcógenos en la posición orto- y meta- así como el ligero incremento en el peso molecular son modificaciones que pueden aumentan la afinidad para el receptor 5-HT$_{1A}$; mientras que las arilpiperazinas con substitución por halógenos en las mismas posiciones además de un pequeño decrecimiento en el peso molecular podrían incrementar la afinidad para el 5-HT$_{2A}$ receptor.

PALABRAS CLAVE: Piperazina; Receptor serotonérgico; Farmacóforos; Afinidad; Selectividad; Diseño de fármaco

---

SUMMARY:

The piperazines are a large family of compounds with an enormous potential for interacting with several serotonin (5-HT) receptors. Those compounds reveal prospect for use as drugs with diverse therapeutic applications, despite the fact that they also show some toxicological effects. Actually, the subtype 1A of 5-HT (5-HT_{1A}) receptor is responsible for efficient treatment of anxiety and depression, while subtype 2A of the 5-HT (5-HT_{2A}) receptor is accountable for several adverse effects. In this study, we applied several computational approaches to better describe the most important chemical and substructure properties that are responsible to influence the affinity of arylpiperazines to the 5-HT_{1A} or the 5-HT_{2A} receptors. In the present work we discuss some properties of the arylpiperazines aromatics structures that are similar for both serotonin receptors. However, consequently, we showed that the chalcogens substitution close to the benzene *ortho*- and *meta*- position as well as a slight increment in the molecular weight showed more affinity to the 5-HT_{1A} receptor. While arylpiperazines with halogens substitution at the same benzene position as well as a minor decrease in the molecular weight had more affinity for the 5-HT_{2A} receptors.

KEY WORDS: Piperazine; Serotonergic receptor; Pharmacophore; Afinitty; Selectivity; Drug design.

INTRODUCTION

The interest in the role of serotonin (5-HT) and the mechanism of action of antipsychotic drugs (APDs) is the result to its direct and indirect effects on various 5-HT receptors, especially the 1A and 2A subtype serotonergic receptors (5-HT_{1A} and 5-HT_{2A}, respectively). Thus, both 5-HT_{2A} antagonism and 5-HT_{1A} agonism may be the most important of the 5-HT receptors for APD action. Postsynaptically, both 5-HT_{1A} and 5-HT_{2A} receptors are localised on the pyramidal neurones in the cortex, where the 5-HT_{1A} receptor inhibits neuronal output by activation of a hyperpolarising potassium current, and the 5-HT_{2A} facilitates output via activation of phospholipase C[1,2].

This opposition between the two 5-HT receptor subtypes suggests that agonists at 5-HT_{1A} receptors may modulate dopaminergic neurotransmission in the brain in a similar fashion to 5-HT_{2A} receptor antagonists. The 5-HT_{1A} receptor agonists can stimulate the release of dopamine (DA) in the prefrontal cortex as well as potentiate the effect of 2 subtype dopamine receptor (D_2) blockers on DA release[3]. These studies suggest that 5-HT_{1A} receptor activation is critically involved in the regulation of DA release in these two brain regions, which are involved in key cognitive function.

5-HT_{1A} receptors are located both presynaptically and postsynaptically. The presynaptic receptors are also known as autoreceptors and are stimulated automatically upon release of serotonin. Activation of the 5-HT_{1A} autoreceptors inhibits the release of serotonin on a global level[3,4]. Several studies suggest that atypical antipsychotics exert their effects on dopaminergic neurotransmission, at least in part, via activation of 5-HT_{1A} receptors[4], presumably due to concomitant potent 5-HT_{2A} and relatively weak D_2 receptor antagonism[5]. Cai *et al.* [6] suggested this may be a mechanism by which 5-HT_{1A} receptors modulate memory and anxiety.

The use of 5-HT_{1A} receptor agonists may substitute for 5-HT_{2A} antagonism and achieve many of the same benefits in combination with weak D_2 receptor blockade. All these studies[1-7] focuses on the regulation of central 5-HT_{1A} receptor function as an ideal target to antidepressant drugs by 5-HT_{1A} receptor agonists underlies the therapeutic efficacy of these drugs.

The 5-HT_{1A} receptor is present in high density in serotonergic cell body areas, in particular the dorsal and median raphe nuclei, as well as in cortical and limbic areas (e.g. frontal cortex, entorhinal cortex, hippocampus, amygdala, septum)[8-10]. It's also present in the hypothalamus where play important roles in the regulation of neuroendocrine function and responses to stress.

Anxiolytic or antidepressant efficacy may be due in part to compensatory changes distal to the 5-HT_{1A} receptor receptor, such as regulation of G protein expression or reduced capacity of the receptor to activate G protein due to regulatory processes (e.g. phosphorylation) at the level of the G protein[7].

The increase in serotonin neurotransmission, due to somatodendritic autoreceptor desensitization, to normo-sensitive 5-HT_{1A} receptors in certain brain regions (e.g. hippocampus or cortex) and to sub-sensitive 5-HT_{1A} receptors in other brain regions (e.g. amygdala or hypothalamus) underlies the therapeutic efficacy of these drugs7 (Figure 1).

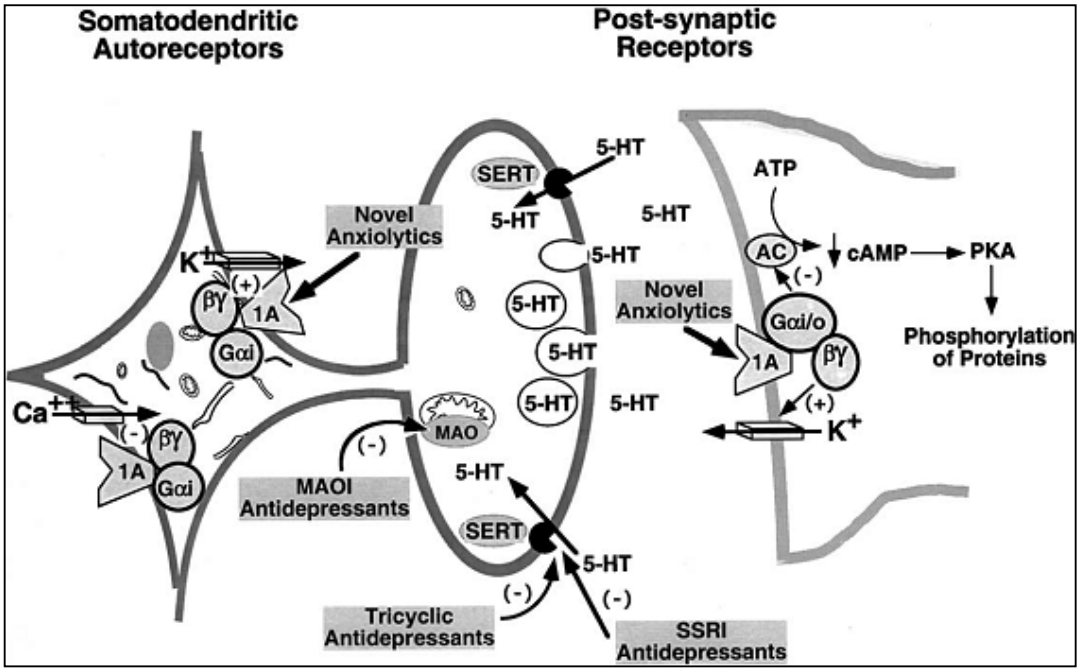

Figure 1. Anxiolytic and Antidepressant Drug Effects on Serotonergic Neurotransmission (Adaptation from Hensler *et al*.[7]). The 5-HT_{1A} receptor is located on serotonergic cell bodies and dendrites, functioning as the somatodendritic autoreceptor. Several compounds are agonists at the 5-HT_{1A} receptor, comprising anxiolytic and antidepressant activity. By blocking the serotonin transporter (SERT) or inhibiting monoamine oxidase (MAO), antidepressant drugs increase the synaptic concentration of the serotonin neurotransmitter (5-HT).

The piperazines (Figure 2) are an important family of compounds with vast pharmacological properties from their interactions with several 5-HT receptors, in particular, the 5-HT_{1A}. However, one assumes that this family has the same mechanism of action and toxicity as amphetamines and ecstasy[11-16]. Actually, the most adverse effects are supposed to be due to the agonist interaction of the piperazines with the 5-HT_{2A}[17]. Indeed, Capela *et al*.[18-19] demonstrated, *in vitro*, that the overstimulation of 5-HT_{2A} receptor is responsible for the cortical neuron's death.

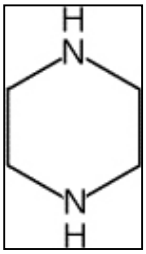

Figure 2. Piperazine functional group.

Furthermore, the adverse effects on 5-HT_{2A} receptors associated with piperazine consumption are usually schizophrenic symptoms and cerebral cortex disorders[18,20], in part, due to the 5-HT_{2A} receptors agonist interaction. On the other hand, the 5-HT_{1A} receptors are responsible for a central modulation of affective disorders, such as anxiety and depression, revealing an enormous potential for antipsychotic drugs[21], as previously referred.

Our goal consisted on identifying the chemical and molecular properties in the piperazine family that determine the selectivity and the affinity for 5-HT_{1A} and 5-HT_{2A} receptors. So, it is possible to improve drug design for compounds with more affinity and selectivity for the 5-HT_{1A} receptor. For this purpose, mathematics and statistics methods were used for our analysis from the arylpiperazines in relation to the two receptor targets.

In the last decade, several Quantitative-Structure Activity Relationships (QSAR) studies were made of arylpiperazines their

chemical and molecular properties relevant to the 5-HT_{1A} and 5-HT_{2A} receptors, as well, to pharmacophores[22-25]. It was shown that electro-topologic structure and substructure distances were the principal factors involved in the structure-activity correlation, although they also were related to the compounds' lipophilicity25.

The structural diversity of 5-HT_{2A} ligands represents a challenge for pharmacophore definition, although some proposed models exist, as we already referred. Tammy *et al.*[26] believed that the presence of a basic nitrogen group as a central point of the ligand-receptor interaction maybe questionable. Indeed, their research illustrated, at least in the piperidine family, that the basic nitrogen group substitution didn't affect ligand's afinity in relation to 5-HT_{2A} receptors and reduced, actually, the possibility of interaction with other receptors. So, our research tried a new biochemical interaction approach that makes possible the design of new drugs and computational simulations.

MATERIALS AND METHODS

Data Set

The pharmacophore characterizations were developed from a group of molecular descriptors (1D, 2D and 3D) obtained from eDragon through a study set of a hundred and twenty four arylpiperazine derivates (Tables 1-7 in the annexes).

Computational methods

Structures of all arylpiperazine derivates were drawn on the ChemDrawn[27] software package, pre-optimized by molecular mechanics using the MM2 force field. The final structure was obtained by subsequent optimization with AM1 semi empirical Hamiltonian, implemented in the MOPAC 6.0 program[28].

Molecular descriptors (n=1666) were calculated for each molecular structure using eDragon software[29-32] while the appropriate descriptor selection was made by means of the genetic algorithm (GA) program designed in Matlab v7.0 for this purpose. After a previous analysis of the initial descriptor set considering: a variability of more than 90% by descriptor and the elimination of the descriptors that present more than 80% of correlation between them, we obtained 572 and 573 final descriptors from eDragon for the 5-HT_{1A} and 5-HT_{2A} receptors, respectively.

For GA analyses[33,34], the procedure was composed of 600 chromosomes chosen by a probabilistic form and the crossover was performed uniformly and with a probability of 50%. This procedure was fixed at 1 000 effective iterations. Next, the mutation procedure was carried out by changing the genes (variables) of the chromosomes with a fixed probability (50%). The crossover/mutation steps were repeated several times until they had reached a fixed stop criterion. In our model, the stop criterion was fixed at 100 000 iterations. The new chromosomes obtained after crossover/mutation procedure were evaluated with the objective function of leave-one-out internal cross-validation (Q^2_{LOO}) and was only included in the population if the Q^2_{LOO} value was higher than any of the chromosomes already considered in the initial population.

To avoid procedure complications by the structural diversity present in the piperazine's data set, we performed the subdivision of the initial number of piperazines into five subgroups by their structural similarity through cluster analysis from Moloc software[35,36]. For each cluster, a model was obtained by the combination of the GA and other parameters for model validation, such as the bootstrap internal cross-validation (Q2BOO) and the leave-multiple-out internal cross-validation (Q^2_{LMO}). The Q^2_{LMO} was calculated considering only a group of molecules (around 33%) for the model construction. While the Q^2_{BOO}, a more accepted internal cross-validation, was calculated by taking samples randomly and repeatedly of size N (where N is the molecules number) for the construction of the model and predicting the remaining molecules. This procedure was repeated several times (we used 5000 repetitions) and the Q^2_{BOO} represented the mean predictability coefficient. For the multiple linear regression (MLR) evaluation we used the Statistical Package[37,38]. Therefore, we elaborated the pharmacophore model of 5-HT_{1A} and 5-HT_{2A} receptors, after flexible alignment. The pharmacophore groups identification as well as the flexible alignment were performed with Moloc software[36].

RESULTS AND DISCUSSION

The vast number of molecular descriptors obtained in eDragon software were simplified by eliminating the low variation and high correlated descriptors in the data set and, therefore, eliminating a large part of background noise from the essential information in our descriptors. The next step consisted of cluster analysis and, consequently, the formation of subgroups from initial piperazines set by the application of the respective method in Moloc software35 (Figure 3). That previous procedure allowed us to get five groups of piperazines with high structural similarity and made possible a better extrapolation of the mutual information shared by the flexible alignment in each group.

Figure 3 presents the initial study set (129 piperazines) division into several clusters by structural similarity shared between them. The number of clusters is proportional to structural similarity shared by the molecules in each subgroup. The selected clusters are pointed out in the figure by the numbers (1-5), which we believe to be a better equilibrium between the chemical characterization and the application domain.

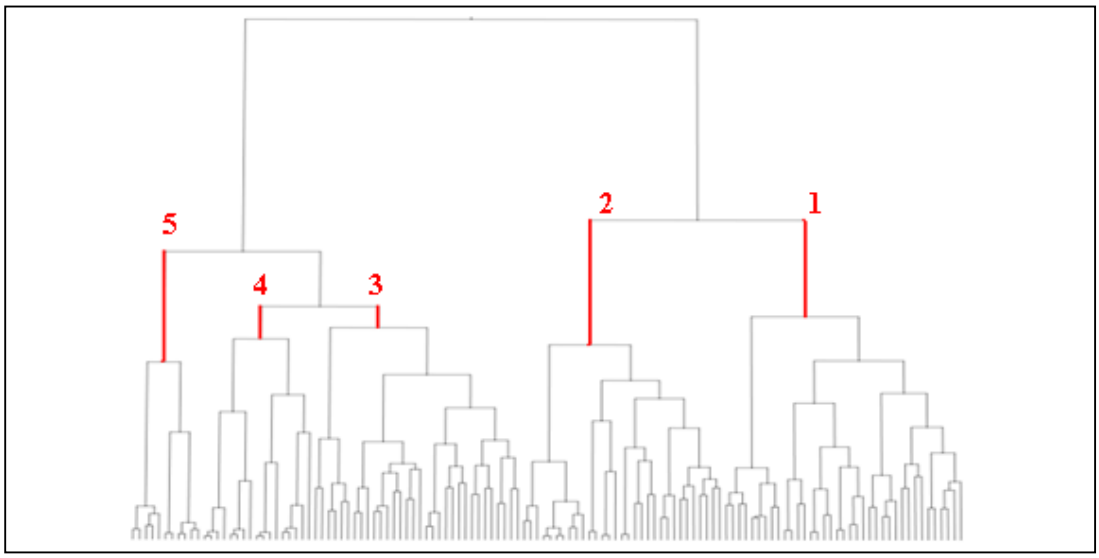

Figure 3. Dendogram obtained by the Moloc cluster analysis of the initial molecular set. The selected subgroups (1 until 5) are marked in red.

It is interesting to note that the five clusters cloud did not point up the same molecules for each receptor (as can be observed in Table 1), because not every molecule from the study set showed simultaneously a known pKi for 5-HT_{1A} and 5-HT_{2A} receptors. For each selected cluster a predictive model was obtained with the combination of the GA and the validation method previously referred. The obtained models and the selected descriptors are presented in the Tables 2 and 3.

Table 1. The number of compounds obtained in each cluster for 5-HT_{1A} and 5-HT_{2A} receptors from cluster analysis in Matlab software.

	Cluster 1	*Cluster* 2	*Cluster* 3	*Cluster* 4	*Cluster* 5	Total
N° of 5-HT_{1A} compounds	32	22	32	17	11	114
N° of 5-HT_{2A} compounds	16	23	24	16	11	90

Table 2. Predictive 5-HT_{1A} models and statistical parameters obtained by GA-MLR.

5-HT$_{1A}$ Models	***Cluster 1***	pK_{1A} = 11.437(1.80) – 4.254(0.73) *GATS4m* + 0.148(0.04) *Mor02m* – 53.062(9.77) *HATS7m*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.845	0.715	23.387	<0. 01	0.621	0.320	0.550
	Cluster 2	pK_{1A} = 7.166(0.175) + 0.688(0.150) *nCs* – 1.094(0.157) *nRNR2* + 0.453(0.125) *C-025*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.912	0.832	29.794	<0. 01	0.745	0.610	0.690
	Cluster 3	pK_{1A} = 5.708(0.117) *RCI* – 0.203(0.046) *RDF140m* – 3.422(0.588) *Mor28v*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.997	0.994	1613.529	<0. 01	0.642	0.400	0.380
	Cluster 4	pK_{1A} = 19.211(1.781) – 0.410(0.100) *MAXDN* – 12.660(1.606) *MATS2v* – 72.323(11.768) *G1e*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.949	0.900	39.087	<0. 01	0.829	0.813	0.670
	Cluster 5	pK_{1A} = – 3.667(0.951) + 2.990(0.361) *PJI3* – 0.021(0.007) *Mor02u* + 20.283(1.301) *Du*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.990	0.980	112.744	<0. 01	0.960	0.898	0.895

Note: The number in (…) is the standard error of the coefficient.

Table 3. Predictive 5-HT_{2A} models and statistical parameters obtained by GA-MLR.

5-HT$_{2A}$ Models	***Cluster 1***	pK_{2A} = 12.301(0.625) + 14.119(1.248) *MATS1v* – 1.489(0.176) *GATS8m* – 0.113(0.024) *RDF045m*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.971	0.942	64.974	<0. 01	0.928	0.870	0.870
	Cluster 2	pK_{2A} = 7.761(0.573) – 1.162(0.182) *Mor15m* + 5.072(1.350) *E3u* + 0.713(0.139) *C-025*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.909	0.827	30.268	<0. 01	0.776	0.740	0.670
	Cluster 3	pK_{2A} = 3.328(0.423) + 0.110(0.013) *RDF065m* + 0.144(0.044) *Mor03m* + 162.926(23.488) *R4v+*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.907	0.822	30.806	<0. 01	0.751	0.570	0.710
	Cluster 4	pK_{2A} = 24.826(1.930) – 24.947(3.033) *P2u* – 31.617(3.391) *Du* – 1.451(0.301) *nHDon*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		0.952	0.906	38.608	<0. 01	0.848	0.870	0.800
	Cluster 5	pK_{2A} = 6.219(0.479) *DISPe* + 20.623(1.163) *G1u* + 0.492(0.034) *nCb-*						
		R	***R²***	***F***	***P***	Q^2_{LOO}	Q^2_{LMO}	Q^2_{BOO}
		1.000	1.000	12893.54	<0. 01	0.975	0.980	0.940

Note: The number in (…) is the standard error of the coefficient.

It's important to remember that the interpretation of molecular properties are extremely difficult, in particular, some classes of eDragon descriptors. Therefore, we used a correlation matrix analysis between the selected descriptors of each cluster and the easily interpretable descriptor family, such as constitutional descriptors, functional groups, atom-centred fragments and few other descriptors, to a better understanding of the structural similarity criteria applied in pharmacophore models by Moloc

software[35]. This procedure allowed us to observe, thought an understanding way, the shared molecular information common in the interaction with both subtypes of serotonergic receptors and compare these molecular aspects with previous researches[22-26].

The GA selected descriptors are strongly related to flexibility (number of rotatable bonds), molecular weight, aromatic substructure and atom type involved in the aromatic substitution; and all they represent the principal factors involved in 5-HT receptor selectivity and affinity. Besides the principal piperazine group, both receptors had affinity to aromatic groups with strong electrotopological or electronegative substituents and the presence of RCONHR bonds in the molecule, which are similar to the peptide bonds observed in several intrinsic proteins in humans. The two subtypes of serotonin receptors shared an empathy with heteroatoms' *alpha* carbons, such as oxygen and nitrogen elements, and aliphatic amines. On the opposition, we observed the lack of empathy with the bromine element and some functional groups, more exactly -$N(CO)_2$ and -CX_3 (where X is a certain chalcogen or halogen element).

On the one hand, the 5-HT_{1A} receptor showed more specificity for arylpiperazines with chalcogens, such as oxygen and sulphur elements, in particular. On the other hand, the 5-HT_{2A} receptors had more affinity for arylpiperazines with halogens, such as fluorine and chlorine elements. Besides all that, both receptors revealed a crescent affinity when those periodic elements were present in the arylpiperazine aromatic structures at *ortho*- and *metha*- positions, in particular. The effect of halogen or chalcogen elements on the arylpiperazine selectivity is probably related to the spatial arrangement of the group in the aromatic ring and its influence on the final arylpiperazine conformation, as observed by Gaillard *et al.*[24] and López-Rodríguez *et al.*[25].

Although molecular weight and saturation index were more or less the same in each cluster, we noted a very small tendency for the 5-HT_{1A} receptor when those factors were slide arises. While 5-HT_{2A} receptor had more affinity for arylpiperazines with less molecular weight and saturation index, the number of substituents in aromatic group of arylpiperazine, which allowed rotative bonds, was more daring for the 5-HT_{2A} receptor.

In pharmacophore construction, we selected one group of molecules that demonstrate an elevated affinity index for one receptor and, simultaneously, a minor affinity index for the other receptor. So those subgroups from the study set should represent the most important intrinsic physico-chemical properties for each serotonin receptor and, therefore, facilitate our interpretation. Next, each previous subgroup was submitted to flexible alignment by a superposition algorithm from Moloc software[35]. This made possible the maximum superposition of molecules and their saturated bonds, in particular. So, common molecular properties were valued in pharmacophore model construction (Figure 4).

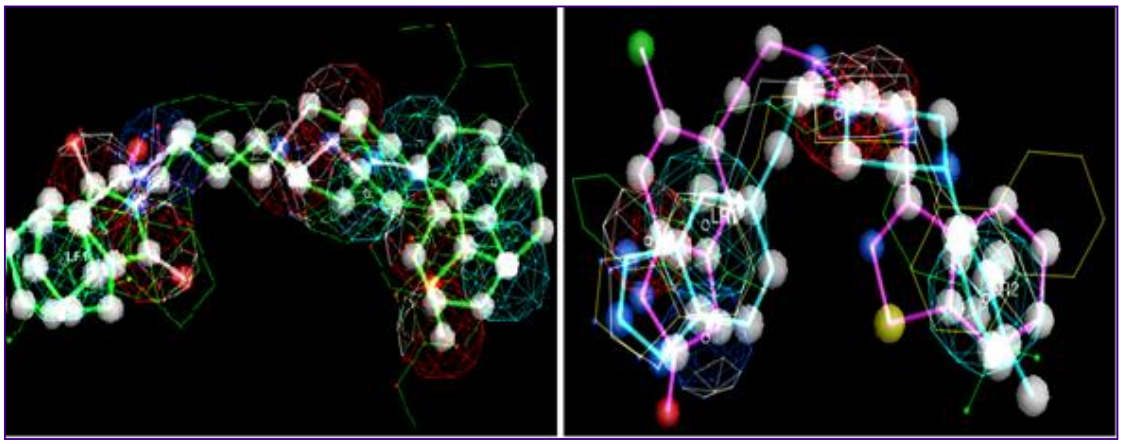

Figure 4. Illustrations of 5-HT_{1A} and 5-HT_{2A} pharmacophores in the left and right pictures, respectively, obtained from Moloc software.
Legend - The pictures of the spheres representing specific three-dimensional conformational characteristics, more exactly: Dark blue sphere - Hydrogen atom donors (acid group). Light blue sphere - Aromaticity. Red sphere - Hydrogen atom acceptors (base group)

Figure 4 show a basic centre in both models, more exactly, the existence of a proton acceptor in the N4 functional group from piperazine (red sphere), while the light blue sphere demonstrated the steric effect due to aromatic substructure predominance around the N4 atom. However, the 5-HT_{2A} model exhibited a more steric shield without losing the N4 atom's basic character. The presence of electronegativity group density, such as the carbonyl group (C=O), shared proportionately a certain basic character in the molecule and it was more visible in 5-HT_{1A} model. Furthermore, acid groups (dark blue sphere) revealed a higher influence in the 5-HT_{2A} receptor interaction, instead of the 5-HT_{1A} receptor, due to their positions in relation to hydrophobic centres of arylpiperazines. These results were in accordance with Chidester *et al.*[35]. In spite of all this, the pharmacophore constituted a standard model from the common properties given by certain number of molecules and, so, was dependent on local molecular properties from some privileged structures. The N4 atom's basic character, the carbonyl group and the aromatic rings' pi (π) density were some examples of such privileged structures present in our study set.

As we can note, both pharmacophore models share some common characteristics, such as aromatic rings and one basic group, mainly a basic nitrogen group. Actually, those structures had the predisposition to form a triangular and linear rearrangement20,26. We observed a major complexity in the 5-HT_{2A} pharmacophore due to the existence of carbonyl group between a N4 atom (see Figure 5) and an aromatic ring in some arylpiperazines (molecules 78, 92, 128 and 129 in the annexes). These circumstances tend to modify the pharmacophore's model itself but preserving its high affinity for the receptor.

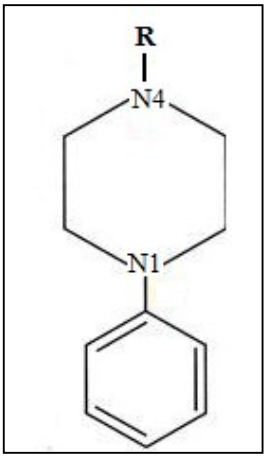

Figure 5. Representation of N4 atom present in the arylpiperazine functional group.

In the performed analysis we studied the properties of the arylpiperazines family principally those aspects related to the aromatic neighbouring. Therefore, the analysis of ligand-receptor interaction needs a further study, such as molecular docking, for a better understanding of how the analysed molecular properties are adjusted with the local environment of the actives sites in the 5-HT receptors.

CONCLUSIONS

We can conclude that arylpiperazines family share similar aromatic structure properties of interaction with 5-HT_{1A} and 5-HT_{2A} receptors, such as i) strong electrotopologic or electronegative substituents, ii) substructures with hydrogen donor and acceptor groups between aromatic group and N4, iii) N and O elements as substituents in benzene (Bz-N1-N4) with the capacity of establish hydrogen bonds, iv) and antipathy with Br element and specific groups ($N(CO)_2$ and CX_3, X = halogen or chalcogen).

Furthermore, there also exits diverse substructures that allowed altering the intrinsic arylpiperazine affinity to select a specific subtype of 5-HT receptor. The 5-HT_{1A} pharmacophore model studied demonstrate that i) a basic group high accessibility, ii) an electronegative substructure near the ortho position aromatic ring and iii) and opposite lipophilic and electrostatic effects in the nitrogen substituent were extremely important for the affinity of arylpiperazines family. In particular, the presence of aromatic substructures at *ortho-* and *metha-* positions, inhibition of their rotative bonds and the attenuated increase of the saturation index and molecular weight are chemical properties that allowed the increase of arylpiperazines' affinity for the 5-HT_{1A} receptor to the detriment of the 5-HT_{2A} receptor.

Finally, arylpiperazines without H-donor groups or halogen atoms and with electronic density or chalcogens (e.g. O and S) close to the benzene *ortho-* and *metha-* substitution position were associated with high increment of 5-HT_{1A} receptor affinity. Our study allows confirming previously experimental data and, more importantly, to understand the electrotopologic and three-dimensional. arylpiperzine's substructures importance in the selectivity of subtype 5-HT receptors.

However, ligand-receptor interaction properties need further investigation thought molecular docking or other computational approach.

REFERENCES

1. Araneda R, Andrade R. 5-Hydroxytryptamine2 and 5-hydroxytryptamine1A receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 1991; 40: 399-412.

2. Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F. Control of serotonergic function in

medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. J Neurosci 2001;21: 9856-9866.

3. Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2003; 27: 1159- 1172.

4. Millan MJ. Improving the treatment of schizophrenia: focus on serotonin 5-HT_{1A} receptors. J Pharmacol Exp Ther 2000; 295: 853- 861.

5. Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY. 5-HT_{2A} and D_2 receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. J Neurochem 2001; 76: 1521-1531.

6. Cai X, Gu Z, Zhong P, Ren Y, Yan Z. Serotonin 5-HT_{1A} receptors regulate AMPA receptor channels through inhibiting Ca2+/calmodulin-dependent kinase II in prefrontal cortical pyramidal neurons. J Biol Chem 2002; 277: 36553-36562.

7. Hensler JG. Regulation of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. Life Sciences 2003; 72: 1665-1682.

8. Hensler JG, Kovachich GB, Frazer A. A quantitative autoradiographic study of serotonin1A receptor regulation: Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 1991; 4:131-44.

9. Kia HK, Miquel MC, Brisorgueil MJ, Daval G, Riad M, El Mestikawy W, Hamon M, Verge D. Immunocyotchemical localization of serotonin1A receptors in the rat central nervous system. Journal of Comparative Neurology 1996; 365: 289-305.

10. Vergé D, Daval G, Marcinkiewicz M, Patey A, El Mestikawy S, Gozlan H, Hamon M. Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. Journal of Neuroscience 1986; 6: 3474-82.

11. Wood DM, Dargan PI, Button J, et al. Collapse, reported seizure-and an unexpected pill. Lancet 2007; 369: 1490.

12. Balmelli C, Kupferschmidt H, Rentsch K, Schneemann M. Fatal brain edema after ingestion of ecstasy and benzylpiperazine. Dtsch Med Wochenschr 2001; 126 (28-29): 809-11.

13. Gee P, Richardson S, Woltersdorf W, Moore G. Toxic effects of BZP-based herbal party pills in humans: a prospective study in Christchurch, New Zealand. N Z Med J 2005; 118 (1227): U1784.

14. Wikstrom M, Holmgren P, Ahlner J. A2 (N-benzylpiperazine) a new drug of abuse in Sweden. J Anal Toxicol 2004; 28: 67-70.

15. de Boer D, Bosman I, Hidvégi E, Manzoni C, Benkö A, dos Reys L, Maes R. Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. Forensic Sci Int 2001; 121 (1-2): 47-56.

16. Sheridan J, Butler R, Wilkins C, Russell B. Legal piperazine-containing party pills - a new trend in substance Misuse. Drug and Alcohol Review 2007; 26: 335-343.

17. Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, Rothman RB. N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or 'Ecstasy'). Neuropsychopharmacology 2005; 30: 550-60.

18. Capela J, Ruscher K, Lautenschlager M, Freyer D, Dirnagl U, Gaio A, Bastos M, Meisel A, Carvalho F. Ecstasy-induced cell death in cortical neuronal cultures is serotonin 2A-receptor-dependent and potentiated under hyperthermia. Neuroscience 2006; 139: 1069-1081.

19. Capela JP, Fernandes E, Remião F, Bastos ML, Meisel A, Carvalho F. Ecstasy induces apoptosis via 5-HT_{2A}-receptor stimulation in cortical neurons. NeuroToxicology 2007; doi:10.1016/j.neuro.2007.04. 005.

20. Rowley M, Bristow LJ, Hutson PH. Current and Novel Approaches to the Drug Treatment of Schizophrenia. Journal of Medicinal Chemistry 2001; Vol. 44: No. 4: pp. 477-501.

21. Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, Touzard M, Verriele L, Audinot V, Millan MJ. Agonist and

antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [35S]GTPgS binding study. Eur J Pharmacol 1998; 355: 245- 56.

22. Hibert MF, McDermott I, Middlemiss DN, Mir AK, Fozard JR. Eur J Med Chem 1989; 24: 31.

23. Kuipers W, Van Wijngaarden I, Kruse CG, Ter Horst-Van AM, Tulp MThM, IJzerman AP. N4-unsubstituted N1-Arylpiperazines as High-Affinity 5-HT_{1A} Receptor Ligands. J Med Chem 1995; 38: 1942-1954.

24. Gaillard P, Carrupt P-A, Testa B, Schambel P. Binding of arylpiperazines, (aryloxy)propanolamines, and tetrahydropyridylindoles to the 5-HT_{1A} receptor: contribution of the molecular lipophilicity potential to three-dimensional quantitative structure-affinity relationship models. J Med Chem 1996; 39: 126-134.

25. López-Rodríguez ML, Ayala D, Benhamú B, Morcillo MJ, Viso A. Arylpiperazine derivatives acting at 5-HT_{1A} receptors. Curr Med l Chem 2002; 9: 443-469.

26. Tammy L, Amanda LB, Andrew M, Caroline Q, Smita P, Kerry Ch, Angus MM. A new class of selective, non-basic 5-HT_{2A} receptor antagonists. Bioorg Med Chem Lett 2006; 16: 3201-3204.

27. ChemDrawn Ultra 9.0. CambridgeSoft. 2004.

28. Frank J, version 2.0, 1993, MOPAC 2.0 Seiler Research Laboratory, US Air Force Academy, Colorado Springs.

29. Tetko IV. Computing chemistry on the Web. Drug Discov. Today 2005; 10: 1497-500.

30. Tetko IV, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko EV, Zefirov NS, Makarenko AS, Tanchuk VY, Prokopenko VV. Virtual computational chemistry laboratory - design and description. J Comput Aid Mol Des 2005; 19: 453-63.

31. Tetko IV, Kovalishyn VV and Livingstone DJ. Volume learning algorithm artificial neural networks for 3D QSAR studies. J Med. Chem 2001; 44: 2411-20.

32. Tetko IV, Luik AI, Poda GI. Applications of neural networks in structure-activity relationships of a small number of molecules. J Med Chem 1993; 36: 811-4.

33. Cavill R, Keun HC, Holmes E, Lindon JC, Nicholson JK, Ebbels TM. Genetic algorithms for simultaneous variable and sample selection in metabonomics. Bioinformatics 2009; 25: 112-118.

34. Yasri A, Hartsough D. Toward an optimal procedure for variable selection and QSAR model building. J Chem Inf Comput Sci 2001; 41: 1218-1227.

35. Chidester CG, Lin C, Lahti RA, Haadsma-Svensson SR, Smith MW. Comparison of 5-HT_{1A} and Dopamine D_2 Pharmacophores. X-ray Structures and Affinities of Conformationally Constrained Ligands. Journal of Medicinal Chemistry 1993; 36: 10.

36. Gerber PR. Topological Pharmacophore Description of Chemical Structures using MAB-Force-Field-Derived Data and Corresponding Similarity Measures. In: Carbó-Dorca R, Giromés X & Merzey P eds. Fundamental of Molecular Similarity; Kluwer Academic/Plenum Publishers, New York, 2001, 67-82.

37. STATISTICA 6.0 Statsoft_Inc. 2001.

38. Young DC. Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems. John Wiley & Sons, Inc, 2001.

Comment of the reviewer Prof. Pilar Muñiz Rodríguez PhD. Titular del Área de Bioquímica y Biología Molecular de la Facultad de Ciencias de la Universidad de Burgos. España.

The piperazines are a family of chemical compounds with different pharmacological properties including those arising from the

result of interaction with serotonin receptors. The authors, by computational methods, establish a relationship between the structure of the interaction with different piperazines with two types of 5-HT receptor antagonists.

The influence of the substituents of benzene ring as the molecular weight of arilpiperizinas was discussed and a model for undestand the substructures importance in the selectivity of subtype 5-HT was established. Further in vivo studies are needed to confirm the data that the autors observed using computational model.

Comment of the reviewer Prof. Amalio Garrido Escudero PhD. Head Environmental Engineering and Toxicology Dpt. Universidad Católica S. Antonio. Guadalupe. Murcia. España.

The authors have developed an extraordinary effort using a highquality set of tools. Bibliography is generous and it is very well updated.

Received: April 21, 2009. Received reviewed: July 15, 2009
Published July 21, 2009

ANNEXES

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

COMPORTAMIENTO DE LAS REACCIONES ADVERSAS A MEDICAMENTOS EN CUBA. AÑO 2007

Ismary Alfonso Orta MD, Giset Jiménez López MD, Jenny Ávila Pérez, Ashley Chao Cardeso.

Unidad Nacional Coordinadora de Farmacovigilancia. CDF, Cuba

isma.alfonso @ infomed.sld.cu

Rev Electron Biomed / Electron J Biomed 2009;2:20-29

Comentario del revisor Dr. Martín de Frutos Herranz PhD. **Fisiología humana. Escuela Universitaria de Enfermería. Universidad de Burgos. Burgos, España**

Comentario del revisor Dr. Enrique Seoane Vázquez. **Doctor en Farmacia. Fármacoeconomista. Profesor de Ohio State University Columbus. Columbus. USA**

RESUMEN:

Introducción. La farmacovigilancia es una actividad de salud pública destinada a la identificación, evaluación y prevención de los riesgos asociados a los medicamentos una vez comercializados. En Cuba existe un sistema de Farmacovigilancia con una tasa elevada de reporte de efectos adversos por medicamentos (7000 a 10 000 casos anuales).

Desarrollo. Se realizó un estudio farmacovigilancia, descriptivo, transversal y retrospectivo, que utilizo la Metodología y Procedimientos de Trabajo de la Unidad Coordinadora Nacional de Farmacovigilancia, donde se analizaron todos los reportes de RAM llegados a la unidad durante el 2007 procedentes de todo el país.

Resultados: Se analizaron 6928 notificaciones de reacciones adversas medicamentosas (RAM), notificándose 12963 RAM a razón de 1.9 RAM por notificación, de ellas 4251 fueron reacciones importantes (61.3%) según criterios establecidos por la unidad coordinadora nacional de farmacovigilancia de Cuba. Los sistemas de órganos más afectados durante el año fueron piel y anejos (1774, 25.6%) seguido del tracto gastrointestinal (1438, 20.7%). Entre los fármacos con mayor numero de reportes se encontró captopril (418/6.03%), el ibuprofeno 289 / 4.2% y ciprofloxacina 259/3.7%. Predominaron las RAM probables (68.7%) y moderadas 47.1% y las más frecuentes fueron erupción cutánea, vómitos y fiebre. Entre las asociaciones fármaco - RAM muy importantes y con baja frecuencia de aparición se reportaron en total unas 2953 (35.9%) en el año, de ellas el 9.1% fueron reacciones no descritas en la literatura revisada.

Conclusiones: se detectaron entre una o dos reacciones adversas a medicamentos por cada notificación realizada. Dejando claro la importancia en la selección de los medicamentos y su uso racional. Los fármacos más asociados a las reacciones adversas notificadas fueron captopril, ciprofloxacina e ibuprofeno, la piel y el sistema digestivo fueron los sistemas más afectados y las reacciones adversas que predominaron fueron las moderadas y probables. Hay que monitorizar las asociaciones fármacos - RAM de baja frecuencia así como las reacciones graves o inesperadas que puedan ocurrir para lograr una adecuada valoración

beneficio - riesgo de los fármacos de que disponemos en el mercado cubano.

PALABRAS CLAVE: reacciones adversas a medicamentos, farmacovigilancia Cuba, efectos indeseables.

SUMMARY:

Introduction: Pharmacovigilance is an activity of public health for the identification, assessment and prevention of risks related to medicines once marketed. In Cuba there is a pharmacovigilance system with a high reporting rate of adverse drug effects (7000 to 10 000 cases annually).

Methods: We developed a descriptive, transversal and retrospective, pharmacovigilance study, which used the methodology and working procedures of the Cuban Coordinating Unit of Pharmacovigilance, and analyzed all reports of ADR received in 2007 country wide.

Results: We analyzed 6928 reports of adverse drug reactions (ADRs) which included 12,963 adverse effects, with a rate of 1.9 ADR per report form. Among them 4251 were significant reactions (61.3%) according to criteria established by the coordinating unit of pharmacovigilance in Cuba. The most affected organ systems during the year were skin and appendages (1774, 25.6%) followed by the gastrointestinal tract (1438, 20.7%). The drug with the highest number of reports was captopril (418/6.03%), followed by ibuprofen (289/4.2%) and ciprofloxacin (259/3.7%). According to intensity, mild ADRs predominated (68.7%) and the other 47.1% corresponded to moderate ones. The most frequent adverse effects were cutaneous eruption, vomiting and fever. Very important associations drug - ADR of low frequency of occurrence were reported (2953/35.9%), from them 9.1% were reactions not described in the literature reviewed.

Conclusions: The Cuban system develops a serious work on drug surveillance; there is no drug without adverse effects, making clear the importance in the selection of medicines and their rational use. The follow up of associations drug-ADR serious and low frequency or unexpected, is necessary to achieve an adequate benefit-risk evaluation.

KEY WORDS: adverse drug reactions, pharmacovigilance, adverse effects, drug surveillance.

INTRODUCCIÓN

La Farmacovigilancia ha sido y es, una materia de las ciencias de la salud con un alto contenido técnico y científico, pero que además impacta directamente sobre la sociedad. Es el conjunto de procedimientos y actividades encaminadas a la detección registro, notificación e información de reacciones adversas, ocasionadas por los medicamentos después de su aprobación y registro. Esto se realiza con el fin de determinar posible causalidad, frecuencia de aparición, gravedad y establecer las medidas preventivas que llevan al uso más racional de medicamentos y a la optimización de la relación Beneficio-Riesgo. Además nos permite identificar nuevos datos sobre riesgos y prevenir daños en los pacientes[1]

La incidencia de reacciones adversas medicamentosas (RAM) encontrada en diversos estudios varía entre el 1% y el 30% debido a la utilización de diferentes metodologías para la detección y evaluación de las RAM. Sin embargo la mayoría de los estudios prospectivos muestran que la incidencia de RAM en los pacientes hospitalizados (excluyendo los pacientes con reacciones leves) es entre el 10% y el 20%[2-3].

La admisión de pacientes a los hospitales por causa de reacciones adversas a medicamentos es entre el 3% y el 7%. Las muertes inducidas por medicamentos son raras y ocurren en el 0.5% y el 0.9% de los pacientes hospitalizados[4-5]. En Cuba el sistema de farmacovigilancia esta integrado por un total de 16 unidades provinciales. Se ha implementado en todo el país un programa de vigilancia para todos los profesionales y técnicos de la salud, con un único método de validación e identificación de los riesgos asociados a los medicamentos comercializados[6].

El desarrollo de la Farmacoepidemiología en Cuba en el año 1996, a través de su centro rector, el Centro para el Desarrollo de la Farmacoepidemiología (CDF) surge por la necesidad de contar con una disciplina que abarcara todos los aspectos relacionados con el medicamento durante su transito, desde la producción hasta el consumo. Descansando su estructura en una red nacional formada por 175 centros municipales de Farmacoepidemiología, ubicados en una farmacia seleccionada en cada municipio y que a partir de ese momento se conoce como Farmacia Principal Municipal (FPM)[6].

El CDF cuenta entre sus principales líneas de trabajo con la Farmacovigilancia y su Unidad Nacional Coordinadora[7]. Para esto se estableció la metodología a seguir en caso de notificación de sospecha de reacción adversa a medicamentos nacionales o importados, así como la Medicina Natural y tradicional[7].

Una reacción, al contrario de un acontecimiento, se caracteriza por la sospecha de una relación causal entre el fármaco y el episodio, atendiendo a la valoración de imputabilidad que haga el profesional sanitario[8-10].

El uso extendido del medicamento establece el alcance definitivo de los riesgos (efectos adversos) y de los beneficios (eficacia terapéutica) en sus diferentes indicaciones terapéuticas. Esto muestra la importancia de la farmacovigilancia para controlar el comportamiento del medicamento una vez que ha sido aprobado para su uso por la autoridad sanitaria[9].

Es reconocido el hecho de que no siempre se utilizan correctamente los medicamentos. Diferentes factores confluyen para generar esta situación, como la innumerable cantidad de fármacos que aparecieron en los últimos años, la información sesgada originada casi siempre en la industria farmacéutica y la falta de un análisis crítico de la información necesaria para una correcta selección de los medicamentos. Todo ello lleva a situaciones, a veces graves, con el uso de medicamentos[8-11].

Es por ello que el sistema cubano de farmacovigilancia permite alertar ante determinadas situaciones al Ministerio de Salud Publica para la toma de acciones específicas por ejemplo: retener lote de un medicamento, retirar un medicamento de la red asistencial por problemas de seguridad, etc. Además de ofrecer información que sirva de base para la docencia del personal de salud y para estudios epidemiológicos de fármacos. También genera señales de alerta sobre el comportamiento de los medicamentos en la población[9-11]. Todas estas informaciones son necesarias y de gran utilidad para la agencia reguladora de medicamentos (CECMED) en la toma de decisiones[7].

Es por ello que esta investigación se propuso analizar el comportamiento de las Reacciones Adversas a Medicamentos en Cuba durante el año 2007, identificándose los fármacos más sospechosos de producir reacciones adversas y clasificando las reacciones adversas según severidad, causalidad y frecuencia.

MATERIAL Y MÉTODOS:

Se realizo un estudio de farmacovigilancia descriptivo, transversal y retrospectivo donde se analizaron todos los reportes de RAM llegados a la base de datos nacional en la unidad nacional coordinadora de farmacovigilancia de Cuba durante el 2007 procedentes de todo el país.

Para ello se utilizo la Metodología y Procedimientos de Trabajo de la Unidad Coordinadora Nacional de Farmacovigilancia[12] la cual citamos a continuación. 1. Método de notificación espontánea de RAM y envío de notificaciones de sospechas:

- **Los profesionales sanitarios en Atención Primaria de Salud (APS), ante un paciente en el que observa un conjunto de signos, síntomas o alteración de algún examen de laboratorio, que hace sospechar una posible asociación con la utilización previa de un medicamento, deben iniciar el proceso de notificación. Pueden comunicarlo mediante el modelo 33-36-1 al jefe de grupo básico de trabajo o al director técnico de la farmacia comunitaria o al director de la farmacia principal municipal, que se lo entregarán al Farmacoepidemiólogo (Presidente del comité farmacoterapéutico municipal).**

- **Los profesionales sanitarios en los hospitales, ante un paciente, tanto ingresado como de cuerpo de guardia, en el que observa un conjunto de signos, síntomas o alteración de algún examen de laboratorio, que hace sospechar una posible asociación con la utilización previa de un medicamento, deben iniciar el proceso de notificación. Pueden comunicarlo mediante el modelo 33-36-1 al farmacoepidemiólogo del hospital o al director técnico-administrativo de la farmacia del hospital, estos se encargaran de velar por la calidad del llenado del modelo y de discutir las notificaciones en el seno del comité farmacoterapéutico**

- **La notificación de las sospechas RAM de frecuencia habitual de aparición se recibirán quincenalmente en la Unidad Coordinadora Nacional de Farmacovigilancia (UCNFv) mediante una base de datos en soporte magnético enviado por las Unidades provinciales, la cual será objeto de revisión por parte de los especialistas de la UCNFv.**

- **Una vez revisada cada sospecha de RAM, si cumple con los parámetros establecidos en la misma pasará a engrosar la base de datos nacional**

- **La notificación de todas las sospechas de RAM graves y mortales se notificarán vía telefónica y/o correo electrónico por la UCNFv, inmediatamente después de haber ocurrido la misma (siempre antes de las 24 horas), enviando posteriormente, y antes de los 10 días posteriores, la planilla electrónica (modelo 33-36-1) y la discusión del grupo de expertos provincial.**

Las reacciones fueron codificadas y clasificadas según sistema de órganos de acuerdo al diccionario de reacciones adversas de la

OMS (WHO Adverse Reaction Dictionary) y los fármacos de acuerdo al sistema ATC (Anatomical Therapeutical Chemical Classification). Se utilizaron los criterios de causalidad nombrados por la OMS (definitiva, probable, posible, condicional y no relacionada) y de acuerdo a su gravedad se clasificaron las RAMs en: leves: cuando los síntomas y signos fueron fácilmente tolerados y no requirieron cambio de terapéutica ni antidoto terapia. Moderadas: cuando hay malestar suficiente que interfiere con la actividad usual, y requirieron observación o cambio de terapéutica y no necesariamente se precisó la suspensión del fármaco. Graves: cuando se puso en peligro la vida del paciente y por tanto requirieron la suspensión del fármaco causante de la reacción y la administración de un tratamiento específico para contrarrestarla. Letales: cuando contribuyeron directa o indirectamente a la muerte del paciente[13]. La información obtenida fue procesada a partir de base de datos nacional de farmacovigilancia en el programa Excel, donde existe un campo que muestra si la reacción adversa reportada fue causa de ingreso hospitalario, lo cual nos aproxima al conocimiento de los reportes de RAM que motivaron ingreso en unidades asistenciales hospitalarias. Se utilizaron como medidas de resumen los números absolutos y porcentajes. Los resultados fueron presentados en tablas y gráficos.

RESULTADOS:

La Tabla 1 muestra la frecuencia de casos de reacciones adversas que motivaron ingreso hospitalario, y la detección de RAM moderadas y graves, reportadas en la base de datos nacional de farmacovigilancia, entre los años 2003-2007.

Tabla 1. Frecuencia de ingresos por RAM y severidad reportadas al Sistema Cubano de Farmacovigilancia. Años 2003-2007.

Año	Total de reportes de RAM / año	Casos de RAM que motivaron ingreso	%	Casos graves y moderados	%
2003	12601	1007	0.8%	6027	47.8
2004	7063	397	5.6%	3651	51.7
2005	7025	272	3.9%	2807	39.9
2006	8261	349	4.2%	3714	44.9
2007	6929	271	3.9 %	3357	48.5

Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia[3].

Durante el año 2007 se recibieron 6929 notificaciones de RAM, notificándose 12963 RAM a razón de 1.9 RAM por notificación, de ellas 4251 fueron reacciones adversas importantes para un 61.3% (según criterios para determinar RAM importantes de la UCNFv, en las Normas y procedimientos de trabajo del Sistema cubano de Farmacovigilancia) 12. La tasa de notificación anual fue de 615 notificaciones por millón de habitantes.

En notificaciones del año 2007, según grupos de edades, predominaron las RAM en adultos 59.8%, seguido de la población geriátrica con un 20.5% y los niños con un 19.4%. La distribución por sexo siguió igual comportamiento que en años anteriores, siendo el sexo femenino el más relacionado con sospechas de RAM (4436 notificaciones, 64,0%).

La relación de causalidad según el algoritmo de Karch y Lasagna[13] se estableció para las RAM (Tabla 2). El 68,7% de las RAM se consideran reacciones probables.

Tabla 2. Distribución de las reacciones adversas según causalidad. 2007.

Causalidad	No	%
Definitivas	182	2.6
Probables	4759	68.7
Posibles	1392	20.1
Condicionales	584	8.4
No Relacionadas	11	0.1

Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia

En la Tabla 3 se muestran la distribución de las notificaciones según severidad durante los años 2005, 2006 y 2007.

Tabla 3. Distribución de notificaciones de RAM según severidad. 2005-2006-2007.

Severidad	2005		2006		2007	
	Nº	%	Nº	%	Nº	%
Leves	4218	55.0	4547	60.0	3550	51.2
Moderadas	2699	42.5	3510	38.4	3262	47.1
Graves	85	2.2	185	1.2	95	1.4
Mortales	23	0.2	19	0.3	20	0.3

Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia

Los sistemas de órganos más afectados durante el año fueron piel y anejos (1774, 25.6%), gastrointestinal (1438, 20.7%), sistema nervioso central (1024, 14.8%), general (801, 11.6), cardiovascular (688, 9.9%) y respiratorio (519, 7.5%).

Las RAM más frecuentes fueron erupción cutánea, vómitos, fiebre, cefalea, epigastralgia, tos, taquicardia, mareos, hipotensión, náuseas, urticaria y disnea. Las RAM fueron en su mayoría leves y conocidas[14-15].

Se reportaron un total de 362 notificaciones a tratamientos de medicina natural y tradicional, representando el 5.2% del total de notificaciones. Este tipo de RAM aumento considerablemente en relación al año anterior, considerandose uno de los logros alcanzado durante el año.

La Tabla 4 muestra los medicamentos que más se reportaron con RAM graves y tipo de RAM.

Tabla 4: Fármacos que produjeron mayor número de RAM graves. 2007.

Fármaco	Notificaciones	RAM
Penicilina RL	13	Anafilaxia, disnea, edema de glotis, shock anafiláctico, inconsciencia
Dipirona	8	Anafilaxia, inconsciencia
Acido acetilsalicílico	5	Hemorragia digestiva
Acido Nalidixico	5	Anafilaxia, convulsiones, edema de la glotis, sincope
Ciprofloxacina	5	Edema angioneurótico, necrolisis tóxica epidérmica, hemorragia digestiva
Espasmoforte	5	Bradicardia, shock anafiláctico, sincope, inconsciencia
Gluconato de calcio	5	Dolor anginoso, hipotensión
Estreptoquinasa recombinante	4	Hipotensión, inconsciencia
Penicilina cristalina	4	Shock anafiláctica, lipotimia, edema de la glotis
Amoxicilina	3	Edema de la glotis, shock anafiláctico
Vacuna pentavalente	3	Cianosis, shock
Digoxina	2	Bloqueo auriculoventricular
Ibuprofeno	2	Shock anafiláctico, síndrome renal rápidamente progresivo
Intacglobin	2	Bradicardia, cianosis
Ringer lactato	2	Cianosis

Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia

Los fármacos aminofilina, ampicilina, anís estrellado, atenolol, captopril, carbamazepina, cefotaxima, ceftazidima, ciclofosfamida, cisdiscloro diamino platino, citoprot P, dextrosa 50%, diatrizoato compuesto, enalapril, ifosfamida, ketamina, metoclopramida, nitroglicerina, penicilina benzatinica, propofol, salbutamol, teofilina, timolol, tioridazina, vancomicina, vincristina y warfarina también presentaron reacciones graves, que en total supusieron 95 notificaciones de RAM graves que

fueron reportadas en las primeras 24 horas de su conocimiento.

En la tabla 5 se muestran las sospechas de reacciones adversas medicamentosas con desenlace mortal en el año 2007.

Tabla 5: Sospechas de Reacciones Mortales Año 2007

FARMACO (No de casos)	REACCIÓN	OTROS FARMACOS	IMPUTABILIDAD
Cisplatino (2)	Mucositis	Bleomicina, metrotexate	Posible
	Leucopenia	Bleomicina, metrotexate, fluouracilo	No relacionada
Penicilina cristalina (2)	Shock anafiláctico	Teofilina	Probable
	Paro cardiorrespiratorio	lidocaína hiperbárica	Posible
Penicilina rapilenta (2)	Shock anafiláctico	-	Definitiva
	Paro cardiaco	Glibenclamida	Posible
L-Asparaginasa (2)	Shock cardiogénico	Prednisona, vincristina, doxorrubicina	Probable
	Coma	Prednisona, vincristina, daunorrubicina	posible
Albúmina (1)	Arritmia	Levamisol, factor de transferencia, fumarato ferroso, multivit (Retinol 5000 U, tiamina 2 mg, nicotinamida 20 mg, riboflavina 5 mg, vitamina D 400 mg)	Probable
Propofol (1)	Broncospasmo	-	Probable
Isoniacida (1)	Insuficiencia hepática	Rifampicina, pirazinamida, prednisona, polivit (tiamina 2.5 mg, riboflavina 1.6 mg, nicotinamida 20 mg, acido folico 0.25 mg, retinol 250 U, cianocobalamina 0.06 mg, piridoxina 2.0 mg)	Posible
Ciclofosfamida (1)	Mielosupresión	Adrianomcina	Posible
Metrotexato (1)	Leucopenia	Ciclofosfamida, 5-fluouracilo, nitropental, enalapril, hidroclorotiazida, verapamilo	Posible
Gentamicina (1)	Paro cardiorrespiratorio	Ciprofloxacina	Posible
Tiopental (1)	Shock anafiláctico	-	Probable
Vacuna Pentavalente (1)	Paro cardiaco	Paracetamol	Condicional
Intacglobin (1)	Shock anafiláctico	-	Posible
Halotano (1)	Paro respiratorio	Succinilcolina	Posible
Propiltiuracilo (1)	Insuficiencia hepática	Propranolol	Probable
Vincristina (1)	Anemia aplásica	Ciclofosfamida,bleomicina, adriamicina, etopósido, morfina	Posible

Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia

En total el presente año cerró con 20 sospechas de reacciones adversas mortales, prácticamente igual número que en el año anterior, de igual manera, los antimicrobianos mantienen su comportamiento.

Entre las asociaciones Fármaco - RAM muy importantes y con baja frecuencia de aparición se reportaron en total unas 2953 (35.9%) en el año, de ellas el 9.1% fueron reacciones no descritas en la literatura revisada. (Formulario Nacional de Medicamentos de Cuba)[16]. Los fármacos que más produjeron estos efectos se muestran en la tabla 6.

Tabla 6. Fármacos que más se relacionaron con efectos adversos de baja frecuencia de aparición. 2007.

FARMACO	Nº Casos (%)*	REACCION ADVERSA
Captopril	125 (29.3)	Disminución de la libido, opresión precordial, edema de la glotis, erupción cutánea
Dipirona	100 (46.5)	Edema angioneurótico, sialorrea, lipotimia, cianosis, eritema multiforme, broncospasmo
Ciprofloxacina	75 (28.5)	Inconsciencia, lipotimia, necrolisis tóxica epidérmica, hemorragia digestiva, glositis, vasculitis, sordera, confusión mental
Metoclopramida	41 (34.4)	Alucinaciones, desviación de la mirada, disnea, inconsciencia, nistagmo, pérdida de la visión, extrapiramidalismo
Vimang	40 (83.3)	Confusión, disnea, epigastralgia, disminución de peso, vértigo, taquicardia
Piroxicam	37 (40.2)	Anafilaxia, disnea, urticaria, confusión
Acido Nalidixico	24 (47.0)	Lipotimia, síncope, fotosensibilidad, fontanela abombada, convulsiones, fotofobia, percepción alterada de los colores
Metocarbamol	21 (67.7)	Alucinaciones, disnea, parestesia, hemorragia digestiva, urticaria
Atenolol	17 (22.4)	Disnea, disminución de la libido, parestesia, broncospasmo, claudicación intermitente
Aminofilina	15 (31.9)	Lipotimia, relajación de esfinter, paro cardiaco
Ajo	13 (30.9)	Hematomas, hematuria, gingivorragia, hipotensión arterial
Aloe	5 (13.9)	Alucinaciones, debilidad muscular, hematuria, hipopotasemia

*** Del total de reportes del fármaco. Fuente: Balance 2007. Unidad Coordinadora Nacional de Farmacovigilancia**

DISCUSIÓN:

En Cuba existe un sistema de Farmacovigilancia con una tasa elevada de reporte de efectos adversos por medicamentos (7000 a 10000 casos anuales). A través de los años el sistema ha perfeccionado la detección y análisis de los efectos adversos y ha aumentado el porcentaje de efectos adversos moderados y graves, que generalmente implican la atención de urgencia y/o ingreso hospitalario. A partir de la definición del concepto de RAM importante en septiembre del 2000 en Cuba se comenzó a ver una tendencia a disminuir la notificación de reacciones leves y aumentar las de moderadas, graves y mortales, y se produce un aumento de la calidad de los reportes enviados por las provincias, lo cual se muestra con los datos mostrados en los resultados de este estudio relacionados con la frecuencia de ingresos por RAM y severidad reportadas al Sistema Cubano de Farmacovigilancia durante los años 2003-2007.

De igual forma más de la mitad de los reportes fueron considerados reacciones adversas importantes que según los criterios del sistema cubano engloba a todos aquellos reportes en niños menores de 1 año y en embarazadas; reacciones adversas relacionadas con la vacunación y con la medicina natural y tradicional; aquellas reacciones adversas que comprometan la vida del paciente o que produzcan la muerte; así como reacciones adversas moderadas que afectan los sistemas nervioso central, cardiovascular, hemolinfopoyetico, piel y anejos, respiratorio, y genitourinario. El tipo de RAM reportado nos habla de un sistema que ha venido ganando en la calidad del reporte.

Los pacientes más afectados fueron del sexo femenino, correspondiendo con lo reportado en la literatura[1,4,14-15]. Esto puede estar relacionado con el mayor número de fármacos a los que están expuestas las mujeres, y por un uso inapropiado de los medicamentos mediante la automedicación.

En relación los grupos de edad, predominaron los reportes de RAM en los adultos; no se comportó de igual forma a lo descrito tradicionalmente en la literatura[1,4,14-15], pues son los ancianos la población de mayor riesgo, debido a que los ancianos consumen más medicamentos y no cuentan fisiológicamente con todas las condiciones necesarias para soportar la agresión inminente de estos fármacos; aunque cabe señalar que muchas de estas reacciones se pueden prevenir si se realiza una adecuada prescripción y vigilancia de estos pacientes. Esto podría ser debido a un subregistro de la notificación de las reacciones adversas en la población de ancianos lo cual es una limitación del método de notificación espontánea de RAM. El problema del subregistro también afecta a las RAM en la población pediátrica que posee características propias como la inmadurez enzimática que favorecen la aparición de reacciones adversas.

Predominaron las RAM probables, debido a que en estos pacientes no existió reexposición ni había una causa alternativa que justificara el cuadro clínico; seguidas de las RAM posibles donde si existió una causa alternativa. Solamente una tercera parte de las RAM se clasificaron como definitivas, hecho que ha sido descrito en la literatura ya que para que la reacción sea clasificada de ese tipo tiene que haber reexposición y reaparición de la reacción, y no es ético reexponer al paciente a un fármaco cuando se sospecha una RAM.

Con respecto a la severidad de las RAM, se reportaron reacciones adversas leves seguidas de las moderadas. Las reacciones adversas graves que pusieron en peligro la vida de los pacientes estuvieron relacionadas en su mayoría con antimicrobianos y antiinflamatorios no esteroideos y reacciones de hipersensibilidad, tipo B, la cuales no son predecibles y están asociadas a una

elevada mortalidad como se constata en la literatura.

Los reportes de reacciones adversas mortales se relacionaron fundamentalmente con fármacos antineoplasicos y antimicrobianos. Al analizar la imputabilidad en cada caso pudimos concluir que en la mayoría de las RAM la relación de causalidad se clasificó como posible por cuanto la patología de base u otro/s fármacos pudieron estar relacionados con la causa de muerte de los pacientes. Hay que señalar que la penicilina rapilenta constituyó el fármaco con mayor número de reportes de reacciones adversas graves manifestadas por reacciones de hipersensibilidad.

En 2009, el sistema cubano ha continuado trabajando en perfeccionar los filtros de revisión de notificaciones a nivel municipal y provincial, en afianzar los grupos de trabajo multidisciplinarios y en desarrollar nuevos métodos de análisis como el método bayesiano de generación de señales, así como en el mantenimiento de la capacitación, investigaciones, publicaciones y retroalimentación a su red[12-13]. Se observa también un aumento en la notificación de efectos adversos de baja frecuencia de aparición. La retroalimentación del sistema y la comunicación del riesgo han llegado hasta los notificadores y profesionales sanitarios en general, y se redactaron notas informativas a profesionales sanitarios dando a conocer las características del problema (por ejemplo nota informativa de piroxican en el 2007[17] y de agonistas dopaminérgicos en el 2008[18]) y las recomendaciones para la prevención de los efectos indeseables.

Este trabajo posee como limitación que el método empleado es la notificación espontánea de reacciones adversas que, como sabemos, es un método de bajo coste pero que depende mucho de la voluntad del personal sanitario para notificar las reacciones adversas, así como de los conocimientos y la importancia que se le atribuya al tema, por lo cual es su principal desventaja la infranotificacion[19-20].

Los objectivos futuros están encaminadas a la detección de RAM moderadas, graves y mortales, además de baja frecuencia de aparición, sobretodo aquellas que se consideren señales, para lograr una adecuada valoración beneficio-riesgo de los fármacos de que disponemos en el mercado cubano, además de la realización de investigaciones donde se combinen métodos de farmacovigilancia activos y pasivos para conseguir un mayor número de reportes. Debe mantenerse la retroalimentación a los profesionales sanitarios y fomentar la discusión de los resultados de este estudio en la comunidad médica.

CONCLUSIONES

Se detectaron entre una a dos sospechas de RAM por cada notificación realizada. Predominando las RAM de pacientes de sexo femenino y en el grupo de adultos. Las reacciones adversas más frecuentes fueron erupción cutánea seguida por los vómitos.

Predominaron las reacciones adversas leves y probables. Entre las asociaciones fármaco - RAM muy importantes y con baja frecuencia de aparición se reporto como fármaco mas frecuente el captopril.

REFERENCIAS

1. Viewpoint Part 2. Watching for safer medicines, the scientific and technical story. Uppsala Monitoring Centre: WHO Collaborating Centre for International Drug Monitoring 2004: 14-27

2. Viewpoint. Parte 1. Vigilando hacia medicinas más seguras. Uppsala Monitoring Centre WHO Collaborating Centre for International Drug Monitoring 2003:3-19.

3. Moore N, Lecointre D, Noblet C, Mabille M. Frecuency and cost of serious adverse reactions in a department of general medicine. Br J Clin Pharmacol 1998; 45: 301-308.

4. Pouyanne P, Haramburu f, Imbs l, Beagud B. Admission to hospital caused by adverse drug reactions: cross sectional incidence study. BMJ 2000; 320:1036.

5. Jiménez L G, Debesa G F, Ávila P J, Bastanzuri V T. Comportamiento de las reacciones adversas a los analgésicos y antiinflamatorios no esteroideos notificados por el sistema cubano de farmacovigilancia. Primer semestre del año 2001. Boletín Fármacos 2002; 5 (2): 1-4.

6. Debesa F, Jiménez G, Pérez J. Vigilancia farmacológica en cuba. Situación actual. Boletin Fármacos, 2001; 4(1) Disponible en: www.boletinfarmacos.org

7. Jiménez L G, Debesa G F, Gonzalez D BE, Avila P J, Pérez P J. El sistema cubano de farmacovigilancia, seis años de experiencia en la detección de efectos adversos. Rev Cubana Farm. 2006; 40 (1).

8. Valsecia M. Farmacovigilancia y mecanismos de reacciones adversas a medicamentos. Disponible en: http://med.unne.edu.ar. Fecha de acceso Abril 2007.

9. Laporte JR, Carné X. Metodología epidemiológica básica en farmacovigilancia. En: Laporte JR, Tognoni G, eds. Principios de epidemiología del medicamento. 2. ed. Barcelona: Masson-Salvat Medicina, 1993:111-130.

10. Laporte JR. Principios básicos de la investigación clínica. 2. ed. Barcelona: Astrazeneca; 2001: 161-190.

11. Lazarou J. Incidence of adverse drug reactions in hospitalised patients: a meta-analysis of prospective studies. JAMA 1998; 279:1200-1205.

12. Normas y Procedimientos de trabajo del Sistema Cubano de Farmacovigilancia". Unidad Coordinadora Nacional de Farmacovigilancia. CDF 2003.

13. Naranjo CA, Busto UE. Reacciones adversas a medicamentos. En: Naranjo CA, ed. Métodos de farmacología clínica. Toronto: El Service Science, 1992: 330-348.

14. Debesa F, Jiménez G, Pérez J, Avila J. Principales resultados del sistema cubano de farmacovigilancia en el año 2002. Boletín Fármacos. 2003; 6(3). Disponible en: www.boletinfarmacos.org .

15. Jiménez G, Debesa F. Medicamentos y Riesgos.: Revista Cubana de Farmacia 2002; 36 (Suplemento especial No 2): 217.

16. Colectivo de autores. Formulario Nacional de Medicamentos. CDF-MINSAP. Cuba 2006.

17. Alfonso I, Jiménez G, Ávila J. Comportamiento de los efectos adversos del Piroxicam. Cuba 2003 - 2007. Boletín Fármacos, 2008;11(2). Disponible en: http://www.boletinfarmacos.org/042008/advertencias_sobre_medicamentos_investigaciones.asp

18. Alfonso I, Jimenez G, Chao A, Ávila J. Seguridad de los Agonistas Dopaminérgicos Ergóticos en Cuba. 2003-2008. Boletín Fármacos, 2009; 12(1). Disponible en:

http://www.boletinfarmacos.org/022009/advertencias_sobre_medicamentos.asp

19. Organización Mundial de la Salud. Vigilancia de la seguridad de los medicamentos. Guía para la instalación y puesta en funcionamiento de un Centro de Farmacovigilancia. The Uppsala Monitoring Centre, Sweden 2001; 23.

20. Goodman & Gilman's The Pharmacological Basis of Therapeutics (CD-ROM) - 11th ed. (2006).

Comentario del revisor Dr. Martín de Frutos Herranz PhD. Fisiología humana. Escuela Universitaria de Enfermería. Universidad de Burgos. Burgos, España

El interés de este artículo se basa en que nos permite conocer el procedimiento de comunicación y registro de los reacciones adversas a los medicamentos en Cuba.

Asimismo, nos permite conocer los procedimientos para el seguimiento que a tales efectos se hace en dicho pais.

Comentario del revisor Dr. Enrique Seoane Vázquez. Doctor en Farmacia. Fármacoeconomista. Profesor de Ohio State University Columbus. Columbus. USA

El perfil de seguridad de un medicamento no se conoce suficientemente en el momento de registro para la comercialización. Los ensayos clínicos previos al registro se realizan en una muestra relativamente pequeña de pacientes que, además, no representa una parte importante de la población, tales como ancianos, pacientes con múltiples patologías y tratamientos, o niños, que utilizara el fármaco. Por ello es necesario la recogida y análisis de información de los efectos del medicamento después de su comercialización.

La farmacovigilancia y, especialmente, el sistema voluntario de notificación de reacciones adversas permiten identificar

problemas de seguridad que suceden después de la comercialización del un medicamento. El estudio realizado por la Dra. Alfonso y colegas provee una descripción del desarrollado sistema de notificación voluntaria de reacciones adversas a medicamentos (RAM) en Cuba y una detallado análisis de la incidencia de las RAM en el país.

El estudio demuestra la existencia de RAM en medicamentos tanto de alta como de baja utilización; la importancia de la polifarmacia como factor de riesgo de la aparición de las RAM; la incidencia de RAM en todos los grupos de población; y la creciente identificación de RAM asociadas con la medicina natural. El artículo también destaca los planes para el futuro desarrollo del sistema de farmacovigilancia en el país.

Recibido: 15 de noviembre de 2008. Recibido revisado: 7 de junio de 2009
Publicado 14 de junio de 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

Inicio Home

Indice del volumen Volume index

Comité Editorial Editorial Board

Comité Científico Scientific Committee

Normas para los autores Instruction to Authors

Derechos de autor Copyright

Contacto/Contact:

SIMPLE AND SENSITIVE METHOD FOR DETERMINATION OF POLYHEXANIDE IN MULTIPURPOSE SOLUTION

A. Arora[1], A. Ali[2], M. T. Zzaman[1],

[1] KIET School of Pharmacy, Ghaziabad.
[2] Jamia Hamdard, Faculty of Pharmacy, Dept of Pharmaceutics. New Delhi. India.

alkamailin @ yahoo.co.in

Rev Electron Biomed / Electron J Biomed 2009;2:30-37

Comment of the reviewer Silvia Albillos García PhD. Inbiotec (Instituto de Biotecnología). León, España

Comment of the reviewer Mónica Cavia Sáiz CD. Research Unint. Complejo Asistencial de Burgos. Burgos. España

---

RESUMEN: MÉTODO SENCILLO Y SENSIBLE PARA LA DETERMINACIÓN DE POLIHEXANIDE EN SOLUCIÓN MULTIUSO.

Un método invertido sencillo de fase HPLC ha sido desarrollado para la determinación de clorhidrato biguanudo de poli-hexametileno en la solución multiuso para las lentillas hidrófilas. La fase móvil fue acetonitrile 1%(v/v) acetato amónico de 20 mmol/L en el agua como 16: 84 (V/V) en una tasa de flujo de 1 ml/min. El clorhidrato biguanudo de poli-hexametileno fue detectado por la absorción de UV a 235 nm. El pH fue mantenido a 4,0 utilizando el ácido acético glacial. El método de Yiping et al ha sido modificado ligeramente según la necesidad. La cantidad detectada es 2 µG/ml y por eso fue llevado a cabo utilizando el método de pre concentración bajo vacio. Desde el cromatograma, fue observado que un pico claro apareció al tiempo de retención 5,883 min para el clorhidrato biguanudo de poli-hexametileno. La recuperación de droga fue encontrada a 99,38% y el método fue sencillo, rápido y propio para la concentración de la droga en la solución multiuso para las lentillas hidrófilas y para el llevar a cabo la estabilidad según la directriz de ICH para valorar la estabilidad de clorhidrato biguanudo de poli-hexametileno en la solución multiuso.

PALABRAS CLAVE: Líquido estándar de lágrima (LEL). Clorhidrato de Polihexanide (CHPH). Área bajo curva (ABC)

SUMMARY:

A simple reversed phase HPLC method has been developed for the determination of polyhexamethylene biguanide hydrochloride in multipurpose solution for hydrophilic contact lenses. The mobile phase was acetonitrile 1% (v/v) ammonium acetate 20 mM in water as 16: 84 (v/v) at a flow rate of 1 ml / min. Polyhexamethylene biguanide hydrochloride was detected by UV absorption at 235 nm. The pH was kept at 4.0 using glacial acetic acid. The method of Yiping et al has been slightly modified as needed. The quantity detected was 2 µg/ml so it was carried out using a preconcentration method under vaccum. From the chromatogram, it was observed that a distinct peak appeared at retention time 5.883 min for polyhexamethylene biguanide

hydrochloride. The recovery of drug was found to be 99.38% and the method was simple, rapid and suitable for the assay of drug in multipurpose solution for hydrophilic contact lenses and for carrying out stability as per ICH guidelines to assess the stability of polyhexamethylene biguanide hydrochloride in multipurpose solution.

KEY WORDS: Standard tear fluid (STF). Polyhexanide hydrochloride (PHNB). Area under curve (AUC)

INTRODUCTION

Polyhexamethylene biguanide hydrochloride (PHMB.HCL) has been utilized as a new antimicrobial providing reliable preservation in multipurpose solution (MPS) for hydrophilic contact lenses[1,2]. It is a polymeric compound with low skin irritancy, low eye toxicity, fast speed of kill, stable and effective over a wide pH range. It is available as 20% aqueous solution (w/v) with a molecular structure as shown in figure 1.

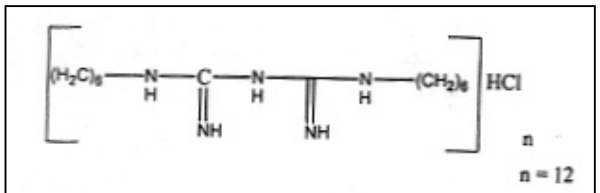

Fig. 1: Molecular structure of PHMB.HCL

The composition of MPS is given in table 1. The MPS is found to be effective against bacteria, fungi, protozoa and viruses even at very low concentration[3,4].

Table 1: Composition of multipurpose solution (MPS)

Ingredient	Concentration
Polyhexamethylene biguanide hydrochloride	0.0002%
Boric acid	0.20%
Sodium tetraborate	0.02%
Sodium chloride	0.80%
Distilled water q.s to	100ml

In order to supervise the quality of MPS containing PHMB.HCl, it is necessary to develop the method to assay the sample of drug accurately. Reversed phase high performance liquid chromatography (RP-HPLC) with photodiode array detector (PDA) is the most frequently used method for routine determination. However, to the best of our knowledge, no RP-HPLC method for the analysis of PHMB.HCl in MPS for hydrophilic contact lenses has been reported except that of Yiping et al.[5] who has tried to establish a simple and rapid HPLC method for the routine analysis of PHMB in compound chemical disinfection.

MATERIALS AND METHODS

Materials

PHMB. HCl was obtained from Avecia Biocides, Manchester, U.K. Acetonitrile and water HPLC grade were from S.D. chemicals, Mumbai, India. Ammonium acetate, glacial acetic acid were of analytical grade and were used as received.

HPLC instrumentation and chromatographic conditions

HPLC was performed with a Shimadzu class VP series manual injector fitted with a 20 µl loop, a model SPD- 10 UV detector, a model LC- ATVP pump operated in an isocratic mode and a 4.6 mm i.d sign 50 mm Shim- pack CLC- ODS C18 column, particle size 5 µm. The mobile phase was 16:84 (v/v) of acetonitrile -1% (v/v) and ammonium acetate 20 mM in water and it was pumped out at 1 ml / min. The mobile phase was filtered through a 0.45 µm membrane filter and ultrasonified before use. The UV absorption spectrum of PHMB.HCl indicated the presence of an analytically useful absorption band with a maximum at 235 nm as shown in figure 2.

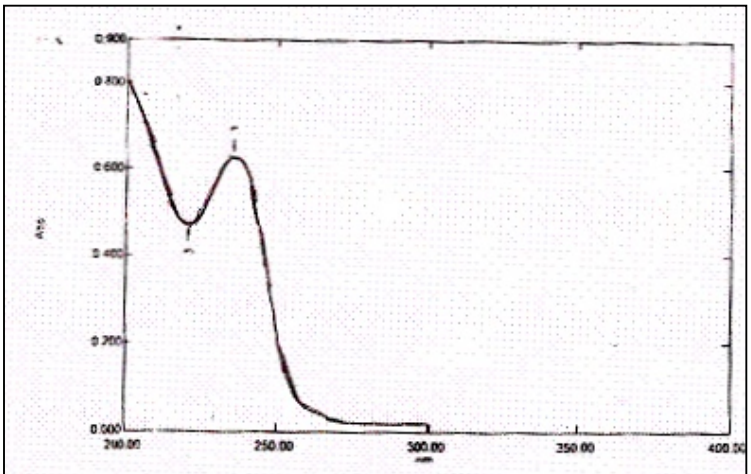

Fig. 2: UV spectrum of PHMB.HCl

The 20 µl samples of MPS which was pre-concentrated under vacuum was injected and chromatogram was obtained. The temperature was maintained at 30 °C. Under this condition, PHMB.HCl was eluted at 5.883 min as shown in figure 3. Interferences from other ingredients of MPS were not observed.

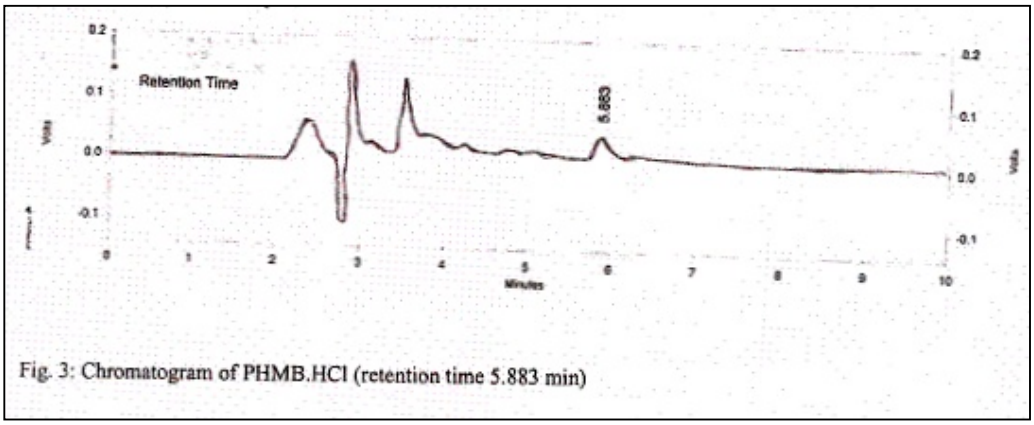

Fig. 3: Chromatogram of PHMB.HCl (retention time 5.883 min)

Preparation of Standard Curve

From the drug sample (20% w/v), 1.0 ml was pipetted and transformed into 100 ml capacity volumetric flask and volume was made up to 100 ml with filtered standard tear fluid (STF) of pH 7.4. The composition of STF was as mentioned in table 2.

Table 2: Composition of standard tear fluid (STF) at pH 7.4

Ingrdients	Concentration
Boric acid	0.2g
Sodium tetraborate	0.02g
Sodium chloride	0.80g
Distilled water q.s.to	100ml

From this solution 1.0 ml was further diluted up to 100 ml with the STF of pH 7.4 in a 100 ml volumetric flask in order to obtain a stock solution of 20 µg /ml. From this stock solution different volumes were withdrawn and diluted with STF of 7.4 for obtaining different concentration from 0.25 µg /ml to 7.5 µg /ml. The volume of each standard solution was 10 ml and all these were filtered through a membrane filter (0.45 µm). The 20 µl of each solution was injected into the HPLC column in order to obtain the chromatogram and AUC (area under the curve) was calculated. The observed values are given in table 3.

Table3 : HPLC reading of standard solution of PMHB.HCl (detection 235nm)

Concentration (µg/ml)	AUC (Average)	Regressed (AUC)	±SD
0.25	110121	114268	0.632455532
0.50	120634	119580	0.894427191
1.00	131147	130204	0.983191563
1.50	141660	140828	0.894427191
2.00	152171	151452	0.894427191
2.50	162686	162076	0.894427191
3.00	173199	172700	0.632455532
3.50	183712	183324	0.894427191
4.00	194224	193948	0.894427191
4.50	204738	204572	0.632455532
5.00	215251	215196	0.983190011
5.50	225764	225820	0.894427191
6.00	236277	236444	0.632455532
6.50	246789	247068	0.816494089
7.00	257302	257692	0.894427191
7.50	267815	268316	0.632455532

n=6, results are the mean of six readings

Coefficient of correlation (r^2) = 0.9994

Equation of regressed line y = 21248x+108956, where y is regressed AUC

Slope(m) = 21248,x is the concentration (µg/ml), intercept (c)= 108956.

The method was repeated for six times i.e. n=6. A standard plot PHMB. HCL was drawn by plotting concentration on X- axis and AUC on Y- axis and is shown in figure 4.

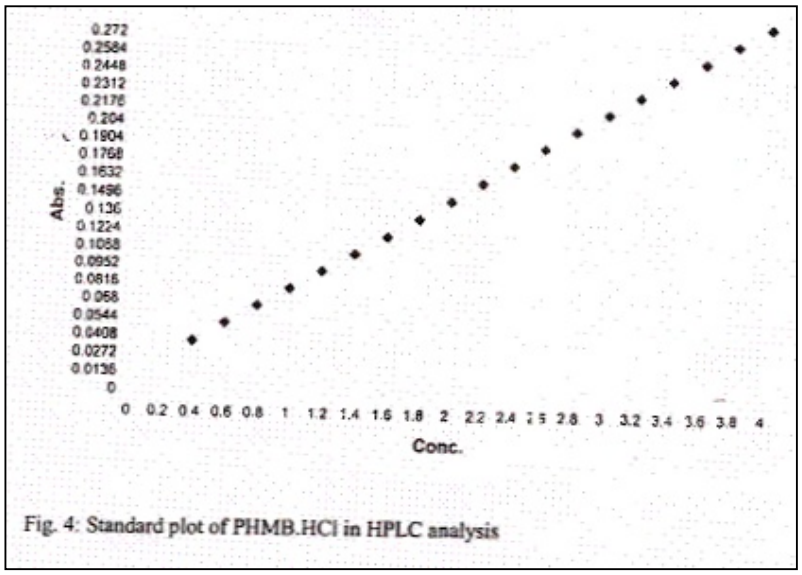

Fig. 4: Standard plot of PHMB.HCl in HPLC analysis

From the point observed the regressed line was drawn and the equation of this regressed line was calculated. The coefficient of correlation (r2) was also determined. The coefficient of correlation (r2) = 0.9994 and equation of regressed line y= 21248x+ 108956, where y is regressed AUC and slope (m) is 21248, x is the concentration (µg / ml) and intercept (c) is 108956.

Determination of PHMB.HCl in MPS under Stability Studies as per ICH Guidelines

Under the stability studies of the formulation, 20 µl of the sample undiluted were injected into an HPLC column, using similar conditions as in the case of the standard curve and AUC was obtained. The drug concentration obtained by extrapolation of the AUC of the sample under the standard curve and the potency of the drug in the sample was calculated.

Sample Stability

The Stability studies were carried out according to the ICH guidelines and for this purpose MPS which were developed on pilot scale were kept at 40°C and 75% RH in humidity chamber for 6 months. The samples were withdrawn at intervals of 0, 1, 2, 4 and 6 months and analyzed for drug content by the HPLC method of analysis as mentioned before. The results are given in table 4.

Table 4: Mean drug content values of stability studies at 40 ± 0.5°C & 75% RH for MPS with PHMB.HCl.

Time (days)	Mean drug content (µg /ml)	Percent drug remaining	Log % drug remaining	Slope	Degradation rate costant K ($days^{-1}$)
0	2.062	100	2.000		
30	2.049	99.37	1.9972		
60	2.028	98.35	1.9928	14.66×10^{-5}	33.76×10^{-5}
120	2.019	97.91	1.9908		
180	2.000	96.99	1.9867		

The results of the stability studies carried out as per ICH guidelines were compared with those of the sample at 0 day. The data converted into log percent drug remaining and it was plotted against time in days as shown in figure 5.

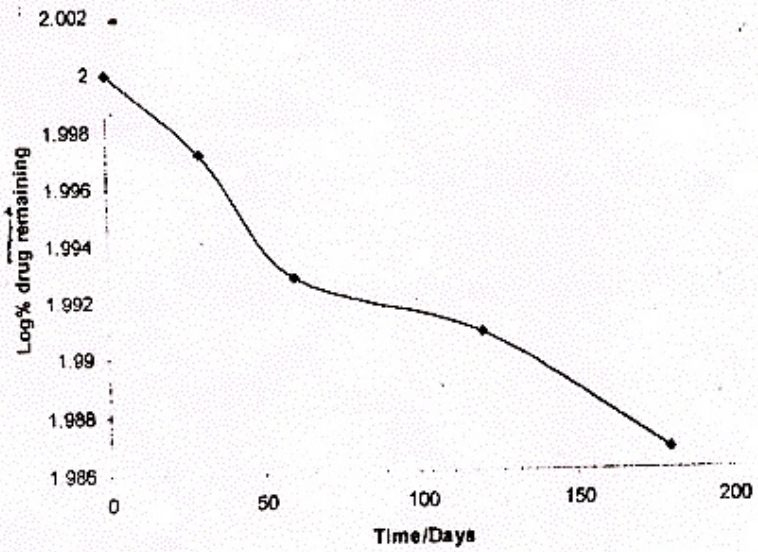

Fig. 5: Degradation kinetic profile of MPS with PHMB.HCl (ICH guidelines)

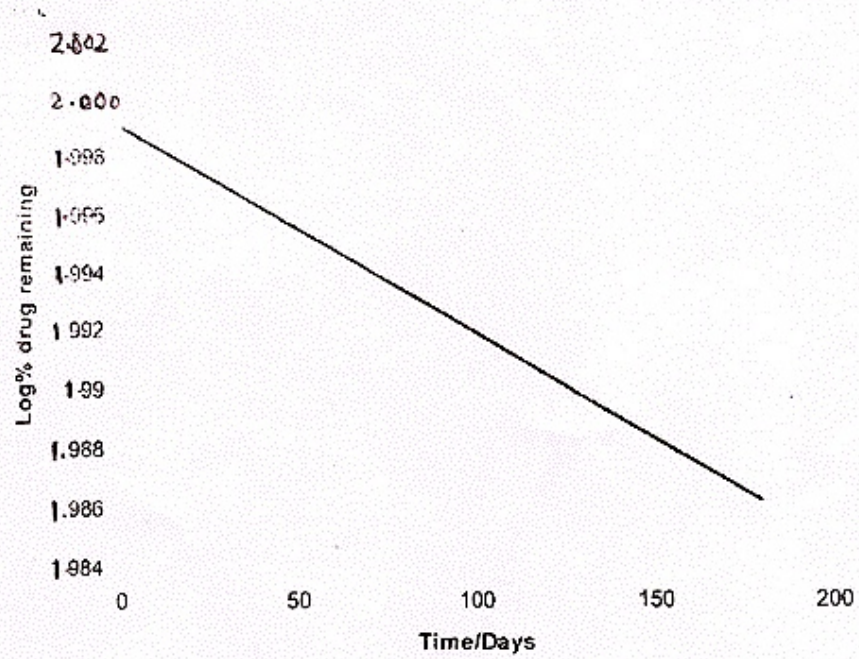

Fig. 5A: Degradation kinetic profile of MPS with PHMB.HCl

The slope of the line was determined, and from this, the degradation rate constant 'K' in days was calculated.

RESULTS AND DISCUSSION.

An HPLC method of analysis of PHMB. HCl was carried using the method of Yiping et al after slight modification. Under the method, UV detection at 235 nm was performed. The UV spectrophotometric determination was carried out in isotonic STF of pH 7.4. For this purpose, the drug solution was scanned in UV range and PHMB.HCl exhibited λ max at 235 nm. The drug was detected accurately with recovery of 99.38%. The standard curve under HPLC analysis between AUC and concentration obeyed Lambert's Beer law between the concentration of 7.5 µg/ml and a high value for the coefficient of correlation was observed. This

method of analysis was found to be simple, rapid and accurate for the determination of PHMB.HCl. The sample was preconcentrated by vaccum before subjecting it to HPLC studies.

Stability studies were carried out as per ICH guidelines in order to see the stability of drug in the MPS. Under the ICH methodology, sufficient number of containers of the product were kept at 40 ± 0.5 °C and RH 75% in a humidity chamber for six months. The samples were withdrawn at different time intervals and analyzed for the drug (PHMB.HCl) by the HPLC method of analysis. The log percent of drug remaining was plotted against time in days and from the curve, the slope was determined. By using the slope, the degradation rate constant was determined and it was found to be 33.76×10?5 days?1. The amount of drug degraded was less than 5 % of the total. Hence an arbitrary shelf life of two years can be assigned to the product as mentioned by ICH guidelines.

CONCLUSIONS

The HPLC method with slight modification of Yiping et al was found to be precise, accurate and suitable. The method was found to be suitable to fix an adequate shelf life for the product.

REFERENCES

1. John T, Desai D, Sahm D. Adherence of *Acanthamoeba castellanii*cysts and trophozoites to unworn soft contact lenses. Am J Ophthalmol. 1989;108:658-664.

2. Silvany RE, Dougherty JM, McCulley JP, Wood TS, Bowman RW, Moore MB. The effect of currently available contact lens disinfection systems on *Acanthamoeba castellanii* and *Acanthamoeba polyphaga*. Ophthalmology. 1990;97:286-290.

3. Levey SB, Cohen EJ. Methods of disinfecting contact lenses to avoid corneal disorders. Surv Ophthalmol. 1996;41:245-251

4. Lever AM, Miller MJ. Comparative antimicrobial efficacy of multi-purpose lens care solutions using the FDA's revised guidance document for industry: stand-alone primary criteria. CLAO J. 1999;25:52-56.

5. Yiping C, Xiaojing D, Pengyani. Rapid determination of Polyhexanide in compound chemical disinfectants by reversed phase HPLC. 2005;8(7):1-5

Comment of the reviewer Silvia Albillos García PhD. Inbiotec (Instituto de Biotecnología). León, España

This study determines the stability of a multipurpose solution (MPS) used as antimicrobial and for preservation of hydrophilic contact lenses by detecting the stability of its main active compound: polyhexamethylene biguanide hydrochloride. This new antimicrobial is said to present advantages such as low eye toxicity, stability over a wide pH range and fast kill speed.

The authors describe a method for quantitation of the active compound found in this MPS by means of HPLC improved from a previously described method by Yiping et al. The improvements achieved a much lower limit of detection for the method. Stability analyses for the MPS at certain storage conditions were performed up to six months proving the methodology valid for determination of the stability of the compound of interest with adequate accuracy and following the ICH guidelines.

Comment of the reviewer Mónica Cavia Sáiz CD. Research Unint. Complejo Asistencial de Burgos. Burgos. España

The HPLC technique used for the determination of drug stability is an essential part of pharmaceutical formulation development. In this article, the authors provides a thorough description of high-performance liquid chromatography (HPLC) for the determination of PHMB.HCL in the multipurpose solution. PHMB.HCL is an antiseptic belong to the biguanide group, which are cationic substances well known for their effective action against microbial infection.

Nowadays, there are few methods for the study of PHMB.HCL. The main reason is the complexity of the sample composition,

that it is always present as mixture of oligomers. Furthermore, the lack of aromatic groups makes that detection via UV-absorption is difficult. The authors in this article development a HPLC method, in terms of throughput, accuracy and cost-effectiveness that it covers essential aspects of enhance efficient separation, such as mobile phase preparation and optimization of HPLC analysis. Analysis of the results showed satisfactory precision ranging. Thus, the detection limits were of 2 µg/mL. It is also demonstrated that the high sensitivity allows the method to be used for development as prophylactic or therapeutic drugs against various eye infections.

Furthermore, this article summarises the stability studies of PHMB.HCL in the multipurpose solution. Results presented show that the amount of drug degraded was less than 5% of the total. These data present new information about of a simple method for the determination of PHMB in eye drops as well as its possible application in pharmacokinetic studies in ophthalmology, since the technique can produce reliable analytical results quickly and relatively cheaply.

Received: May 4, 2009. Received reviewed: July 17, 2009.
Published August 31, 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

ATTEMPTING TO PREDICT THE FATE OF AN ONGOING EPIDEMIC. LESSONS FROM A(H1N1) INFLUENZA IN USA.

José Luis Hernández Cáceres, Karel Vazquez Argote[1], Reyder Risco Soria, Miguel Martín Martínez

Center for Cybernetics Applications to Medicine, Havana Higher Institute of Medical Sciences. Ciudad de La Habana.
[1]Dirección Provincial de Salud. Victoria de Las Tunas. Cuba.

cacerjlh @ infomed.sld.cu

Rev Electron Biomed / Electron J Biomed 2009;2:38-48

Comment of the reviewer Prof. Fernando Tricas García. **PhD in mathematics. Professor of Languages and Systems. Department of Computer and Systems Engineering. University of Zaragoza. España.**

Comment of the reviewer Prof. José María Eirós Bouza. **Professor of Microbiology, Faculty of Medicine, University of Valladolid. Head of Virology, Hospital Clínico Universitario. Advisory Committee of the WHO Influenza. Member of Working Group National Influenza Center. España.**

RESUMEN:

Se hace un intento de estimar los parámetros más importantes del brote de influenza A(H1N1) en los Estados Unidos de América en 2009 sobre la base de la información pública emitida por los Centros para el Control de Enfermedades (CDC) norteamericano durante los días iniciales de la epidemia. Por tratarse de un problema estadístico mal planteado, se combinó la estimación no lineal (método de Gauss-Newton y de Hooke-Jeeves) con procedimientos de linealización que permitieran establecer un conjunto adecuado de valores iniciales para comenzar la estimación recursiva de los parámetros.

Sobre la base de los datos disponibles hasta el 13 de mayo de 2009, se predicen los siguientes valores para el brote en los Estados Unidos. Tau (tiempo hasta el pico de incidencia) 32 días; R_0 (numero de infecciones secundarias por individuo infectado)1.7; K (numero total de casos) 20000 (15000-35000). Estos resultados concuerdan con lo reportado por el "WHO's Rapid Assessment Team" para la epidemia en México. El método puede aplicarse en cualquier locación donde se registren adecuadamente el número cumulativo de casos de una epidemia o brote.

PALABRAS CLAVE: Inluenza A(H1N1). Modelos Matemáticos para epidemias. Predicción de la evolución de un brote.

SUMMARY:

An attempt is made to estimate the main parameters of the 2009 Influenza type A(H1N1) outburst in USA based on public information provided by Centers for Disease Control (CDC) during the early stage of the epidemic. Given the ill-posed nature of the statistical problem, a nonlinear fuction estimation method (Gauss-Newton and Hooke Jeeves) was combined with linearization procedures that allowed to set adequate initial guess values for estimation.
Based on data until May 13th, 2009, the following values are predicted for the USA outbreak: Tau (time to the peak of incidence) 32 days; R_0 (number of secondary infections per infected individual) 1.7; K (total number of cases) 20000(15000-35000). These results are in good agreement with the values reported by the WHO's Rapid Assessment Team for the outburst in Mexico. The method can be applied in any setting where cumulative number of cases are properly recorded.

KEY WORDS: A(H1N1) Influenza. Mathematical models for epidemics. Outburst evolution prediction.

INTRODUCTION

HIV/AIDS, Ebola, SARS, Avian Flu, and the Swine Flu A(H1N1) are examples of infectious diseases, completely unknown to the human immune system, that appeared in the last years. The dates of appearance of these cited above suggest that in the next ten years al least several new similar scenarios will emerge[1].

It is uncertain whether public health systems are ready to face a pandemic of a new disease[2]. Evidences from the spread of AIDS in Southern Africa, to just present an example, cast doubt on a positive answer.

Among the questions that are important for individuals, public and authorities there is the prediction of an ongoing epidemic[3]. SARS affected more than 7000 persons worldwide[4], whereas H1N1 probably will surpass that number several times[5].

A crucial issue on an ongoing epidemic is to have estimates for values such as the expected total number of cases, the moment when the peak will be attained, as well as the number of persons infected by a single primary case[6].

Public health services are mobilized the very first days of an outbreak, since early planning is decisive. Unfortunately these are the days when the prediction is poorest; from the large number of models that could be used all of them are highly nonlinear, and having few data points can lead to false values, and subsequently, spoil the whole process of resource planning.

A very important point is which model to select for making the approximations. With available computer technology models can be as complicated as wished, and literature can provide wide evidence on that point[7].However, when little is known about the mechanisms of the ongoing disease, it is advisable to select the simplest models being capable of adapt to different possible variants. Picking a complicated and shrewd model in this case is comparable to the well known case of attempting to describe the growth curve of a dog with a miligram-precision scale.

At the same time, experience shows that for a large variety of models, the incidence I(t)during an outburst can be nicely approximated by the following function, that was proposed 70 years ago by Kermack and McKendrick as an fairly accurate solution to their seminal "SIR" model[8].
$I(t)=A*sech^2(Bt-C)$ (1)

In this case A gives the peak value of incidence, that appears at time Tau= C/B

If the dates of onset of cases are properly documented, this can be a good candidate function to be fitted.

Thus, for the H1N1 outbreak in a New York school in April 2009, where epidemiological information was under strict scrutiny[9], a good approximation was obtained applying a Gauss-Newton algorithm to model (1) (fig 1). This school-based outbreak is the largest cluster of H1N1 flu cases reported in the United States thus far, and indeed is a valuable source of information.

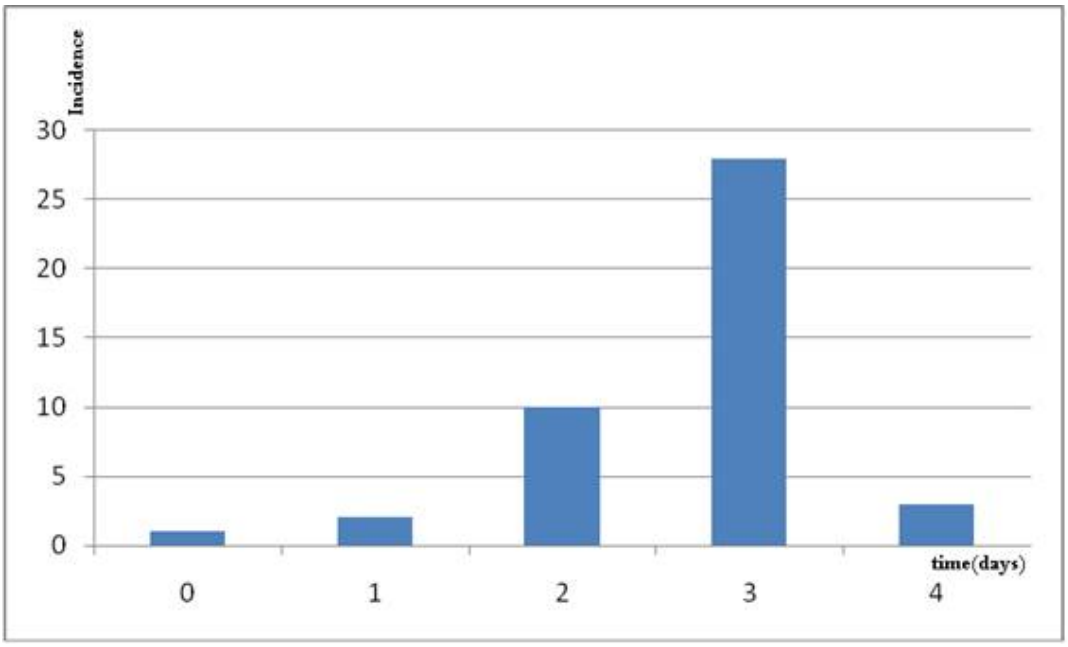

Figure 1. Data from an H1N1 outburst in a school in New York in April 2009.
Data were fitted with model (1) yielding the following values A=30.93 (vs. 28 observed) B=1.48 C=4.11; C/B=2.77 (vs. the observed value of 3). 99.43% of the variance was explained with the model.

However, it is difficult to have reliable data about real incidence in practical situations. Thus, from the 642 cases reported between April 15th 2009 and May 5th 2009 in the US, it was possible to identify the date of the onset of symptoms only for 394 patients (61%) [10]. In places with more fragile health infrastructure no better reliability is to be expected.

It seems preferable to use cumulative data S(t), for which the Richards model can be valid [2]:
$S(t)=K/(1+\exp(r(Tau-t)))$ (2)
K corresponds to the total number of cases and equals
$K= (S(0)*(1+\exp(r*Tau))$ (3)

Tau has the meaning of the peak time for incidence, and the basic reproductive number R0 (defined as the average number of secondary cases generated by one primary case) can be estimated as
$R_0=\exp(T_g*r)$ (4)

Where T_g is the transmission time, or the mean time between the appearance of symptoms in the primary case and the appearance of symptoms in a secondary case [2]. To use for assessing an epidemic cumulative confirmed cases data presents several limitations, including the lag between starting of the window of viral shedding and the laboratory report, as well as complications related to the uneven speed of laboratory confirmation of suspected cases, but there are reasons to assume that these are the best data publically available at this moment.

The other limitation is due to finding parameters from a data set that obviously is not the best suited for "academical" approximation purposes. For illustration, on how difficult this task can be, in figure 2, the best expected prediction for the Richards model is represented in the solid blue line whereas the cumulative cases numbers for USA until (12 of May, 2009) are represented as red crosses.

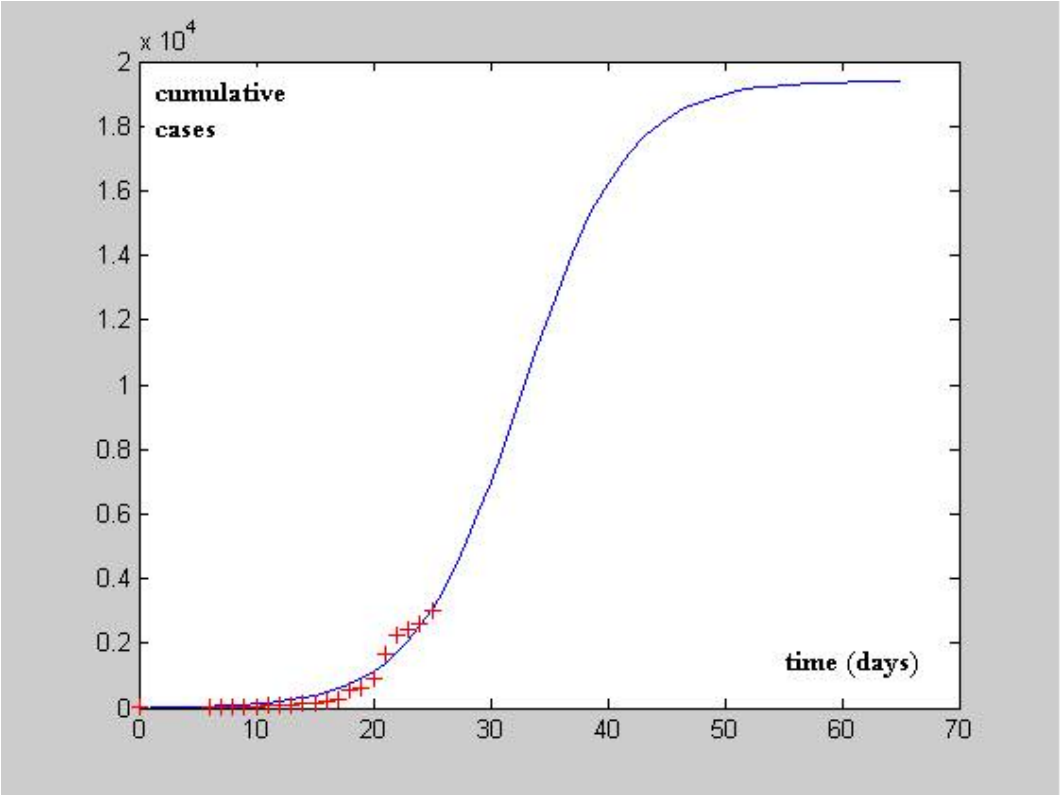

Figure 2 CDS confirmed data for USA since April 21 2009 until May 12th (+) and putative evolution curve estimated using the methods described in this paper (solid blue line)

There are several approximation methods to fit nonlinear data into models, such as Simplex, Hooke-Jeeves, Gauss-Newton, that have been implemented in different commercial statistical packages. These allow, in principle to simultaneously estimate several parameters from a data set. However, straightforward estimation beyond the domain of observed values with a highly nonlinear function, is not always reliable. Thus for the case of USA A(H1N1) data set, estimates for k, using all parameters at a time, yielded values of k equal to 160000, 3241 or 6217 in all the three cases showing "excellent" fits with explained variances higher than 95%. In other words, data are behaving as those typical for ill-posed inverse problems. A practical way to try to deal with this kind of problems is limiting the space of possible solutions, and imposing the solutions certain plausible requirements. In this case, the use of linearizations and manual stepwise estimation of values seems to be recommended.

We are testing this approach in the case of H1N1 cases confirmed by CDC until May 13th, 2009 (Figure 1)

The first attempt to linealize data is as follows. If r(Tau-t)>>1, the inverse of (1) can be seen as
1/S~exp (r(t-tau)),

From a Taylor expansion of the exponential function till the fourth power, it can be obtained the following approximation for the fourth root if the inverse of S(t)
(1/S(t))^(1/4) ~ -(r(t-tau))

The right side becomes equal to zero when t=Tau; thus from the relationship between the inverse of the cumulative data and elapsed time, it is possible to obtain a good guess for the time to the peak of the outburst.

As it can be seen from Figure 3, data for USA fit well into this approximation and a guess value for Tau= 32.56 is assumed

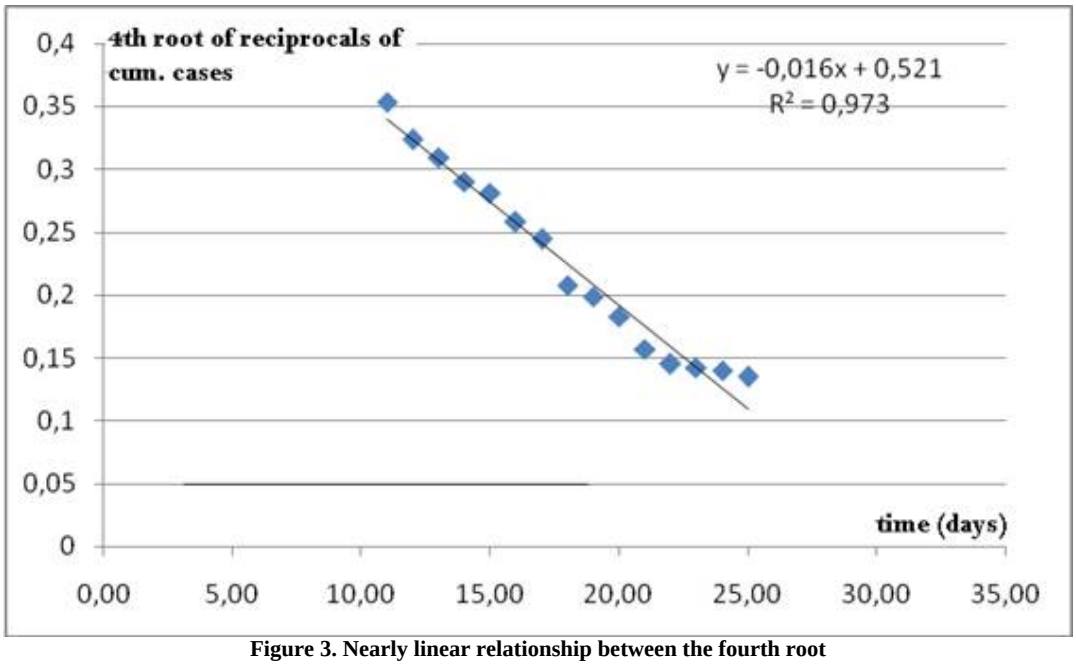

Figure 3. Nearly linear relationship between the fourth root of the reciprocals of cumulative data and time. The time when the line crosses the axis of abscissas is taken as a rough initial estimate for Tau in Richards model (2).

This seems to be a reasonable guess, since cumulative data are increasing after 27 days from the onset of the outburst.

Inspection of (1) suggests that a nearly linear relationship must be between ln(S) and time.
In this case the intercept will depend on all the 3 parameters of the model and has little practical use. However, the slope can bring a good initial estimate for r. As it can be seen from figure 4, a value of r= 0.324 is suggested.

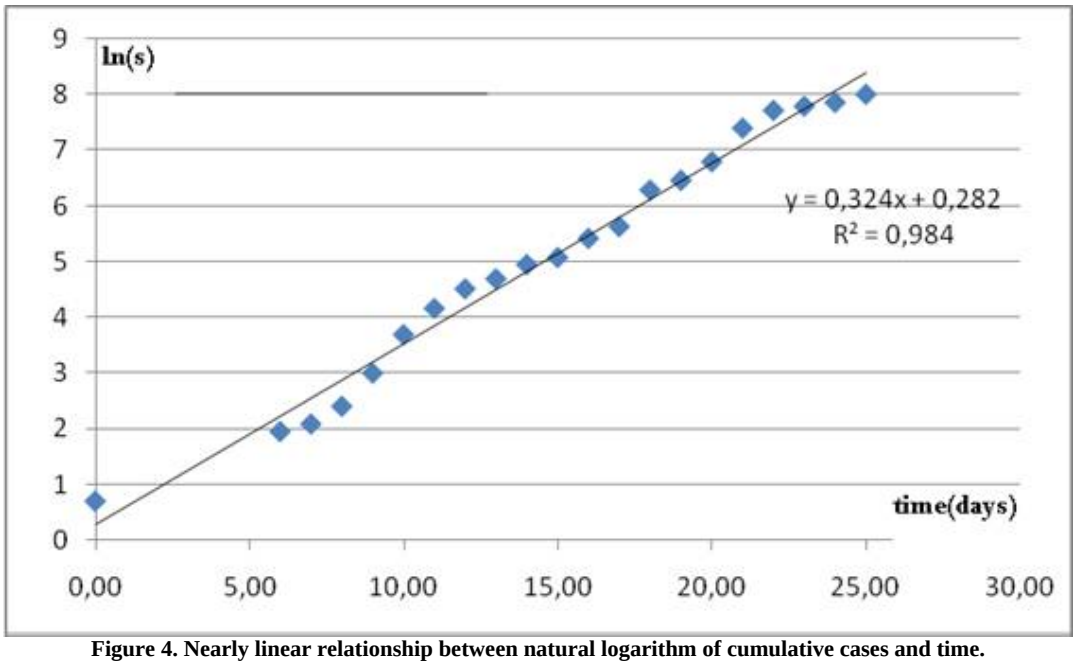

Figure 4. Nearly linear relationship between natural logarithm of cumulative cases and time. The slope of the regression line is taken as a guess value for parameter r in model 2.

If a value of generation time T_g=2.3 is accepted as the most plausible for H1N1 [11,12], this suggests a value of R0=2.1, which is in agreement for the first early report in the range of R_0=1.4-1.6 made for H1N1 in Mexico[5].

Since S_0=2, from expression (3) it can be obtained, a value of k=56004 cases. Obviously, the power function (3) is very sensitive to

errors in the estimates. Refining the approximation can provide a more reliable datum.

If these values were true, we must see the peak of incidence on May 21st, the basic reproductive number (R_0) is 2.1 which is similar to the value of 1.4-1.6 reported by the WHO rapid assessment team for Mexico[5], and about 28.000 cases will be reported for May 21st.

Having these initial estimates, a refinement can be found using a nonlinear function estimation method such as Gauss Newton or Hooke-Jeeves.

For coming to the final value we recursively refined the model's parameters. For that, we started with the two estimates obtained from linearization and applied the Gauss Newton algorithm to estimate k. After this the estimation procedure was repeated each time feeding the model with new updates for several times (from 4 to 19). The stop criteria were the highest explained variance with reliable estimates and similarity in subsequent estimation of parameters. In this example, the explained variance rose from 92% to 96%.

The following "final values" were found:

Tau=32.65 days
r=0,232
K=19826
96% of the variance explained.

This corresponds to an estimate of R_0=exp(2.3*0.232)=1.7, which is closer to the above mentioned estimate obtained by the WHO Rapid Assessment Team for Mexico.

In Mexico, an estimate of 23000 cases (between 6000 and 32000) has been reported for parameter K. Due to the similarity in population between the two countries, our estimate seems to be in agreement.

Publically available information from the Centers for Disease Control[13] also allow studying data from different states.

In table 1 data for some states are provided based on reports until May 15th, 2009. The agreement between values of r is noticeable.

State	Onset of Outburst	Tau	r	K	% of explained variance
California	17/04/2009	34.926	0.2015	1987	94.61
Illinois	01/05/2009	23.03	0.1545	3036	86.67
Texas	23/04/2009	20.87	0.213	2506	93.96

Table 1. Estimates obtained from H1N1 cumulative data until May 13th for 4 different states from USA.

Early estimates

The main question we are addressing here is how reliable it can be an estimate obtained from early data of an outburst. Apparently, comparing the evolution of estimates as the outburst proceeds can help in clarifying this question.

We analyzed, starting from day 7 from onset (with only 3 values) how different parameters looked like. It should be payed attention to the fact that the second report was 6 days after the first report, thus on the day 7th only 3 data points were available. This would suggest that early estimates can have only a very rough approximative value, but, in any case, are of great value when very little is known about the epidemic.

In figure 5 it can be appreciated that predictions for Tau close to 30 days appear since day 12 (with only 8 data points available). From all observations the mean and standard deviation for Tau were 31.0±5.2

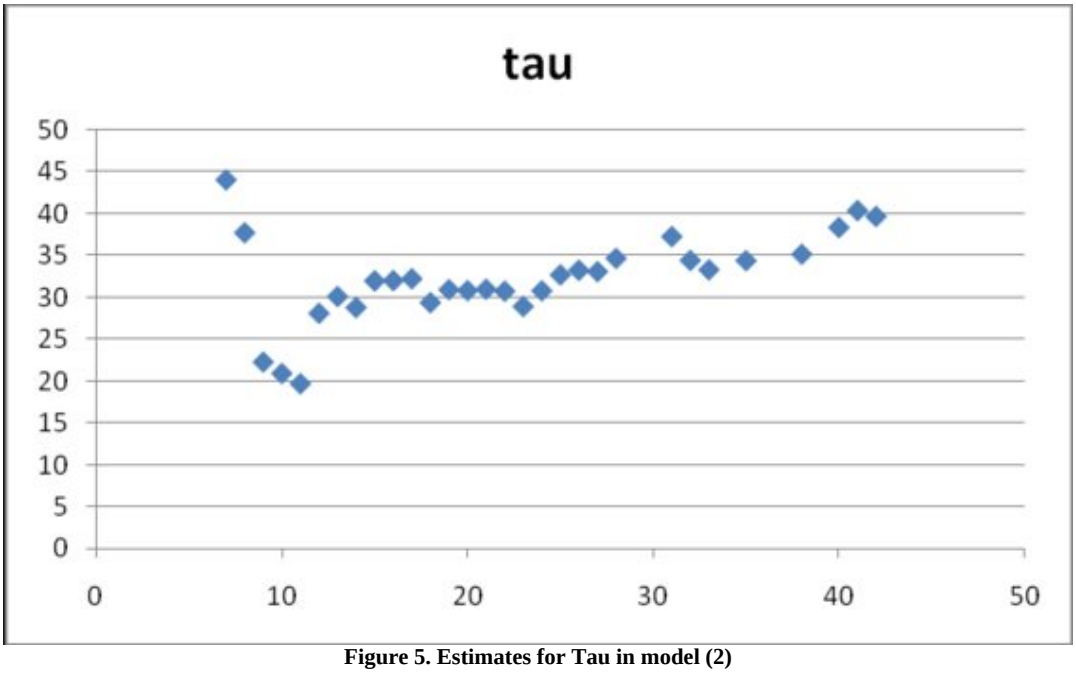

Figure 5. Estimates for Tau in model (2) obtained from cumulative data gathered at different dates after the onset of data collection on April 17, 2009.

In figure 6 values for parameter r are shown. After day 12 the parameter keeps nearly constant values around 0.33 and at the end they go to smaller values, below 0.20 (0.31±0.12).

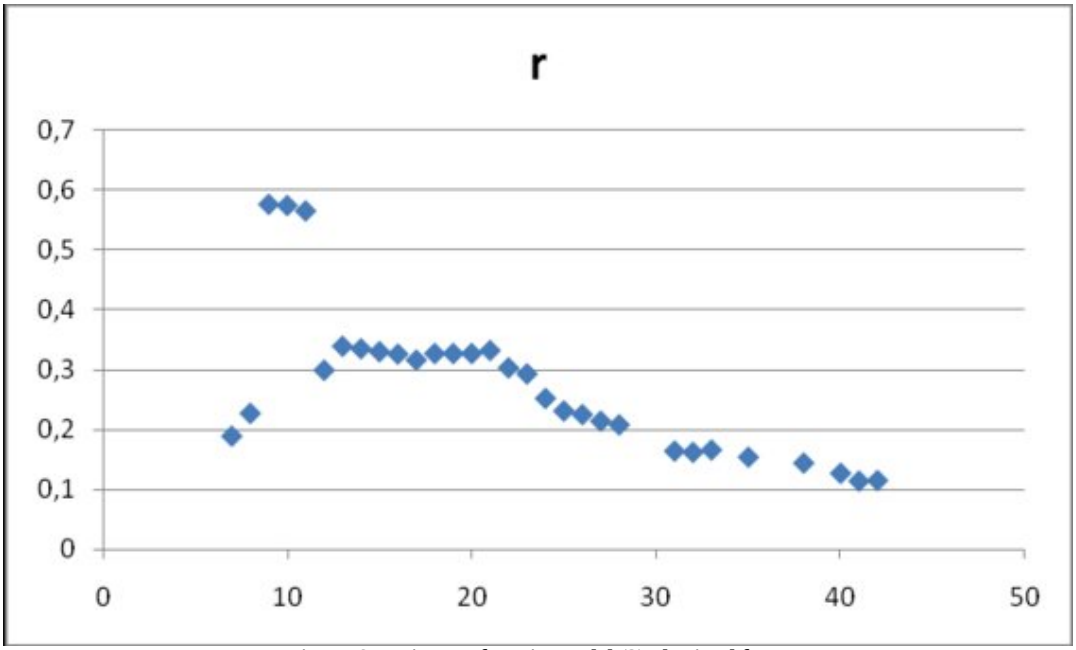

Figure 6. Estimates for r in model (2) obtained from cumulative data gathered at different dates after the onset of data collection on April 17, 2009.

Finally, the parameter K (fig 7) takes values between 5000 and 50000, with an apparent tendency to lie between 10000 and 20000 on later days (25294±11965).

The full set of values is in table 2

Days	tau	r	k	% Variance
7	43.98	0.19	9197	99.44
8	37.68	0,228	9477	98.86
9	22.26	0.577	43541	90.0
10	20.9	0.575	20584	97.8
11	19.68	0.566	8108	99.17
12	28.07	0.30	9990	91.02
13	30.07	0.34	38919	96.86
14	28.76	0.336	21442	97.44
15	31.94	0.331	47121	96.29
16	31.97	0.327	44800	96.49
17	32.20	0.317	38013	96.95
18	29.35	0.328	20252	96.34
19	30.89	0.328	32863	98.05
20	30.78	0.328	31685	99.00
21	30.94	0.333	40386	96.97
22	30.71	0.304	30476	96.41
23	28.89	0.294	16425	96.90
24	30.75	0.253	18207	95.60
25	32.65	0.232	19826	95.50
26	33.2	0.226	22477	95.95
27	33.04	0.215	19990	97.05
28	34.62	0.209	24809	97.51
31	37.22	0.165	23290	93.93
32	34.38	0.163	15169	94.67
33	33.26	0.167	13126	95.19
35	34.35	0.155	13953	95.35
38	35.12	0.145	12450	94.80
40	38.306	0.128	16001	93.90
41	40.32	0.115	17704	94.67
42	39.62	0.116	16683	95.57
43	41.29	0.110	18277	95.85
44	43.79	0.106	20724	96.01
45	43.42	0.104	20125	96.55
46	42.90	0.106	19737	96.75
47	46.66	0.1002	24480	96.87
52	42.80	0.106	19844	97.61
54	40.56	0,119	18328	97.53
57	40.9684	0.11937	19046	97.55

Table 2. Evolution of parameters estimates for US H1N1 cumulative data as the outburst proceeds.

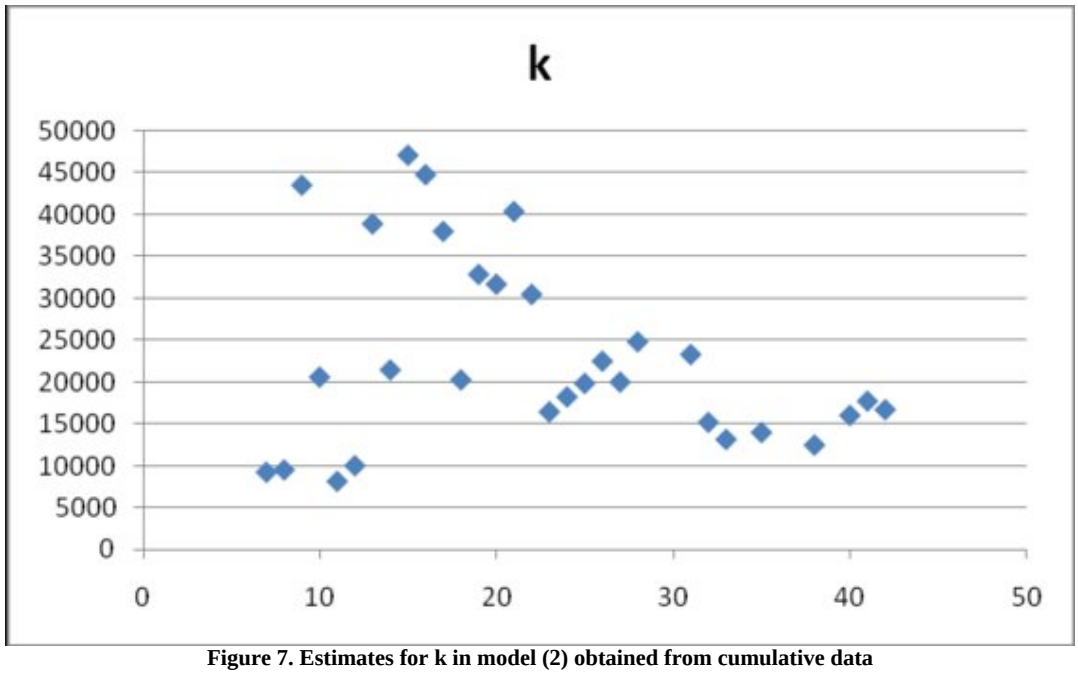

Figure 7. Estimates for k in model (2) obtained from cumulative data gathered at different dates after the onset of data collection on April 17, 2009.

DISCUSSION.

Overall, these results suggest that USA H1N1 data may be described with the Richards model, and reliable estimates for both the peak of incidence as well as for R_0 can be obtained from early data.

If we accept the value of Tau=38 as a true value, our results suggest that with as little as 6 observations, corresponding to less than half of Tau, quite acceptable estimates were observed for all the three parameters.

The estimates for the total number of cases give only rough orientation using this approach, but judging by the ample interval of possible values provided by the WHO Rapid Assessment Team, it seems that better precision is difficult to be attained. The simplest explanation for this fact comes from the errors in Tau and r. Since K depends on the exponential of the multiplication of these two parameters, a high span of values can be observed, in this sense, changing by a factor of 4 from lowest to highest value is not a very large dispersion.

There are large data sets about epidemics that have been modeled using different approaches, and valuable conclusions were drawn from them. However, the case when few data are at hand and it is necessary to maximally squeeze information from them is not uncommon in many places. This report has been an attempt to address this last situation.

We assume that due to the simplicity of this method and its possibility to bring early estimates of important parameters, its implementation could be of use practically in any setting where data can be properly collected.

The general Model of Richard includes a sigmoidicity parameter that apparently changes while analyzing real data. In USA flu data this parameters is practically 1 (0.999307 with 96.75% of explained variance when 35 data points corresponding to the fist 45 days of the outbreak were analyzed), which substantiate the choice of the simple version of the model.

CONCLUSIONS

- **Traditional methods for nonlinear approximation are of little use if are applied straightforward for simultaneous parameter estimation from the beginning of an epidemic outburst.**
- **Properly transforming cumulative data can provide good initial estimates for the time to peak as well as for the basic reproductive number.**
- **Initial estimates of the total number of cases have only an orientative value, and can be between a half and the double of more reliable estimates.**
- **Stepwise use of classical approximation methods can yield acceptable estimates at the beginning of an outburst.**

- **The following parameters were predicted from the USA H1N1 cumulative cases data:**
  - **Time to peak=32 days (19th of May)**
  - **R_0 close to 1.7**
  - **Total number of cases 20000 (Ranging from 9000 to 48000, and being close to 10000 on may 21st)**

ACKNOWLEDGEMENTS

Authors would like to express their gratitude to the following professors for their valuable suggestions: George Duncan, Alicia Juarrero, Tomas Johnson and Mario Estévez. MSc. Sonia Hernández Camacho contributed with a in-depth revision of the manuscript.

REFERENCES

1. Graeme Laver and Elspeth Garman. The Origin and Control of Pandemic Influenza. Science 2001:293:1776-1777.

2. Richard Coker. Swine flu. Fragile health systems will make surveillance and mitigation a challenge. BMJ 2009;338:b1791. Published 30 April 2009, doi:10.1136/bmj.b1791

3. World Health Organization. Epidemic and Pandemic Alert and Response (EPR). Assessing the severity of an influenza pandemic. 11 May 2009

4. Zhou G, Yan G. Severe acute respiratory syndrome epidemic in Asia. Emerg Infect Dis. 2003;9:1608-1610.

5. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, Griffin J, Baggaley RF, Jenkins HE, Lyons EJ, Jombart T, Hinsley WR, Grassly NC, Balloux F, Ghani AC, Ferguson NM, Rambaut A, Pybus OG, Lopez-Gatell H, Apluche-Aranda CM, Chapela IB, Zavala EP, Guevara DM, Checchi F, Garcia E, Hugonnet S, Roth C; The WHO Rapid Pandemic Assessment Collaboration. Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings. Science. 2009 May 11. [Epub ahead of print]

6. Cohen J, Enserink M. Infectious diseases. As swine flu circles globe, scientists grapple with basic questions. Science. 2009;324:572-573.

7. Riley S. Large-Scale Spatial-Transmission Models of Infectious Diseases. Science 2007;316:1298-1301

8. Kermack WO, McKendrick AG. Contributions to the mathematical theory of epidemics. Proc Royal Soc A 1927;115:700-721.

9. CDC MMWR dispatch. Swine-Origin Influenza A (H1N1) Virus Infections in a School - New York City, April 2009. April 30, 2009 / 58(Dispatch);1-3

10. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans www.nejm.org May 7, 2009

11. Center for Disease Control. Interim Guidance for Clinicians on Identifying and Caring for Patients with Swine-origin Influenza A (H1N1) Virus Infection May 4, 2009 4:45 PM ET. Available at Available at http://www.cdc.gov/h1n1flu/identifyingpatients.htm

12. Carrat F, Vergu E, Ferguson NM, Lemaitre M, Cauchemez S, Leach S, Valleron AJ. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol 2008;167:775-785

13. Center for Disease Control. H1N1 Flu. Available at http://www.cdc.gov/h1n1flu/

Comment of the reviewer Prof. Fernando Tricas García. PhD in mathematics. Professor of Languages and Systems. Department of Computer and Systems Engineering. University of Zaragoza. España.

The article shows how epidemic data can be adjusted to a well known model. With the available data the authors can estimate some parameters that can serve as early predictors for the incidence of A(H1N1) influenza.

It is still pending to see if these estimations will work well as early estimators with future epidemic outbursts

---

Comment of the reviewer Prof. José María Eirós Bouza. Professor of Microbiology, Faculty of Medicine, University of Valladolid. Head of Virology, Hospital Clínico Universitario. Advisory Committee of the WHO Influenza. Member of Working Group National Influenza Center. España.

Los modelos estocásticos aplicables a enfermedades infecciosas epidémicas revisten un innegable interés. Sus asunciones teóricas necesitan posterior comprobación, pero ello no resta oportunidad al diseño de los mismos.

En el presente trabajo Hernández Cáceres et al plantean la estimación de los parámetros que consideran importantes en el brote de gripe A H1N1 de la nueva variante en los Estados Unidos de América en 2009, con referencia a la información pública emitida por los CDC durante los días iniciales de la epidemia. De la lectura del mismo cabe apuntar su potencial aplicación a otras situaciones en las que se registren adecuadamente el número acumulativo de casos de una epidemia o brote.

Es notorio el esfuerzo metodológico que realizan los autores desde el Centro de Aplicaciones Cibernéticas de La Habana, que debe constituir un estímulo para cuantos desarrollan su actividad en modelos predictivos.

Received: May 17, 2009. Received reviewed: June 17, 2009.
Published July 26, 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

LA HIPERQUERATOSIS CITOLOGICA COMO PARAMETRO INDIRECTO DE LA INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO.

Ángel García Iglesias, Silvia García Valle[1], Joaquín López Marcos[2], Victoria Martín Gómez, David Holgado Sánchez

Departamentos de Obstetricia y Ginecología, [1]Farmacia y [2]Cirugía. Facultad de Medicina y Hospital Clínico Universitario. Salamanca. España

aiglesias @ usal.es

Rev Electron Biomed / Electron J Biomed 2009;2:49-55

Comentario del revisor Dr. Oscar Marín. **Jefe de Servicio de Anatomía Patológica y Citología. Hospital Pablo Soria. Jujuy. Argentina.**

Comentario del revisor Dr. Rodrigo Valdés Annunziata. **Centro de Diagnóstico Histopatológico y Citopatológico Histonor Ltda. Antofagasta. Chile.**

RESUMEN:

Introducción. La hiperqueratosis es una lesión epitelial que histológicamente se caracteriza, por presentar un aumento del grosor de la capa epitelial superficial cornificada, con ausencia de núcleos. Entre las causas que favorecen la aparición de hiperqueratosis, se puede resaltar la reacción del epitelio a los estímulos locales, mecánicos, químicos o infecciosos, siendo más relevante en estos casos el virus del papiloma humano.

Objetivo: Conocer la relación que existe entre la presencia de hiperqueratosis en los estudios citológicos de cribado y la posible relación con la infección del virus del Papiloma Humano.

Material y métodos: Se han estudiado un total de 2372 extendidos citológicos realizados durante un año en el Servicio de Obstetricia y Ginecología del Hospital Clínico de Salamanca. La edad de las pacientes estaba comprendida entre los 16 y 65 años. La toma citológica realizada mediante la técnica de triple toma se depositaba en medio líquido. Se descartaron las citologías que presentaban alteraciones citológicas, y se valoraron 1125 estudios citológicos considerados como negativos. De estos, 316 presentaron hiperqueratosis. A las pacientes que presentaban hiperqueratosis se les realizó estudio colposcópico y determinación de VPH por PCR.

Resultados: En el 27% de las hiperqueratosis, la PCR de VPH fue negativa, el resto, positiva. Se ha podido determinar un VPP del 72% y un VPN del 39%. La sensibilidad fue del 46% y la Especificidad del 81%.

PALABRAS CLAVE: Hiperqueratosis citológica. Papilomavirus. Neoplasia cervical intraepitelial

SUMMARY:

Introduction: Hyperkeratosis is an epithelial lesion with a histology that consists of a thickening of the cells of the stratum corneum that lack nuclei. There are some factors that can cause hyperkeratosis, such as a reaction of the epithelium to local, mechanical, chemical or infectious stimuli. The most common cause is the human papillomavirus.

Objetives: We want to establish the relation between the presence of hyperkeratosis in the cytological screening test and a possible relation with the infection of human papillomavirus.

Materials and methods: We studied 2372 cytological samples that were taken during one year in the Unit of Gynaecology and Obstetrics of the University Hospital of Salamanca. The age range of the patients was between 16 and 65 years of age. The cytological sample was taken with a Pap smear and it was kept in a liquid medium. All samples with cytological alterations were rejected and 1125 studies were considered negative. From these, 316 samples showed hyperkeratosis. All patients who presented that condition underwent a colposcopic study, and the HPV was detected by PCR.

Results: In 27% of the cases, the PCR test for HPV was negative, and it was positive in the rest of them. A PPV of 72% and a NPV of 39% were established. Sensitivity value was 46% and specifity was 81%.

KEY WORDS: Hyperkeratosis. Papillomavirus. Cervical intraepithelial neoplasia

INTRODUCCIÓN

Resulta difícil establecer estimaciones entorno al número de mujeres portadoras de infecciones ocultas por el virus del papiloma humano, y el volumen de lesiones asociadas[1]. Estas infecciones siguen habitualmente un curso silente, que tienden a establecer alteraciones citológicas características que se englobarían mayoritariamente en la neoplasia cervical intraepitelial[2].

Teniendo en cuenta que el ciclo vital de los papilomavirus se desarrolla de forma coordinada con la diferenciación y maduración celular de los queratocitos, la infección se localiza en las células escamosas epiteliales de la capa basal del epitelio.

Pero no siempre que se produce una infección por el virus del papiloma aparecen alteraciones celulares de bajo o alto grado, dependiendo de las características de la integración viral[3], o de la respuesta inmunológica[4], de tal forma, que en la citología de una infección por el virus del papiloma, puede que no aparezcan alteraciones celulares, que hagan sospechar la infección por HPV.

Como consecuencia de la integración viral y sin aparecer alteraciones celulares significativas, en ocasiones se detectan modificaciones celulares conocidas como hiperqueratosis debido a la infección por el virus del papiloma, no apreciándose otros factores orientativos de dicha infección[5].

Nuestro objetivo es demostrar que, la hiperqueratosis citológica puede considerarse un factor de sospecha de infección por el virus del papiloma humano, en extendidos citológicos en los que no se detectan otros tipos de alteraciones citológicas.

MATERIAL Y MÉTODOS:

Se han valorado los resultados de la citología de 2373 pacientes realizadas durante el periodo de un año, comprendido desde el 1 de Enero al 31 de diciembre de 2007 y que acudieron a la consulta de ginecología del Hospital Clínico de Salamanca, por diversos motivos, procesos ginecológicos y revisiones. La edad estaba comprendida entre los 16 y 65 años.

No se incluyeron en este estudio las portadoras de cánceres de cervix y los resultados citológicos de pacientes gestantes que forman parte de otro estudio. La metódica de recogida de las muestras citológicas fue en todos los casos igual, obteniendo el material citológico de las paredes vaginales, exocervix y endocervix, que seguidamente se depositaba en medio líquido THIN PREP, para posterior tinción e interpretación citológica.

Según los resultados obtenidos, se descartaron los estudios citológicos identificados como: Asc-us 68 (2,86%), Ag-us 29 (1,22%), L-SIL 834 (35,14%), H-SIL 317 (13,35%). 1125 (47,40%) presentaron citologías negativas, de las cuales en 316 (28,08%) se determinó la presencia de hiperqueratosis. En 809 (71,91%) se identificaron otros hallazgos, que quedaron distribuidos: 658 (58,48%) como citologías sin ningún tipo de alteración y 151 (13,42%), como inflamatorias. Se incluyeron en el protocolo, las 316 pacientes que presentaban hiperqueratosis. A todas ellas, se les realizó recogida de muestra cervical para determinación de HPV mediante técnica de PCR, complementándose con la exploración colposcópica que incluía: Visión del cervix sin preparar, visualización después de limpiar con suero fisiológico y visualización del cervix, después de aplicar ácido acético al 5% y una solución de lugol.

La eficacia queda establecida por el tamaño de la muestra estadística, la efectividad de las técnicas utilizadas y el intervalo de confianza (95%). El nivel de significación se estableció en 0,05.

Los parámetros estudiados fueron evaluados mediante el Test de CHI cuadrado. Se valoraron los porcentajes de falsos positivos y negativos, para poder determinar los valores predictivos positivos y negativos, así como la sensibilidad y especificidad. El tratamiento estadístico se realizó mediante el paquete SPSS versión 11 para PC.

RESULTADOS:

En la tabla I se describen los hallazgos en pacientes con citología negativa. El 58,48% no presentan ningún otro componente (P< de 0,05), mientras el 28,08% presentaban hiperqueratosis. El resto correspondía a hallazgos bacteriológicos.

Tabla I: Distribución de los hallazgos en citologías negativas.

HALLAZGOS EN CITOLOGIA NEGATIVA	F. ABSOLUTA	F. RELATIVA
HIPERQUERATOSIS	316	28,08
CANDIDIASIS	78	6,93
F. INESPECIFICA	61	5,46
FLORA MIXTA	12	1,06
SIN OTROS HALLAZGOS	658	58,48
TOTAL	1125	

$X^2 = 178,6$. $P<0,05$.

Tabla II. Se representan las imágenes colposcópicas identificadas en las pacientes con hiperqueratosis. Predominan las colpitis a puntos blancos en el 43,35%, seguida de la mucosa originaria en el 22,78%. La imagen menos frecuente correspondía a la zona de transformación con el 3,48%. (P <0,05).

Tabla II: Hallazgos colposcópicos en pacientes con hiperqueratosis citológica

COLPOSCOPIA	F. ABSOLUTA	F. RELATIVA
M. ORIGINARIA	72	22,78
P. BLANCOS	137	43,35
C. MOSAICIFORME	33	10,44
C. FLORIDA	39	12,34
C. MICROPAPILAR	24	7,59
Z. TRANSFORMACION	11	3,48
TOTAL	316	

X^2= 316. P<0,05

Tabla III. Se establece la relación entre la Hiperqueratosis y el VPH. El 54,43% eran positivos para el papilomavirus de alto riesgo, siendo negativo el 27,84% (P<0,05). Tabla IV. Se describen los resultados estadísticos de la sensibilidad que fue del 46% y la especificidad del 81%. Así como el valor predictivo positivo y negativo.

Tabla III: Identificación de VPH en pacientes con hiperqueratosis citológica

IDENTIFICACION VPH	F. ABSOLUTA	F. RELATIVA
NEGATIVO	88	27,54
ALTO RIESGO	172	54,43
BAJO RIESGO	56	17,72
TOTAL	316	

X^2= 212. P< 0,05.

DISCUSIÓN:

A pesar de que el mecanismo de la integración viral del VPH en las células del epitelio pavimentoso es bien conocido y referenciado[6], la posible relación entre la hiperqueratosis citológica y la presencia del virus del papiloma humano no está lo suficientemente establecida. Está claro que la hiperqueratosis citológica puede aparecer por variadas causas, como puede ser el arrastre celular del epitelio queratinizado de la vulva[7], o de lesiones condilomatosas[8] que puede inducir a errores y hacer sospechar la presencia de epitelio queratósico en el cervix, cuando en realidad la procedencia es distinta, aunque se ha señalado la posibilidad de relación entre la hiperqueratosis y la presencia del virus del papiloma humano .

Estudios previos[9,10] han demostrado que el hallazgo de hiperqueratosis, hace necesaria la realización de una colposcopia debido a la mayor incidencia de HPV o displasias encontrada en las pacientes portadoras de estas alteraciones[11]. Se ha demostrado en estudios histológicos que el gen E7 del genotipo 16 del virus del papiloma humano, causa una hiperplasia epidérmica, caracterizada por una expansión del compartimento proliferativo, y de las células queratin 10 positivas que se traduce en hiperqueratosis[12]. De esta forma podríamos deducir, que el mecanismo por el que el VPH produce hiperqueratosis esta mediado por los mismos genes que inician el proceso de la carcinogénesis, siendo esta por tanto, un estadio precoz de las lesiones displásicas producidas por el virus del papiloma humano[13].

La presencia de queratinización en una citología con atípia escamosa inflamatoria y, sobre todo en la atípia escamosa, justifica el seguimiento y control de las mismas debido a su posible asociación con la infección por el VPH.

Se puede afirmar que la colposcopia en mujeres con hiperqueratosis pone de manifiesto un alto porcentaje de alteraciones inflamatorias y/o alteraciones cervicales[14]. Pero lo que realmente establece la posible relación entre la hiperqueratosis y el VPH, es sencillamente el estudio histológico de la biopsia de las lesiones cervicales, habiéndose podido observar, un alto porcentaje de pacientes con hiperqueratosis que presentaban displasia de distintos grados, lo que establece una estrecha relación entre ambas entidades[15]. Williamson[16], asocia la hiperqueratosis en un 9,4% a anormalidades celulares, al Ascus en un 17,4% y a las displasias en un 2,1%. Bajo el mismo criterio, se ha comunicado la asociación entre el grado de hiperqueratosis y la presencia de SIL en la biopsia[17].

Por otra parte hemos encontrado un estudio que no establece relación entre la hiperqueratosis y la infección por el virus de papiloma humano[18]. En ellos se utilizaron técnicas de radiodiagnóstico sin amplificación previa de ADN para la detección del virus, circunstancia que hay que tener en consideración, ya que estos métodos no poseen la misma sensibilidad que la PCR a la hora de detectar el DNA viral. No se han encontrado estudios que hagan referencia a la sensibilidad de la técnica para sospechar la presencia del VPH, pero son similares o algo inferiores a los de la citología de cribado, 53,8% de sensibilidad. En cambio, la prueba combinada citología y ADN- VPH alcanza una sensibilidad del 91%, siendo baja la especificidad del ADN-VPH y la citología[19]. Pero a pesar de que en los estudios citológicos se pueden detectar alteraciones celulares sugestivas de infección por HPV, se ha recomendado[20,21] utilizar la prueba de detección de HPV como única prueba de cribado sin efectuar citologías de forma sistemática a todas las mujeres. Si se utiliza la citología junto con la prueba de detección del VPH para la identificación de mujeres HPV positivas, se puede conseguir una mayor sensibilidad y solo una pequeña pérdida de especificidad, en comparación con la citología convencional incluso en mujeres en edad comprendida entre 25 a 34 años, en la que la presencia de infección es muy frecuente[22]. De los resultados obtenidos podría considerarse a la hiperqueratosis citológica, como un factor más de sospecha de infección del virus del papiloma humano.

REFERENCIAS

1.- Buechler EJ. Pap Test and HPV infection. Advances in screening and interpretation. Postgrad Med 2005 118:37-40.

2.- Ahmed AS, Goumalattsos G, Akbar N, Lawton, FG, Savvas M. Outcome analysis of 4 years follow-up of patients referred for colposcopy with one smear showing mild dyskaryosis. Cytopathology 2008 19:94-105.

3.- Lopez J, Aristizaban FA. Integración viral y cancer de cuello de utero. Rev Col Cienc. Quim. Farm. 2006 35:5-32.

4.- Paavonen J. Human Papillomavirus infection and the development at cervical cancer and related genital neoplasias. Int j infect Dis 2007 11: Suppl 2:3-9.

5.- Trottier H. Franco EL. The epidemiology of genital human Papillomavirus infection. Vaccine 2006 30:24 Suppl 1: 1-5.

6.- Yu T, Ferber MJ, Cheung TH. The role of viral in the development of cervical cancer. Cancer Gent Cytogenet 2005 158: 27-30.

7.- Hadzic B, Djurdjevic S, Hadzic M, Jerant-Patic V. Morphologic Manifestations of human papillomavirus infection in the vulvar and anogenital region Ded Pregl 1998 51:265-270.

8.- Uezato H, Haiwara K, Maruno M, Ramuzi ST, Khaskhely NM et al. Detection of human type 58 in a case of a perianal Bowen's disease coexistent with adult T-cell leuquemia J Dermat. 1999 26:168-73.

9.- Lindeque BG. Managemen of cervical of cervical premalignant lesions. Best Pract Res Clin Obste Gynaecol. 2005 19:545-561.

10.- Al-Nafussi A, Rebello G, Al-Yusif R, McGoogan E. The borderline cervical smear : Colposcopic and biopsy outcome. J Clin Pathol.2000 53:439-44.

11.- Syrjamen K, Naud P. Derchain S, Roteli C,Longatto A, Tatti S, et al. Comparing PAP smear cytology, aided visual inspection screening colposcopy, cervigraphy and hpv testing as optional screening toois in Latrin America. Study design and baseline data pf the LAMS study. Anticancer Res 2005 25:369-3480.

12.- Herber R, Liem A, Pitot H, Lamber PF. Squamous epithelial hyperplasia and carcinomas in nice transgenic for the human papillomavirus type 16, E7 Oncogene. J Virol 1996 70: 1873-1881.

13.- DiMaio D: Human papillomaviruses and cervical cancer. Adv Virus Res 2006 66: 125-159.

14.- Pruitt SL, Parker PA, Peterson SK, Le T, Follen M, Basen-Engquint K. Knowledge of cervical displasia and human papillomavirus among women seen in a colposcopy clinic. Ginecol Oncol 2005 99:236-244.

15.- Zahn CM, Askew AW, Hall Kl, Barth WH Jr. The significance of hyperkeratosis/paraqueratosis on otherwise normal Papanicolau smears. Am J Obst Gynecol 2002 187:997-1001.

16.- Williamson Ba, De Frias D, Gunn R, Tarjan G, Nayar R. Significance of extensive hyperkeratosis on cervical/vaginal smears. Acta Cytol 2003 47: 749-752.

17.- Navarro R, Furlani B, Songco L, Alfieri ML, Nuovo GJ. Cytologic correlatos of bening versus dysplatic abnormal keratinizacion. Citopathol 1997 17:447-451.

18.- Kiviat NB, koustsky LA, Critchlow CW, Lorincz AT, Cullen AP, et al. Pce and cytology manifestation of human papillomavirus types 6,11,16,18,31,33, 35,42,43,45,51,52, and 56 among 500 consecutive women. Inter J ginecol Patol. 1992 15:327-341.

19.- Baseman JG, Kulasingan SL, Harris TG, Hughes JP, Kiviat NB. DNA testing and cervical cytology Amer J Og Gynecol. 2008 199:261-268.

20.- Ronco G. Human Papillomavirus testing and liquid-based cytology: results at recruitment fron the new technologies for cervical cancer randomized controlled trial. J Nat cancer Inst 2006 98: 765-774.

21.- Riorgi Rossi P: The impact of new technologies in cervical cancer screening: resultads of the recruitment phase of a large randomised controlled trial from a public health perspective. Int J Cancer 2007 121:2739-2734.

22.- Ronco G. HPV triage for low grade (L-SIL) cytology is appropriate for women over 35 in mass cervical cancer screening using liquid based cytology. Eur J Cancer 2007 43:476-480.

CORRESPONDENCIA: Dr. A. García Iglesias
Hospital Clínico Universitario.
Dpto. de Obstetricia y ginecología
Paseo de san Vicente, 58-182
37007 Salamanca
E-mail: aiglesias @ usal.es

Comentario del revisor Dr. Oscar Marín Jefe de Servicio de Anatomía Patológica y Citología. Hospital Pablo Soria. Jujuy. Argentina.

La hiperqueratosis, lesión epitelial histológicamente caracterizada por aumento del grosor de la capa córnea epitelial superficial con ausencia de núcleos, es analizada por los autores en su relación a los casos de infección por HPV (Human Papiloma Virus) en el cuello uterino humano, mediante estudios de citología.

Considerando los autores aquellos casos en los cuales existen infecciones silentes, que no producen alteraciones celulares atribuibles a HPV, en las cuales la hiperqueratosis puede considerarse factor de sospecha de infección por este virus, siendo este un tópico poco analizado en la literatura, si bien se sugiere una relación causal por mecanismo genético inducida por HPV16.

Los autores nos muestran que los resultados de su estudio -determinando un 72% de hallazgos positivos por PCR, con un 54% de cepas de alto riesgo-, describiendo además las alteraciones colposcópicas encontradas asociadas a estos casos y la confirmación histopatológica de lesiones epiteliales, permiten de los resultados obtenidos, considerar a la hiperqueratosis

citológica, como un factor más de sospecha o de alarma de infección del virus del papiloma humano, a tener en cuenta en los tamizajes de citología, especialmente por aquellos que trabajamos en zonas de alta incidencia de neoplasias de cuello uterino.

Comentario del revisor Dr. Rodrigo Valdés Annunziata. Centro de Diagnóstico Histopatológico y Citopatológico Histonor Ltda. Antofagasta. Chile.

Hasta ahora se ha propuesto que la hiperqueratosis se asocia más frecuentemente con condiciones benignas de tipo inflamatorio, incluyendo el prolapso, cervicitis crónica y otras. Se considera una condición reactiva y los frotis citológicos se consideran obviamente negativos para efectos del screening.

Según lo aceptado en la práctica, la hiperqueratosis se considera como un hallazgo relativamente infrecuente (menos de 1% de los frotis rutinarios) y poco relevante, conociéndose que tiende a ser consistente en el seguimiento de las pacientes en que está presente. Está también presente en frotis con atipias de significado incierto y podría indicar la presencia de enfermedad en pacientes con una historia de patología cervical.

El trabajo aborda un tema que podría ser muy discutido y en caso de demostrar lo propuesto podría ampliar los criterios citológicos universalmente aceptados, con implicancias diagnósticas individuales y ciertamente con impacto en campañas poblacionales.

Recibido: 19 de mayo de 2009. Recibido revisado: 5 de julio de 2009
Publicado 31 de agosto de 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

TOURNIQUET: LACUNAE OF PREANALYTICS

Smita S. Sonoli MD, Anuradha B. Patil MD, P. B. Desai MD, M. V. Kodliwadmath MD, C. V. B. Prasad PhD, Shivprasad MD.

**Department of Biochemistry,
KLE University's Jawaharlal Nehru Medical College.
Belgaum, Karnataka, India.**

drsmitasonoli @ yahoo.co.in

Rev Electron Biomed / Electron J Biomed 2009;2:56-61

Comment of the reviewer Pilar Calmarza, PhD. **Laboratorio de Bioquímica. Hospital Miguel Servet. Zaragoza. España.**

Comment of the reviewer Dra. Pilar García-Chico Sepúlveda. **Jefe del Servicio de Análisis Clínicos del Hospital General de Ciudad Real. España.**

RESUMEN:

Objetivo: Este estudio tuvo como objetivo investigar la influencia del estancamiento prolongado, creado durante la recogida de la muestra de sangre, sobre cinco parámetros bioquímicos comunes.

Metodología: La sangre fue recogida por punción venosa de 20 individuos sanos. Cinco muestras fueron recolectadas, una antes de aplicar presión y cuatro después de aplicar presión estándar de 60 mm de Hg (1 y 3 minutos) y 90 mm de Hg (1 y 3 minutos) con la ayuda del esfingomanómetro. Los valores de proteínas totales, albúmina, glucosa, urea y creatinina se calcularon por el método de kit y análisis en un semiautoanalizador, fabricado por Transasia en colaboración con Erba Diagnostic (Alemania).

Resultados: No hubo aumento en los niveles de proteínas totales y albúmina y la disminución de los niveles de glucosa y urea ($p < 0,05$) con aumento de la presión y el tiempo. Sin embargo, la creatinina no mostró cambios en los valores de estancamiento a corto plazo, pero la estasis prolongada a 90 mm de Hg durante 3 minutos mostró una disminución en los valores ($p < 0,02$).

Conclusión: A partir de nuestros resultados, podemos concluir que los parámetros en cuestión son influenciados por la duración y la magnitud de la presión aplicada, por lo tanto, el torniquete se debe utilizar de manera meticulosa. El personal del Laboratorio debe ser educado sobre el uso de torniquete, para que los errores de laboratorio puedan ser identificados y prevenidos.

PALABRAS CLAVE: Errores preanalíticos. Estasis prolongado. Flebotomía. Torniquete.

SUMMARY:

Aim / Objective: This study was aimed to investigate the influence of prolonged stasis, created during blood sample collection, on five common biochemical parameters.

Methodology: Blood was collected by venepuncture from 20 healthy individuals. 5 samples were collected. One before applying standard pressure and four after applying standardized pressure of 60 mm of Hg(1 and 3 minutes) and 90 mm of Hg (1 and 3 minutes) with help of sypgmomanometer. Total protein, albumin, glucose, urea, creatinine were estimated by kit method and analysed on semiautoanalyzer manufactured by Transasia in collaboration with Erba Diagnostics (Germany).

Results: There was increase in the levels of total protein and albumin and decrease in levels of glucose and urea ($p < 0.05$) with increase in pressure and time. However creatinine did not show change in values on short term stasis, but prolonged stasis at 90 mm of Hg for (3 minutes) showed decrease in values ($p < 0.02$).

Conclusion: From our results, we conclude that the parameters in question are influenced by the duration and magnitude of pressure applied, hence tourniquet should be used meticulously. Phlebotomists should be educated regarding the usage of tourniquet so that laboratory errors can be identified and prevented.

KEY WORDS: Phlebotomy. Pre-analytical errors. Prolonged stasis. Tourniquet.

INTRODUCTION

Quality assurance is the focus of current concern in laboratory, as laboratory errors are of great impact on medical diagnosis and therapies. Errors might originate from pre analytical, analytical and post analytical sources, hence laboratorians have realised the importance of monitoring all the steps in laboratory tests to detect and prevent defects.

Advances in instrument technology and automation have simplified tasks in laboratory diagnostics and improved the quality of test results, thereby decreasing the analytical defects to less than 7-10%[1]. Improvement now should be targeted to extra-analytical phase (pre analytical and post analytical phase). However difficulties to monitor the pre analytical variables are that, they lie outside the direct control of laboratory personnel, one such being phlebotomy. In developing countries like India, there is lack of understanding about good laboratory practices and inadequate training to phlebotomists, compelling them to make errors during phlebotomy. The improper venous accesses, or prolonged venous stasis created by tourniquet application will result in collection of unsuitable blood sample.

Ideally, the tourniquet should be used only if necessary and removed as soon as the needle is within the vein. A normal healthy individual with a systolic blood pressure of 120-130 mm of Hg, the pressure from the tourniquet should be around 100 mm of Hg and should not last longer than 1 minute[2]. Unfortunately this is never practised; the amount of time and pressure created by tourniquet is often under looked by the phlebotomist leading to prolonged stasis, and the amount of pressure far exceeds, which may reach up to 90-100 mm of Hg, or even more which is never analysed.

Keeping this in view, the aim of our study, was to assess the effect of prolonged stasis created by sypgomanometer of about 60 mm of Hg(1 and 3 minutes) and 90 mm of Hg (1 and 3 minutes) on five biochemical parameters routinely done in all laboratories.

MATERIAL AND METHODS

The Study was conducted in Department of Biochemistry, Jawaharlal Nehru Medical College, Belgaum.

Inclusion Criteria:

20 healthy males in the age group of 28 to 32 years were selected as participants of this study. Morning fasting sample was taken using 21 gauge straight needle and collected in vacutainer by single experienced ,expert phlebotomist using same sphygmomanometer every time to create the required standardized pressure. Sphygmomanometer mimicked as tourniquet. The study was approved by Ethical Committee of the Jawaharlal Nehru Medical College, Belgaum. Consent was taken from all participants.

Exclusion Criteria:

Elderly males, children, obese individuals, smokers, alcoholics and individuals with systemic diseases were excluded from this

study.

Procedure for Blood Collection:

All Samples were collected in sitting position. Venipuncture was done on different arms of anticubital site to exclude any interference originating from the previous tourniquet. The first phlebotomy was carried out without stasis, while the subsequent four with stasis. Second and third samples were collected after application of standard external pressure of 60 mm of Hg using sphygmomanometer for one and three minutes while fourth and fifth samples were collected after application of standard external pressure of 90 mm of Hg for 1 and 3 minutes. The pressure was released only after the blood was drawn and after every prick rest time of 10 min was given to the volunteers. 3 ml of Blood was collected every time out of which 2 ml was evacuated into the vacuum tube containing gel of the same lot and 1 ml was collected in fluoride containing tubes for analysis of glucose.

Serum was obtained after allowing it to clot for 30 minutes at room temperature, followed by centrifugation at 3000 rpm for 10 minutes. All Specimens were processed within 2 hours of collection. Total protein, albumin, glucose, urea, creatinine were estimated by kit method and analysed on semiautoanalyzer manufactured by Transasia in collaboration with Erba Diagnostics (Germany).

The Instrument was calibrated against appropriate reference standard material & controlled daily by the use of control sera, there by maintaining Quality control.

Statistical Analysis: The data were expressed as mean ± standard deviation (M ± SD). Student 't' test was used to detect significant differences between no stasis and other groups. The difference was considered significant at $p < 0.05$.

RESULTS

Results of our present evaluation are summarized in Table 1.

TABLE 1: Statistical analysis of 5 routine biochemical analytes tested in blood specimen collected without stasis(No stasis) and after application of a standardized external pressure of 60 mm of Hg and 90 mm of Hg for 1 minute(1 min stasis) and 3 minute(3 min stasis) respectively

Parameters	Non Stasis (n= 20)	60 mm Hg (n= 20)		90 mmHg (n= 20)	
		1 min	3 min	1 min	3 min
Total Protein (g/dl)	6.99±0.98	7.17±0.63* (P=0.006)	7.14±0.7* (P=0.000)	7.32±1.66* (P=0.000)	7.56±0.67* (P=0.002)
Albumin (g/dl)	3.88±0.28	3.96±0.32* (P=0.031)	4.03±0.38* (P=0.035)	4.09±0.34* (P=0.000)	4.15±0.31* (P=0.000)
Glucose (mg/dl)	89.10±11.72	86.45±12.75* (P=0.003)	87.15±11.76* (P=0.04)	85.80±10.71* (P=0.05)	85.30±9.98* (P=0.05)
Urea (mg/dl)	21.15±3.64	22.1±3.78* (P=0.004)	22.2±3.30* (P=0.009)	23.2±3.57* (P=0.000)	21.1±3.82* (P=0.000)
Creatinine (mg/dl)	0.97±0.23	0.98±0.23^{NS} (P=1)	0.97±0.23^{NS} (P=0.163)	0.99±0.23^{NS} (P=0.359)	1.03±0.24* (P=0.021)

Values are expressed as mean±SD. Difference between samples were evaluated by a paired student 't'test (p).
n is number of subjects; * Significant difference; NS- Nonsignificant change

Statistically significant differences according to the paired student 't'- test could be observed between the samples collected with stasis after a standardized pressure of 60 mm and 90 mm of Hg was applied for 1 and 3 minutes with that of Non stasis($p<0.05$).

Although statistical analysis was satisfactory for total protein, albumin, glucose and urea, the clinical acceptability was somehow lower for creatinine.

DISCUSSION

Today's era of modern medicine focuses to achieve total quality in laboratory testing. Earlier investigations which have surveyed the causes of laboratory errors found that out of total error, 46% are contributed by pre-analytical phase, 47% by post-analytical & 7% by analytical[3]. To err is human, but to persevere in error is only the act of a fool. This is also reasonably true when dealing with laboratory errors, unless such errors can actually be identified[4] and rectified. Only awareness of the mistake is not enough, vigorous steps to reduce them is mandatory, to improve the overall quality of laboratory services.

In course of time analytical errors have been reduced drastically. So our prime focus being pre-analytical phase. Phlebotomy, is one of the pre-analytical phase, which is least supervised. Although there are standard guidelines, for blood sample collection, but none are mandatory in India[5], leading to errors by phlebotomist. Errors are due to their ignorance about the methods of sample collection as they are lacking proper education regarding the standard guidelines. Actually, tourniquet should be used only in cases of non accessibility of veins. But, tourniquet is tied before the blood is drawn and till the blood is drawn, immaterial of veins visibility and without accessing the amount of time and pressure being created by it. So keeping these observations in view, in our study we have created both short term venous stasis and prolonged stasis, for blood sample collection.

We analysed that there was statistical significant difference($P<0.05$) between the samples collected by without stasis and with stasis of 60 and 90 mm of Hg for 1 & 3 minutes respectively created by sphygmomanometer in cases of 4 parameters those are total proteins, albumin, glucose and urea. Total protein and albumin levels increased with increase in pressure. While glucose and urea level decreased with increase in pressure.

The result is in accordance with study done by Lippe G et al[6]. Contradictory results have been observed by Mc Mullan et al[7], Jung B et al[8], McNair P et al[9] and Serdar MA et al[10]. In our study in case of creatinine, statistically significant difference ($P<0.02$) between the sample collected by non stasis and with stasis at 90mm of Hg for 3 minute was observed, but no statistical significant difference between the sample collected at 1 & 3 minutes for 60mm of Hg and 1 min for pressure of 90mm of Hg with that of non stasis was observed. This result shows that, short term venous stasis has no effect on creatinine values, but long term do has attributed to the extravasation of creatinine from the strained localised muscle tissue[11] caused by tourniquet application.

Hypothesis states that the application of tourniquet, by reducing pressure below systolic pressure, maintains the effective filtration pressure within the capillaries. As a result, small molecules and fluid are transferred from intravasal space to the interstitium. Application of the tourniquet for longer than 1 minute and up to 3 minute can result in hemoconcentration, causing an increase in the concentration of large molecules that are unable to penetrate the capillary wall[12]. However this hypothesis does not hold good for creatinine in our study.

We can conclude from our study that application of tourniquet for a prolonged time influences the reliability of the results, for instance, glucose levels keep on decreasing with increase in pressure, then there are chances of missing cases of borderline diabetes mellitus. Urea level decreased with increase in pressure, and then we may faultily report a renal diseased person to be in a recovery state. So one of the remedial measure to access the amount of time and pressure created is usage of re/de-inflatable devices as suggested by Lippi G et al[6]. Even we can formulate some correction factors for the parameters showing changes with change in pressure as one being done for the estimation of calcium by Mc Mullan et al[7].

Our suggestion are manufacturing of new type of tourniquet which can show pressure changes digitally, when applied. Better is to avoid tourniquet, when not required, as in case of visible veins. Lastly, phlebotomists have to be educated regarding the proper and timely usage of tourniquet. However further studies have to be done with a larger sample size and involvement of patients with diseases to see the changes in the levels of parameter in question.

CONCLUSION

Results from our investigation, confirms that, the biochemical parameters, tend to vary with change in duration and pressure created by tourniquet. Just awareness is not enough, but vigorous action to reduce the errors is mandatory.

REFERENCES

1.- Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med 2006; 44: 750-759.

2.- Dittmann M. Blood collection techniques. An Informative guide to blood collection 6th edition. Greiner Bio-One GmpH, Kremsmunster Austria 2008: 1-24.

3.- Plebani M, Carraro P. Mistakes in a stat laboratory:types and frequency. Clinical Chem 1997 Aug; 43(8 Pt 1):1348-1351.

4.- Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006; 44: 358-365.

5.- Ashavaid TF, Dandekar SP, Keny B, Bhambhwani VR. Influence of blood specimen collection method on various preanalytical sample quality indicators.Indian J Clin Biochem 2008; 23: 144-149.

6.- Narayanan S. The Preanalytic Phase- An Important Component of Laboratory Medicine. Am J Clin Pathol 2000; 113: 429-452.

7.- Lippi G, Salvagno GL, Montagnana M, Brocco G, Guidi GC. Influence of short-term venous stasis on clinical chemistry testing. Clin Chem Lab Med 2005; 43: 869-875.

8.- McMullan AD, Burns J, Paterson CR. Venepuncture for calcium assays: should we still avoid the tourniquet? Postgrad Med J 1990; 66: 547-548.

9.- Junge B, Hoffmeister H, Feddersen HM, Rocker L. Standardisation of obtaining blood samples: influence of tourniquet application on 33 constituents of blood and serum. Dtsch Med Wochenschr 1978; 103: 260-265.

10.- McNair P, Nielsen SL, Christiansen C, Axelsson C .Gross errors made by routine blood sampling from two sites using a tourniquet applied at different positions. Clin Chim Acta. 1979; 98(1-2): 113-118.

11.- Young DS, Bermes Jr. EW. Preanalytical variables and biological variation. Tietz Text Book of Clinical Chemistry and Molecular Diagnostics 4th edition. Elsevier, New Delhi 2006: 449-473.

12.- Serdar MA, Kenar L, Hasimi A, Kocu L, Türkmen YH, Kurt I, Akman S , Erbil Mk. Tourniquet Application Time During Phlebotomy and TheInfluence on Clinical Chemistry Testing; Is It Negligible? Turkish J Biochem 2008; 33: 85-88.

CORRESPONDENCE: Dr. Smita S. Sonoli
Assistant Professor. Department of Biochemistry
Jawaharlal Nehru Medical
Nehru nagar. Belgaum- 590 010
Karnataka, INDIA
E mail: drsmitasonoli @ yahoo.co.in

Comment of the reviewer Pilar Calmarza, PhD. Laboratorio de Bioquímica. Hospital Miguel Servet. Zaragoza. España.

The pre-analytical phase is a vitally important part of the process for accurate results in any biochemical analysis.

A large number of errors can occur within the wide spectrum of this phase, and knowledge of them and their detection is the responsibility of the clinical laboratory professionals.

Although there are many studies on the quality of the analytical phase in clinical analysis, the same cannot be said of the pre-analytical and post-analytical phases. This may be because their great importance in the final result of the biochemical analysis has only been recently recognised.

It is the responsibility of the laboratory to take the necessary measures to minimise the sources of error in the pre-analytical phase, in aspects regarding the correct filling in of the analysis request form, as well the correct drawing and collection of the sample, developing standard procedures for preparation of the patient and obtaining the sample.

As regards obtaining the blood sample, as is demonstrated in this work, the time of applying the tourniquet is fundamental, attempting to minimise its use as far as is possible.

The standardisation of all these parameters will lead to a higher quality of the analytical process itself; this process being considered as a single entity which in turn consists of the pre-analytical, analytical and post-analytical phases.

Comment of the reviewer Dra. Pilar García-Chico Sepúlveda. Jefe del Servicio de Análisis Clínicos del Hospital General de Ciudad Real. España.

The most common causes of laboratory error can be classified into 3 phases: pre-analytical errors (46 %), analytical errors (7 %) and post-analytical errors (47 %). Phlebotomy and the training of the phlebotomists is an important step in achieving quality in the pre-analytical phase. This article sets out the way make a blood sample withdrawal, avoiding venous stasis, controlling the pressure applied by the tourniquet by measuring with a sphygmomanometer.

The scope of the preceding article is narrow, since it only looks at five parameters in 20 patients. It is an article with well set out methodology, and well documented bibliographically. The results and conclusions add value.

Received: June 24, 2009.
Published August 31, 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

HERIDA PENETRANTE INTRACRANEAL CAUSADA POR NAVAJA: PRESENTACIÓN DE UN CASO

Carlos Fernando Lozano MD, Luis Rafael Moscote Salazar MD, Alejandro Celemin Nieto MD, Fredy Llamas Cano MD.

Facultad de Medicina de la Universidad de Cartagena. Cartagena. Colombia

neuromoscote @ gmail.com

Rev Electron Biomed / Electron J Biomed 2009;2:62-65

RESUMEN:

El traumatismo craneoencefálico es una entidad común en el servicio de urgencias y se ha vuelto un problema de salud pública. El trauma penetrante de cráneo ocupa un lugar especial por su rareza.

Se presenta el caso de un joven de 17 años que sufrió un trauma penetrante de cráneo durante riña por arma blanca, fue sometido a extracción de la misma en quirófano y cuya evolución y pronóstico fueron favorables.

El conocimiento del trauma penetrante de cráneo es fundamental para el médico general y todo el personal que trabaja en los servicios de urgencia.

PALABRAS CLAVE: Trauma craneoencefálico. Arma blanca. Edema cerebral. Traumatismo penetrante de cráneo.

SUMMARY: Head injury is a common entity in the emergency department and has become a public health problem. The penetrating skull trauma has a special place for its rarity.

A case of a young man of 17 who suffered a penetrating skull trauma during a knife fight by is presented. The patient underwent removal of knife in the operating room. The evolution and prognosis was favourable.

Knowledge penetrating skull trauma is essential for general practitioners and all staff working in emergency services.

KEY WORDS: Head trauma. Stabbing. Cerebral edema. Penetrating skull trauma.

INTRODUCCIÓN

El trauma constituye un importante problema de salud pública, debido a su carácter epidémico actual con sus consecuentes implicaciones económicas, sociales y morales. Su incidencia varía según cada país, así como las consecuencias para el individuo y su entorno social. La diversas manifestaciones de la violencia en nuestro país, entre ellas las agresiones con armas cortopunzantes son frecuentes en nuestros servicios de urgencias.

El trauma craneoencefálico penetrante en un tópico importante de neurocirugía. El manejo en los servicios de urgencias y su posterior resolución son cruciales para una evolución óptima de los pacientes. Se consideran heridas intracraneales penetrantes a aquellas que presentan orificio de entrada en contraposición a las heridas perforantes que son aquellas con orificios de entrada y salida[1,2]

CASO CLÍNICO:

Paciente masculino de 17 años, vendedor ambulante, quien fue agredido por desconocido con arma cortopunzante en región temporoparietal izquierda.

Trasladado de inmediato al servicio de urgencias del HUC. Se valora paciente con frecuencia cardiaca de 70 pulsaciones/m, TA 110-80. Glasgow 12/15, AO 3, RV 3, RM 6. Con disfasia y lenguaje coprolálico, se observa objeto metálico en cuero cabelludo que protruye medio centímetro. (Fig. 1a y b)

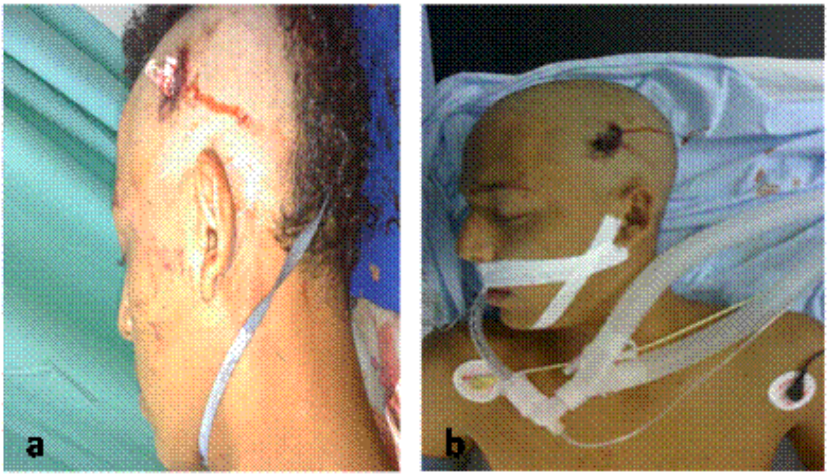

Fig. 1a y b. Presencia de arma blanca aun insertada dentro del cráneo del paciente. Se observa elemento en región temporoparietal izquierda-

En radiografía de cráneo y TAC cerebral simple se confirma la presencia de objeto extraño: hoja de navaja, 5 cms. dentro del parénquima cerebral en el girus angular asociado a hematoma epidural laminar (Fig.2 a, b y Fig. 3).

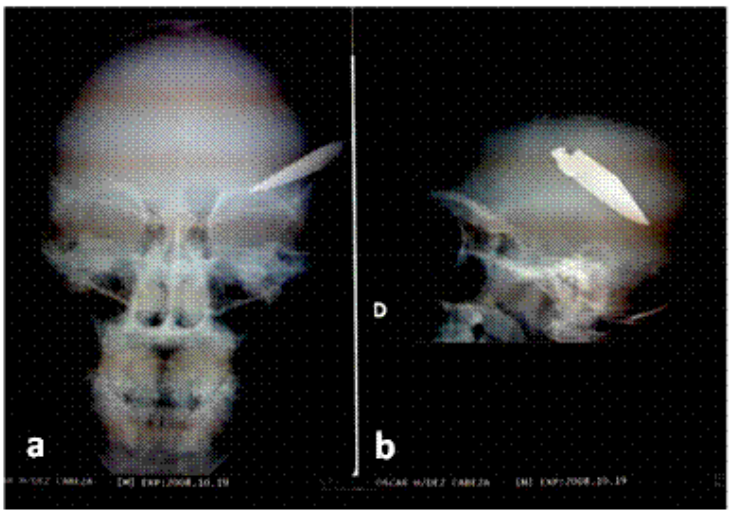

Fig 2a y b. Radiografía AP y LAT donde se observa el arma blanca.

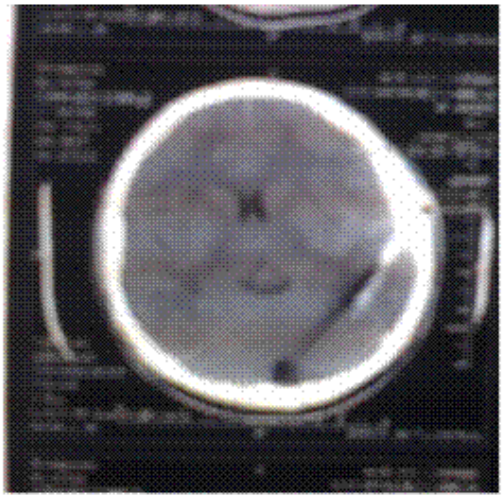

Fig 3. TAC de cráneo simple, cortes axiales, se evidencia lesión hiperdensa en región Parietal izquierda, compatible con hematoma epidural adyacente a arma blanca. Se Observa edema cerebral hemisférico y discreta desviación de la línea media.

Se somete a cirugía de craneotomía y resección de cuerpo extraño con hemostasia profunda y drenaje del hematoma epidural. El paciente fué cubierto con antibioticoterapia intravenosa por una semana y vía oral por 15 días.

Última valoración al cabo de un mes del evento: paciente sin signos de focalización neurológica, con lenguaje fluido y coherente, sin signos de lesión parietal. Resto del examen neurológico normal.

DISCUSIÓN:

Las heridas por arma blanca han sido definidas como "aquellas causadas por un arma con una pequeña área de impacto y de baja velocidad". Este tipo de lesión ha sido llamado como síndrome de Jael, por la forma en que Jael asesinó a Sissera como se menciona en el antiguo testamento.

Las heridas por arma blanca producen un tipo de lesión basada en el impacto mecánico que origina la degeneración neuronal mediante tres mecanismos básicos: Mecanismo lesional primario que es el responsable de las lesiones nerviosas vasculares, mecanismo secundario que es el responsable de las lesiones cerebrales producidas por alteraciones sistémicas y mecanismo neuroquímico que se inicia inmediatamente al trauma[3,4].

De los efectos y consecuencias de un traumatismo sobre el cráneo, tienen mayor jerarquía e importancia los que afectan al parénquima del SNC. El manejo de los pacientes incluye una estabilización en los servicios de urgencias, antibióticoterapia, anticomiciales, realización de imagenología (radiografia de cráneo y tomografía cerebral simple con ventana ósea) como también una valoración urgente por el servicio de neurocirugía o la remisión a un nivel superior sino se cuenta con un neurocirujano y una respectiva unidad de terapia intensiva[5,6].

CONCLUSION

Los pacientes que sobreviven a un trauma craneoencefálico penetrante potencialmente pueden presentar múltiples complicaciones, incluyendo el déficit neurológico persistente, infecciones, epilepsia, fistula de liquido cefalorraquídeo, déficit de nervios craneales, pseudoaneurismas, fistulas arteriovenosas, e hidrocefalia. En nuestro paciente la rápida y oportuna atención conllevo a un pronóstico favorable.

REFERENCIAS:

1. Cosar A, Gonul E, Kurt E, et al. Craniocerebral gunshot wounds: results of less aggressive surgery and complications. Minim Invasive Neurosurg. 2005;48:113-118.

2. Siccardi D, Cavaliere R, Pau A, et al. Penetrating craniocerebral missile injuries in civilians: a retrospective analysis of 314 cases. Surg Neurol. 1991;35:455-460.

3. Trask TW, Narayan RK. Civilian Penetrating Head Injury. In: Narayan R, Wilberger J, Povlishock, J, eds. Neurotrauma. New York, NY: McGraw Hill; 1996:868-889.

4. Merino F, Rego J, Vega J, Goldberg D, Mercuri J. Patología traumática. Herida por arma blanca penetrante en cráneo, Reporte de un caso. Rev Argent Neuroc. 2004, 18: 157.

5. Dante AF, Alfaro AM, Alvarez GH, Toxtle MA. Traumatismo penetrante de cráneo, caso clínico, Bol Med Hosp Infant Mex, 2005;268:272.

6. Zazpe I, Vázquez A, Beaumont C, Bardón A, Azcona J, Gallo-Ruiz A, Portillo E. Heridas múltiples penetrantes intracraneales causadas por pistola de clavos: caso clínico. Neurocirugía 2006;17:544-549

CORRESPONDENCIA:

Correspondencia: Dr. Luis Rafael Moscote Salazar. Sección de Neurocirugía. Universidad de Cartagena. Cartagena de Indias, Bolívar, Colombia.
E-mail: neuromoscote @ gmail.com

Recibido: 28 de julio de 2009.
Publicado 31 de agosto de 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

PROVISION OF HEALTH CARE SERVICES IN CANADA: CHALLENGES AND OPPORTUNITIES

Rosa Rodriguez-Monguio, Ph.D.[1,2], Enrique Seoane-Vazquez, Ph.D. [3,4], Fernando Antoñanzas Villar, Ph.D.[5]

[1] School of Public Health and Health Sciences, [2] The Institute for Global Health, University of Massachusetts, Amherst.
[3] College of Pharmacy, [4] College of Public Health, The Ohio State University
[5] Department of Economy and Business, University of La Rioja

rmonguio @ schoolph.umass.edu

Rev Electron Biomed / Electron J Biomed 2009;2:66-75

Comment of the reviewer Prof. Jaime Espin Balbino LLB, MBA, MSc. **Andalusian School of Public Health (Escuela Andaluza de Salud Pública). España**

Comment of the reviewer Daniel A. Sepulveda Adams. **Research Scientist, PRIME Institute. College of Pharmacy, University of Minnesota. Minneapolis, USA**

RESUMEN:

El sistema sanitario de salud canadiense provee completo acceso a servicios hospitalarios y de atención ambulatorio, incluyendo servicios terapéuticos diagnósticos y preventivos. El nivel de cobertura de los servicios varía en el país. Este estudio examina las características principales de los sistemas canadiense de salud y de cuidados de larga duración; presenta un análisis estructurado del aseguramiento, financiación y provisión de los servicios sanitarios y de cuidados de larga duración; describe los principales desafíos de dichos sistemas; y concluye con una lista de oportunidades para la política sanitaria pública.

Los principales desafíos del sistema Canadiense están relacionados con el envejecimiento de la población, la prevalencia de enfermedades prevenibles causadas por hábitos no saludables, la cobertura y financiación de los cuidados de larga duración, la financiación de nuevas tecnologías y medicamentos de alto coste, y la escasez y inadecuada distribución geográfica de los profesionales sanitarios. Las oportunidades para la política sanitaria canadiense incluyen: fortalecer la política de salud pública, continuar con la transferencia de la atención al nivel ambulatorio, mejorar la coordinación entre los servicios de atención primaria y especializada, implantar un sistema nacional de planificación de recursos humanos, e integrar la atención domiciliaria como parte de la atención primaria de salud.

PALABRAS CLAVE: Canadá, política pública, salud pública, servicios sanitarios, cuidados de larga duración.

SUMMARY

The Canadian health care system provides comprehensive coverage of hospital and outpatient care, including therapeutic, diagnostic and preventive services. The level of coverage of services varies across the country. This study examines the key characteristics of the Canadian health and long-term care systems; presents a structured analysis of the insurance, financing and provision of health and long-term care services in Canada; describes the main challenges of the Canadian health and long-term care systems; and concludes with feasible opportunities for the Canadian health policy.

Main challenges to the Canadian system are related to population ageing; prevalence of avoidable diseases caused by poor health habits; coverage and financing of long-term care services; financing of expensive new technologies and pharmaceuticals; and the shortage and unbalanced geographic distribution of health care professionals. Opportunities for the Canadian health policy are: strengthening public health policy, continuing shifting care to the ambulatory level; improving the coordination between primary care and specialist services; implementing a system-wide national human resources planning; and integrating home-based care as part of overall primary health care.

KEY WORDS: Canada, public policy, public health, health care, long-term care.

---

1. INTRODUCTION

Canada had 31.6 million inhabitants in 2006[1], a population which is expected to reach 37.0 million by 2020[2]. The crude birth rate was 11.3 per 1,000 population in 2000, and it is expected to decrease to 10.4 by 2020 (Table 1). The total fertility rate among women aged 15 to 49 declined to 1.53 in 2004[3]; this rate is below the replacement level fertility for the total population without immigration (i.e. 2.11 per 1,000 women). The combined effects of the reduction of births, the net number of migrants and the increase in deaths are projected to reduce the population growth rate from 1.0% in 2000 to 0.7% in 2020[2]. In 2006, Aboriginal people (i.e. First Nations, Métis and Inuit) accounted for 4% of the total population of Canada[4]. Canada's population as a whole is growing older. The proportion of people aged 65 years and over was 13.7% in 2006[1], and it is projected to increase to 18.2% in 2020[2].

Table 1. Vital Rates. 2000-2020.

	2000	2005	2010	2015	2020
Births per 1,000 pop.	11.3	10.8	10.7	10.7	10.4
Deaths per 1,000 pop.	7.4	7.7	8.0	8.4	8.8
Net number of migrants per 1,000 pop.	6.2	5.9	5.6	5.4	5.2
Rate of natural increase (percent)	0.4	0.3	0.3	0.2	0.2
Growth rate (percent)	1.0	0.9	0.8	0.8	0.7

Source: U.S. Census Bureau, International Data Base.

The percentage of the Canadian population reporting good health status decreased from 89.2% to 88.2% for both genders and most age groups during the period 1994-2004[3]. Circulatory diseases, including heart disease, were the leading causes of death, representing 31.4% of all deaths in 2002. Obesity and obesity-related morbidities are major public health problems. In 2005, 22.4% of the population aged 18 years and over was obese and 35.1% was overweight.

In 2003, First Nations reported difficulties in accessing health care including long waiting lists (33.2%); access to health services not covered under the Non-Insured Health Benefits Program (NIHB) (20.0%); availability of a health professional in rural and remote areas (18.5%); inadequate provision of health care (16.9%); and denial of prior approval for services under NIHB (16.1%)[5].

The Canadian federal government's responsibilities include setting and administering national principles for the health system, providing financial support to the provinces and territories, and delivering primary and supplementary services to certain groups, including persons of Aboriginal ancestry, veterans of the Armed Forces and members of the Royal Canadian Mounted Police. The federal government is also responsible for public health protection and regulation, consumer safety, and disease surveillance and prevention. It also provides support for health promotion and health research. Constitutionally health care is a matter of provincial jurisdiction. The provincial and territorial governments have most of the responsibility for organizing and delivering health care and other social services[6]: their role includes administering their health insurance plans, planning, paying for and evaluating hospital and outpatient care, and negotiating fee schedules for health professionals[7].

This study examines the key characteristics of the Canadian health and long-term care systems and analyzes major challenges

associated with the provision of health care, including the ageing population, prevalence of chronic diseases, and coverage of long-term care services. The paper provides a structured analysis of the regulation, insurance, financing and provision of health and long-term care services in Canada and assesses the major strengths and weakness of the country's provision of health and long-term care services. Finally, the paper describes the main challenges confronted by the Canadian health care system to overcome its current gaps, and presents health policy opportunities.

2. HEALTH CARE INSURANCE AND FINANCING

Under the Canada Health Care Act (1984), all residents of a province or territory are eligible to receive free medically necessary health services[8]. The Canada Health Act does not define the specific health services eligible for public coverage but sets general principles for the health care system. According to these principles, health care in Canada should be comprehensive in coverage; accessible without financial barriers; portable within the country and during travel abroad; and publicly administered.

Canada's health care system is organized in ten provincial and three territorial health insurance plans. Each provincial and territorial plan covers medically necessary hospital and outpatient medical care free of charge. Provinces and territories also provide coverage to certain population groups for health services that are not generally covered under the publicly funded health care system.

Private insurance for services insured under the Health Care Act is prohibited by provincial legislation in six provinces and discouraged in the other four through prohibitions of the subsidizing of private practice by public plans. Private insurance may covers the cost of supplementary services such as drugs, dental care, vision care, and complementary and alternative medicines and therapies[6], [9]. In 2003, 53.6% of dental care, 33.8% of prescription drugs and 21.7% of vision care was funded through private health insurance[8]. Most of the private health insurance is employment-based and is part of compensation packages, rather than privately purchased by individuals. Private health insurance for long-term care and home care is limited.

Canada has a predominantly public health care system financed through federal, provincial and territorial taxation. In 2004, 69.8% of total health care revenue came from public sources (mainly taxation) (Table 2). The federal government provides cash and tax transfers to the provinces and territories to finance health care services through the Canada Health Transfer[6].

Table 2. Health Care Financing. 1990-2004.

Percent Distribution	1990	1995	2000	2001	2002	2003	2004
Public Expenditures	74.5	71.4	70.3	69.9	69.6	70.1	69.8
General Government Excluding Social Sec.	73.5	70.3	70.3	68.5	68.2	68.6	68.3
Social Sec. schemes	1.1	1.1	1.4	1.4	1.4	1.5	1.5
Private Expenditures	25.5	28.6	29.7	30.1	30.4	29.9	30.2
Private insurance	8.1	10.3	11.5	12.4	12.7	12.7	12.8
Out-of-pocket payments	14.4	15.9	15.9	15.2	15.2	14.5	14.9
All other private funds	2.9	2.5	2.3	2.5	2.6	2.6	2.5

Source: OECD Health Data 2006.

All provinces manage single-payer systems for the delivery of hospital, physician and diagnostic services; provinces fund hospitals through health districts. Hospitals are paid through annual global budgets negotiated with the provincial and territorial ministries of health, or with a regional health authority[10]. The vast majority of specialists and general practitioners work under fee-for-service schedules; fees for health care providers are negotiated directly with the provincial ministry in a contractual relationship with regional health authorities (RHAs). General practitioners operate largely outside the RHA system8. Most health care personnel, including nurses (i.e. registered nurses, licensed practical nurses, psychiatric nurses and nurse practitioners), are salaried.

Canada spent US$97,202 million on health in 2004 (Table 3). Health expenditures represented 9.9% of its GDP in 2004, which was above the OECD average for health expenditures3. Expenditures per capita were US$3,043 in the same year[11].

Table 3. Health Expenditures. 1990-2004.

	1990	1995	2000	2001	2002	2003	2004
Health Expenditures (Million US$)							
Public	38,412	38,130	44,739	46,358	48,869	59,157	67,868
Private	13,120	15,304	18,876	19,934	21,391	25,185	29,334
Total	51,532	53,433	63,615	66,293	70,259	84,343	97,202
Health Expenditures per Capita (US$)							
Public	1,387	1,301	1,458	1,494	1,558	1,869	2,124
Private	474	522	615	643	682	795	918
Total	1,861	1,824	2,073	2,137	2,239	2,664	3,043
Health Expenditures as a % of GDP							
Public	6.7	6.5	6.3	6.6	6.7	6.9	6.9
Private	2.3	2.6	2.7	2.8	3.0	2.9	3.0
Total	9.0	9.2	8.9	9.4	9.7	9.9	9.9

Source: OECD Health Data 2006.

Also in 2004, hospital services, excluding capital expenditures, accounted for 31.3% of health expenditures (Table 4), ambulatory care providers for 27.5%, pharmaceutical expenditures for 16.9%, and nursing and residential care facilities for 9.8%[3]. Health expenditures are projected to reach $147.0 billion (inflation-adjusted) in 2020. Health expenditures vary across the provinces and territories due to differences in the services that each province and territory covers, and in demographic factors such as the population's age.

Table 4. Current Health Care Expenditures by Provider as a Percentage of Total Expenditures. 1990-2004

	1990	1995	2000	2001	2002	2003	2004
Public							
Ambulatory Care Providers	26.8	27.3	26.9	26.7	26.6	26.3	26.1
Hospital Services	50.2	46.1	42.1	41.4	41.2	40.8	41.0
Nursing and Residential Care Facilities	9.7	10.1	10.7	10.7	10.7	10.6	10.7
Retail Sale & Other Providers of Medical Goods	6.0	7.6	9.3	9.8	10.3	10.6	10.9
Public health org. Public Current	4.4	6.1	7.6	8.0	8.0	8.5	8.2
Health Care Administration	2.3	2.3	2.9	3.0	2.9	2.9	2.9
Health Services of Other Industries	0.6	0.5	0.4	0.4	0.4	0.4	0.4
Total Public	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Private							
Ambulatory Care Providers	37.6	37.4	33.1	32.9	32.8	31.5	30.8
Hospital Services	15.1	11.7	9.4	9.4	9.6	9.4	9.1
Nursing and Residential Care Facilities	10.6	10.4	8.8	8.3	8.1	8.0	7.9
Retail Sale & Other Providers of Medical Goods	32.2	34.2	43.1	42.6	42.3	43.2	44.5
Public health org. Private Current	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Health Care Administration	4.5	6.3	5.6	6.8	7.2	8.0	7.8
Health Services of Other Industries	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Private	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total							
Ambulatory Care Providers	29.5	30.2	28.8	28.6	28.5	27.9	27.5
Hospital Services	41.2	36.2	32.3	31.8	31.5	31.4	31.3
Nursing and Residential Care Facilities	9.9	10.2	10.1	10.0	9.9	9.8	9.8
Retail Sale & Other Providers of Medical Goods	12.7	15.2	19.4	19.7	20.1	20.3	21.0
Public health org Total Current	4.4	5.5	5.3	5.6	5.5	5.9	5.7
Health Care Administration	1.7	2.3	3.7	4.1	4.2	4.4	4.4
Health Services of Other Industries	0.4	0.4	0.3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Source: OECD Health Data 2006.

3. PROVISION OF HEALTH AND LONG-TERM CARE

Provinces in Canada work through, or contract with, a large range of health care organizations and providers, from RHAs to

individual hospitals and physicians. RHAs act as both purchasers and providers: the majority of acute care facilities, including their salaried employees, are managed directly by RHAs, and some RHAs contract with private providers for the provision of specialized ambulatory care services. Nursing homes and other long-term care facilities are either run directly by RHAs or have a contractual relationship with them. Family physicians provide the majority of primary medical care services in Canada. Most physicians work in their own private clinics. Patients have freedom of choice in selecting a family physician, and such general practitioners act as gatekeepers to access to specialist and hospital services.

Recent reforms in Canada have focused on increasing the number of community primary care centers that provide services around the clock; placing greater emphasis on promoting health, preventing illness and injury, and managing chronic diseases; increasing coordination and integration of comprehensive health services; and improving the work environment of primary care providers6. There is also an increasing interest in expanding the roles of nurses and pharmacists in primary care. Many provinces are changing their laws to enable nurse practitioners to deliver a broader range of primary care services. Moreover, some jurisdictions are setting targets concerning the replacement of fee-for-service remuneration by alternative payment mechanisms, encouraging physicians' engagement in the provision of essential services around the clock, and accelerating the development of medical telephone call centers.

Publicly owned hospitals perform the majority of the secondary, tertiary and emergency care, as well as the majority of specialized ambulatory care and elective care. Although hospitals provide mainly acute care, some comprehensive hospitals also provide extended or chronic care, rehabilitation and convalescent care, and psychiatric services; hospitals also manage nursing stations and outpost hospitals in remote areas. Canadian hospitals are organized and administered on a local basis, with almost all hospitals operating at arm's length from provincial and territorial governments.

Canada has experienced a decline in the number of hospitals. The total number of hospital beds was relatively stable until the mid-1980s, but began to decline after 1985-1986. Hospital beds fell rapidly in the 1990s. Trends appear to be stabilizing at present[12]. There were 746 hospitals with 115,120 beds in 2002-2003[13]. Approximately 8 in 100 Canadians were hospitalized in 2005-2006, representing a decrease of 24.6% since 1995-1996. The decline in hospital beds and the rate of hospitalization are due to a number of factors, including clinical practice changes and new surgical techniques[14]. The number of days spent in acute care hospitals was 20.3 million in 2005-2006, representing a 13.1% decrease since 1995-1996[15].

A considerable amount of hospital care has been shifted from inpatient settings to ambulatory clinics[12]. In 2003, 35.9% surgical procedures were done in the hospital and 64.1% in ambulatory settings[3]. An increasing number of surgeries are being performed in a day surgery setting (up by 30.6%) compared to an inpatient hospital setting (down by 16.5%) over the past decade[15].

Between 1990 and 2004, the number of general practitioners for every 1,000 people decreased from 1.1 to 1.0, and the number of practicing specialists increased from 1.0 to 1.13. In 2006, the number of physicians for every 100,000 population was 190 (98 family medicine physicians, 92 specialists). In 2006, the ratio of physicians per 100,000 population among the provinces ranged from 36 in Nunavut to 226 in Yukon Territory[16].

Nurses are the largest group of health care workers in Canada, though in the last 20 years, the supply of nurses has fluctuated significantly[17]. Between 1990 and 2004, the number of practicing nurses for every 1,000 people decreased from 11.1 to 9.93. In 2005 the ratio of registered nurses employed in nursing among the provinces and territories ranged from 1:76 in Northwest Territories to 1:153 in British Columbia[18], [19]. The average age of registered nurses in 2006 was 45.0 years, 4.4 years older than that of the rest of the country's workforce[20], [21].

Canada Health Act does not cover long-term care services. Nevertheless, all provinces and territories provide and pay for certain long-term care services. In 2001, provincial programs and subsidies for long-term care, home care, community care, and public health and prescription drugs dispensed in long-term care facilities amounted 23% of total health expenditures. Home care expenditures increased from $26 million in 1975 to approximately $2.7 billion in 2001, and nursing home expenditures also increased from $800 million to $6.8 billion over the same period[17]. Referrals to long-term care institutions can be made by doctors, hospitals, community agencies, families, and the patient him/herself; needs are assessed and services are coordinated to provide comprehensiveness and continuity of care.

There is large variability across Canada in the type of services offered in long-term care facilities. In general, long-term care facilities provide living accommodation for people who require on-site delivery of continuous supervised care, including professional health services, personal care and services such as meals, laundry and housekeeping. Provincial and territorial governments finance health care services provided in long-term care institutions, and individuals pay out-of-pocket for room and board; in some cases, the provincial and territorial governments subsidize these out-of-pocket payments.

Some nursing homes are managed directly by the RHAs, but a large number remain independent companies, often in a contractual relationship with the RHA to provide a defined level of long-term care. There is also a for-profit nursing home sector providing various levels of care, mostly to the elderly.

Demand for home care has increased due to an ageing population and other societal developments. The availability of family members for informal care is expected to continue to decline due to reductions in family size and increasing mobility. Home care services generally include nursing, physiotherapy, occupational therapy, speech therapy and personal care including assistance

with the activities of daily living. Some provinces and territories provide complete coverage for certain services while others provide more limited home care services according to acuity of illness, financial means, dollar limits, or other criteria. With the exception of British Columbia, where eligibility requires 12 months of residency, general eligibility requirements for home care across Canada require three months' residency; a health insurance card; a health or medical need for care; a suitable home for care; and a physician referral.

Since the 1970s, home care services have been funded through a combination of provincial and territorial funds, federal funds, private insurance, and out-of-pocket payments. The federal government provides funding support through transfer payments for health and social services, and some provinces use means testing to determine access to home care services; on average, provinces and territories spend between 4% and 5% of their health budgets on home care. Between 1980 and 2000, the average annual rate of growth for these expenditures was 14%, compared with 7.1% for all provincial-territorial health expenditures17.

Informal caregivers play an essential role in the delivery of home care services in Canada and also provide ongoing care, support and advocacy for people with physical disabilities[17, 22]. Overall 18% of the Canadian population 15 years of age or older provide unpaid care or assistance to seniors[23] and 85% to 90% of home care is provided by family and friends [24, 25]. Each province and territory has its own policies and programs concerning support and services for informal caregivers. Respite services for informal caregivers are provided through a home care worker coming to the home, a patient being placed in a respite bed in a long-term care facility for a short-term stay or a mixture of services[22, 26]; the availability of such services across Canada varies widely depending on provincial/territorial financial resources and the availability of qualified workers. Additionally, the Compassionate Family Care Benefit was introduced in 2004 to support those who need to leave their job temporarily to care for a gravely ill or dying child, parent or spouse. Policies also vary across Canada with respect to allowances for the cared-for person and the caregiver.

4. CHALLENGES AND OPPORTUNITIES FOR THE CANADIAN HEALTH POLICY

Canada's health care system faces major challenges related to demographic changes; prevalence of avoidable diseases caused by poor health habits; coverage of health care services, especially long-term care services; and the shortage and unbalanced geographic distribution of health care professionals. Demographic patterns in Canada over the long-term are characterized by demographic ageing, the slowing of the increase of the natural rate of population and cultural diversity resulting from high rates of immigration. Aboriginal health is also a matter of concern of federal jurisdiction[27, 28]. The demographic and epidemiological trends represent a growing threat for the Canadian welfare state. Prevalence of avoidable diseases caused by poor health habits is a threat to the health of Canadians. Obesity is becoming more prevalent among Canadians, and it is increasing among children.

Health coverage levels vary across the country, from full financial protection for all necessary healthcare services to some exclusions and cost-sharing arrangements. Timely access to health care also remains as a concern associated with the provision of care to rural and remote populations, and with the cultural diversity resulting from high rates of immigration[29].

Challenges to the Canadian system are also related to the coverage and financing of long-term care services. Canada has established long-term care programs under the auspices of health and welfare services: across the country, provincial and territorial governments offer a different range of services covered under a variety of cost-sharing arrangements and eligibility requirements. However, lack of minimum standards and coverage criteria across the country could erode the equity of the welfare system.

The demand for long-term care services will continue to increase due to a combination of economic and socio-demographic factors, including improvements in treatment outcomes, bed closures, and reductions in length of stay, improvements in homecare services, and the ageing of the population. Demographic ageing, the increased participation of women in the labor market, higher levels of geographical mobility, a lower ratio of working age people to elderly, and changes in family structure may also affect the extent to which long-term care is provided.

Changes in how health care services are organized and delivered in hospitals and other settings have had a direct effect on the workload of health professionals, particularly nurses, and their competencies. Professional dissatisfaction and unbalanced distribution of health care professionals have led to increased problems of health care delivery in Canada[30].

Canada also faces a number of challenges in terms of supply, distribution, retention, recruitment, and training of health care professionals[16, 19, 31]. Based on existing trends, the proportion of family physicians is expected to decrease over time and forecasting studies predict shortages in family physicians in Canada; some specialties will experience shortages as well (e.g. geriatricians)[32]. There are also nursing shortages in certain practice areas and an uneven distribution of nurses across geographic regions, especially in rural and remote areas. The problem of attracting health care providers to rural communities is exacerbated by competition among provinces and territories. The current shortage of nurses could also worsen: at a time when an ageing population will require more nursing services, a large cohort of nurses will be retiring and will not be replaced by a similar number of new graduates[21].

Long-term care is a labor-intensive sector and the sector is also likely to suffer from acute labor shortages related to ageing of health professionals. Additionally, as home care services continue to expand, there will be growing demands for trained home

care workers, and population ageing and labor shortage could drive up the cost of providing long-term care services. To some extent, shortages may also explain the lack of capacity in institutional long-term care, which results in elderly people occupying hospitals' acute care beds for longer than necessary, as well as a decline in the quality of services provided.

Alongside its challenges, the Canadian health care system has valuable opportunities that can be grouped under three main areas: epidemiological trends, delivery of health care and human resources. The country could strengthen public health measures to tackle poor health habits of the population: in 2002, the federal, provincial and territorial health ministers launched the Integrated Pan-Canadian Healthy Living Strategy, an intergovernmental plan which attempts to improve the state of knowledge and coordinate governmental and voluntary initiatives, all encouraging physical activity, healthier eating and tobacco cessation[8, 33].

Canadian public health activity focuses on the traditional roles of health protection and behavioral approaches to health promotion[33, 34]. A broader approach to public health policy has been proposed based on the following strategies[35, 36]: reducing the prevalence of risk factors (tobacco, nutrition, physical activity, alcohol consumption, overweight and obesity, hypertension, high cholesterol) associated with disease; reducing the burden of chronic disease; advancing comprehensive population-based interventions; reducing inequities in risk factors and in access to services due to cultural, social, economic and geographic disparities, especially for Aboriginal populations; engaging researchers, policy-makers and practitioners for policy and program development proposes; raising public awareness of the broader determinants of health; aligning national public health policies with those of the provinces and territories.

As of result of task transfers from secondary care, there is a trend towards expanding responsibilities in primary care, as well as a stronger involvement of primary care professionals in screening, prevention and health promotion services. Continuing shifting care to an ambulatory level and improving coordination between primary care and specialist services could improve the effectiveness of health-care delivery, reduce fragmentation of the delivery process and contain health expenditures without jeopardizing health outcomes.

Additionally, informal caregivers play a growing role in providing care in Canada. Informal care and self-care are substituting for services otherwise provided in institutional settings and by professionals. It is essential to provide appropriate institutional support to caregivers and to integrate effective home-based and other long-term care as part of the country's health and social systems. It is also important for the future sustainability of the Canadian health and long-term care systems to ensure that home-based care is an integral part of overall primary health care.

One major policy issue for workforce development is the extent to which countries allow nursing assistants to perform certain tasks currently performed by nurses (e.g., administering medications, providing wound care and changing catheters). Giving long-term care workers added responsibility and autonomy might motivate them to remain in the job, or to encourage others to seek these positions.

There is no a national agency responsible for system-wide national human resources planning in Canada - most planning is done within the ministries of health at the provincial level. The Canadian Institutes for Health Research and the Canadian Institute for Health Information is a step forward towards implementation of a comprehensive information system to monitor the actual number of health care workers and to forecast the stock and distribution of health care professionals by practice settings[37].

It is also recommended that the country identify and support the implementation of retention strategies for the health care workforce[32]. Key strategies could include addressing appropriate nurse/patient ratios; encouraging efficient team-based working relationships; reducing non-nursing duties; preventing workplace injuries and illness; implementing improved flexibility and family-friendly scheduling options; and increasing childcare support. It is also essential to increase training and employment opportunities for graduating students, and to provide appropriate support within the workplace to ensure their integration within the nursing profession[38]. Like other countries experiencing health care personnel shortages, Canada is also considering salary increases[39], but policies focusing heavily on such increases have only limited success[40, 41].

The Canadian International Medical Graduate (IMG) Taskforce made six recommendations which were endorsed by the federal/provincial/territorial Ministers of Health in 2004[42]. These recommendations include: to increase the capacity to assess and prepare IMGs for licensure; to work towards standardization of licensure requirements; to expand programs to assist IMGs with the licensure process and requirements in Canada; to develop capacity to track and recruit IMGs; and to develop a national research agenda. Many countries rely on immigration of health professionals to address labor shortages, but while inward migration could partially mitigate the shortages of health care professionals in the short term, these are still narrow approaches, particularly in rural areas.

REFERENCES

1. U.S. Census Bureau. International Database, 2007. http://www.census.gov/ipc/www/idb/

2. Canada's National Statistical Agency. Statistics Canada, 2006 Census. Canada, 2008. http://www12.statcan.ca/english/census06/data/popdwell/Table.cfm?T=101.

3. OECD . Heath Data. Organization for Economic Cooperation and Development (OECD), 2006.

4. Minister of Industry. Statistics Canada. Aboriginal Peoples in Canada in 2006: Inuit, Métis and First Nations, 2006 Census. Census year 2006. Canada, 2008. http://www12.statcan.ca/english/census06/analysis/aboriginal/pdf/97-558-XIE2006001.pdf

5. Minister of Health (2006a), Healthy Canadians. A Federal Report on Comparable Health Indicators. Minister of Health. Canada, 2006.

6. Health Canada, Canada's Health Care System, 2005. Available online at: http://www.hc-sc.gc.ca

7. Evans R, Roos NP. What is Right about the Canadian Health Care System?. The Milbank Quarterly. 1999; 77:393-399.

8. Marchildon GP. Health Systems in Transition: Canada. European Observatory on Health Systems and Policies. Copenhagen, Denmark, 2005.

9. Canadian Life and Health Insurance Association. The Role of Supplementary Health Insurance in Canada's Health System. Submission to the Commission on the Future of Health Care in Canada. Toronto, Canada, 2001.

10. Sheils JF, Young GJ, Rubin RJ. O Canada: Do we Expect too much from its Health System?. Health Affairs. 1992; 11, 7-20.

11. European Observatory on Health Care Systems. Health Care Systems in Eight Countries: Trends and Challenges. Edited by Dixon A. and Mossialos E. Commissioned by the Health Trends Review, HM Treasury. London, UK. 2002.

12. Canadian Institute for Health Information. Hospital Trends in Canada. National Health Expenditure Database. March 25, 2005. Ottawa, Ontario, 2008. http://secure.cihi.ca/cihiweb/dispPage.jsp?cw_page=AR_1215_toc_e.

13. Canadian Institute for Health Information. Canadian MIS Database. 2008.

14. Evans RG, McGrail KM, Morgan SG, Barer ML, Herzman C. Population Aging and the Future of Health Care Systems. Can J Aging. 2001;20:160-191.

15. Canada Institute for Health Information. Inpatient Hospitalizations and Average Length of Stay Trends in Canada, 2003-2004 and 2004-2005. Hospital Morbidity Database and the Discharge Abstract Database. Canada, 2005. http://secure.cihi.ca/cihiweb/dispPage.jsp?cw_page=bl_hmdb_3jan2007_e. Accessed June, 2008

16. Canadian Institute for Health Information. Supply, Distribution and Migration of Canadian Physicians, 2006. Health Human Resources Database. Ottawa, Ontario, 2007. http://secure.cihi.ca/cihiweb/splash.html

17. Commission on the Future of Health Care in Canada. Building on Values the Future of Health Care in Canada. Roy J. Romanow Commissioner. Canada, 2002. http://www.cbc.ca/healthcare/final_report.pdf

18. Canadian Nurses Association. Workforce Profile of Registered Nurses in Canada. Canada, 2005. http://www.cna-aiic.ca/CNA/resources/bytype/statistics/default_e.aspx.

19. Canadian Institute for Health Information. Distribution and Internal Migration of Canada's Registered Nurse Workforce. Health Human Resources Database. Ottawa, Ontario, 2007. http://secure.cihi.ca/cihiweb/splash.html.

20. Canadian Institute for Health Information. Statistics Canada and Health Canada. Findings from the 2005 National Survey of the Work and Health of Nurses. Statistics Canada, Ministry of Industry. Ottawa, Ontario, 2006.

21. Canadian Institute for Health Information. Workforce Trends of Registered Nurses in Canada. Health Human Resources Database. Ottawa, Ontario, 2006. http://secure.cihi.ca/cihiweb/splash.html

22. Keefe JM, Manning M. The Cost Effectiveness of Respite: A Literature Review. Health Canada Home and Continuing Care Policy Unit Health Care Policy Directorate. Halifax, Nova Scotia, 2005. http://www.hc-sc.gc.ca/hcs-sss/alt_formats/hpb-dgps/pdf/pubs/2005-keefe/2005-keefe-eng.pdf

23. Statistics Canada. Unpaid Work. Census Technical Report. Catalogue No. 97F0013XCB2001003 Topic-based Tabulations, Canada's Workforce: Unpaid Work. Statistics Canada 92-397-XIE, Ottawa, Ontario 2005. http://www.statcan.ca

24. Ontario Coalition of Senior Citizens' Organizations. Presentation to the Commission on the Future of Health Care in Canada, Sudbury Public Hearing. Sudbury, Ontario, 2006.

25. Family Caregivers Association of Nova Scotia. Presentation to the Commission on the Future of Health Care in Canada, Halifax Public Hearing. Halifax, Nova Scotia, 2002.

26. Dunbrack J. Respite for Family Caregivers an Environmental Scan of Publicly-funded Programs in Canada. Health Canada, 2003. http://www.hc-sc.gc.ca/hcs-sss/alt_formats/hpb-dgps/pdf/pubs/2003-respite-releve/2003-respite-releve-eng.pdf

27. Frohlich KL, Ross N, Chantelle R. Health Disparities in Canada Today: Some Evidence and a Theoretical Framework. Health Policy. 2006; 79: 132-143

28. Statistics Canada. Projections of the Aboriginal Populations, Canada, Provinces and Territories. Statistics Canada, Catalogue no. 91-547-XIE. Canada, 2005

29. Wilson K, Rosenberg MW. Accessibility and the Canadian health care system: squaring perceptions and realities. Health Policy. 2004;67: 137-148

30. Eike-Henner WK. The Canadian Health Care System: An Analytical Perspective. Health Care Analysis. 1999; 7: 377-391.

31. Sameer R, Kisalaya B. Interprovincial Migration of Physicians in Canada: Where are they Moving and Why? Health Policy. 2006;79: 265-273

32. Canadian Institute for Health Information. Canada's Health Care Providers, 2007. Ottawa, Ontario, 2007. http://www.cihi.ca.

33. Raphael D, Bryant T. The State's Role in Promoting Population Health: Public Health Concerns in Canada, USA, UK, and Sweden. Health Policy. 2006;78: 39-55

34. Dennis R. Barriers to Addressing the Societal Determinants of Health: Public Health Units and Poverty in Ontario, Canada. Health Prom Int. 2003;18: 397-405.

35.Chronic Disease Prevention Alliance of Canada. Primary Prevention of Chronic Diseases in Canada: A Framework for Action. Canada, 2008. http://www.cdpac.ca/media.php?mid=451

36. Low J, Thériault L. Health Promotion Policy in Canada: Lessons Forgotten, Lessons Still to Learn. Health Prom Int. 2008;23: 200-206

37. Bloor K, Maynard A. Planning Human Resources in Health Care: Towards an Economic Approach: An International Comparative Review. Canadian Health Services Research Foundation. Ottawa, Ontario, 2003. http://www.chsrf.ca

38. Advisory Committee on Health Delivery and Human Resources. The Nursing Strategy for Canada. Ottawa, Ontario, 2003. http//www.hc-sc.gc.ca/english/nursing

39. Scanlon WJ. Nursing Workforce: Recruitment and Retention of Nurses and Nurse Aides Is a Growing Concern. Testimony before the Committee on Health, Education, Labor and Pensions, U.S. Senate, GAO-01-750T, U.S. General Accounting Office, Washington DC, 2001. http://www.gao.gov/new.items/d01750t.pdf

40. Buchan J, Maynard A. The Health Care Workforce in Europe: Learning from Experience. In Bernd R, Carl-Ardy D, Martin M, editors. World Health Organization; European Observatory on Health Systems and Policies. Copenhagen, Denmark, 2006.

41. Docteur E, Oxley H. Health-Care Systems: Lessons from the Reform Experience Organization for Economic Co-operation and Development. OECD. Paris, France, 2003.

42. Minister of Health. Pan-Canadian Health Human Resource Strategy 2005-2006 Annual Report. Canada, 2006.

Correspondence:
Rosa Rodriguez-Monguio, Ph.D.
Assistant Professor
University of Massachusetts. Amherst -School of Public Health and Health Sciences. USA
E-mail: rmonguio @ schoolph.umass.edu

Comment of the reviewer Prof. Jaime Espin Balbino LLB, MBA, MSc. Andalusian School of Public Health (Escuela Andaluza de Salud Pública). España

This present analysis of the health care services in Canada is a good example of description of a national health system taking into account, not only the present situation, but also the future opportunities of improvement.

It is very important to point out that this type of analysis will help policy makers to look for other policy initiates than help them to confront the common challenges that we facing now: population ageing, sustainable financing, etc. And in this case, with the Canadian example, we can learn from a complex decentralized health system (federal in this case) in order to manage resources in an efficient way.

Comment of the reviewer Daniel A. Sepulveda Adams. Research Scientist, PRIME Institute. College of Pharmacy, University of Minnesota. Minneapolis, USA

The article has more comments related to the structure rather than the background
1. The authors made an extended and systematic literature review, which helps support their ideas in a sustained way.

2. The article is based on a literature search; consequently the authors were able to express clearly both the strengths and weaknesses of the Canadian Health System and the possible challenges that the Canadian Government has currently and will have in the future.

3. The article has significance for further research in countries that have the same Health System as Canada; researchers evaluating those systems will be able to use the same analysis and structure used by the authors here.

Received: February 19, 2009.
Published: June 14, de 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

Letters to the Editor / Cartas al Editor

ABORDAJE BASICO DEL PACIENTE CON CERVICOBRAQUIALGIA

Rosmery Villa Delgado[1], Luis Rafael Moscote Salazar MD[2], Carlos Fernando Lozano Tangua MD[2], Rubén Sabogal Barrios MD[3]

[1] Estudiante de Medicina, [2]Residente de Neurocirugía, [3] Docente de Neurocirugía
Universidad de Cartagena. Cartagena de Indias. Colombia

neuromoscote @ gmail.com

Rev Electron Biomed / Electron J Biomed 2009;2:76-78

Sr. Editor:

La cervicobraquialgia o síndrome cervicobraquial es el dolor originado en la región cervical que se irradia al miembro superior a través del territorio correspondiente a una raíz nerviosa cervical. Este síntoma constituye una causa importante de consulta en todas las edades[1]. Las raíces más frecuentemente afectadas son C7 y C6, por este orden. La causa más frecuente de cervicobraquialgia es la compresión de una raíz nerviosa cervical baja a nivel de su emergencia radicular[2].

Es fundamental conocer la anatomía del plexo braquial, pues interviene en la inervación sensitiva y motora de todo el miembro superior exceptuado una parte adyacente al hombro cuya inervación depende del plexo cervical y la parte interna del brazo que depende de los nervios intercostales. El plexo braquial está formado por 5 raíces C5, C6, C7, C8 y T1 que conectan la médula espinal con los nervios más periféricos del brazo. Esta conexión con el sistema nervioso central permite que las órdenes originadas en el cerebro se trasmitan a los músculos del brazo y de la mano y a su vez la información recibida por las terminaciones nerviosas sensitivas se trasmitan al cerebro. Las ramas de C5 y C6 se unen y forman el tronco superior, la rama de C7 permanece individual y constituye el tronco medio y las ramas de C8 y T1, se unen y forman el tronco inferior. Al nivel de la clavícula, cada tronco se divide en dos ramas una anterior y otra posterior que luego se unen entre sí. La unión de las tres divisiones posteriores forma el cordón posterior o radiocircunflejo. La unión de las divisiones anteriores del tronco superior y el medio constituye el cordón lateral. La división anterior del tronco inferior forma el cordón medial. Estas ramas dan a su vez colaterales y terminales para inervar a los diversos músculos del brazo, antebrazo y la mano. Las variaciones anatómicas son comunes[3].

Etiología: La cervicobraquialgia generalmente se debe a irritación de una raíz nerviosa que puede obedecer a diferentes causas dentro de la cuales se encuentran: hernias discales cervicales, trastornos degenerativos de la columna, inflamación, tumores (neurinoma, tumor de pancoast), canal medular estrecho, cervicobraquialgia postraumática (accidentes de tránsito), atrapamiento de los nervios o de los vasos que pasan a través de los músculos escalenos y clavícula[4-5]. Se pueden mencionar como otras causas de cervicobraquialgia, las enfermedades neuromusculares, lesiones del nervio torácico largo que provoca una escapula alada con la consiguiente flexión anterior del hombro, parálisis del nervio espinal accesorio, osteocondromas de la clavícula, inestabilidad glenohumeral, infecciones por herpes zoster[4]. En pacientes jóvenes generalmente se debe a cuadros

agudos como hernia discal traumática, y en pacientes de edad avanzada la comprensión es más crónica y suele deberse a espondiloartrosis[1].

Cuadro Clínico: La cervicobraquialgia se caracteriza por dolor cervical irradiado a lo largo del miembro superior, en ocasiones se irradia a región escapular; el dolor puede ser unilateral o bilateral (en raras ocasiones); constante o intermitente; la intensidad del dolor se evalúa mediante la escala visual análoga del (EVA), cuya puntación oscila entre 0 a 10 puntos, siendo 0 sin dolor, 1-3 dolor leve, 4-6 dolor moderado y 7-10 dolor severo. Al dolor se le agregan otros signos y síntomas como trastornos de la sensibilidad, debilidad muscular, parestesias, hipoestesias en hombro y brazo que se extienden hasta la mano y en algunos casos disminución de los reflejos tendinosos del brazo[1]. El dolor puede empeorar al levantar peso o hacer esfuerzo físico y mejorar al levantar los brazos, con los codos flexionados y abducción del hombro. Es importante definir de qué raíz nerviosa se origina el dolor que se esquematiza en el siguiente cuadro:

Raíz comprometida	C5	C6	C7	C8
Dolor y perdida de la sensibilidad	Base del cuello, hombro y deltoides	Cara lateral del brazo, antebrazo, dedo pulgar e índice	Región medial del brazo, antebrazo, tercer y cuarto dedo	Cara cubital de brazo, antebrazo, dedo anular y meñique
Déficit motor	Deltoides	Músculos flexores	Músculos extensores	Músculos intrínsecos de la mano
Reflejo afectado		Bicipital y estiloradial	Tricipital	

El paciente además del cuadro clínico anterior también puede referir cefalea, limitación de los movimientos del cuello y miembro superior. En ocasiones el cuadro clínico o el dolor pueden simular un dolor de tipo anginoso sin traducción en el electrocardiograma[2].

Diagnóstico
Anamnesis: se debe interrogar al paciente acerca de antecedentes personales de lesión traumática, enfermedades reumáticas, neoplasias. Es necesario identificar las características del dolor (inicio, localización, intensidad, irradiación, acompañantes, tipo, que lo alivia y que lo exacerba), igualmente tratamientos previos[5].
Exploración física: se realiza la exploración general del paciente, esto incluiría evaluación de la postura (antialgica), movilidad del cuello, fuerza muscular (por grupos musculares), sensibilidad, reflejos tendinosos, se debe valorar la maniobra de spurling (con el paciente sentado se hace presión de la cabeza hacia abajo del lado del dolor en el plano vertical, se considera positiva para cervicobraquialgia si se genera dolor y parestesias), en caso de hernia discal si se realiza la maniobra de valsava se generara dolor, igualmente se palparan las zonas de dolor.

Pruebas diagnósticas:

- **1. Estudios de laboratorios: están indicados para descartar enfermedades sistémicas, infecciosas, enfermedades reumáticas o patologías tumorales.**
- **2. Estudios radiológicos: para determinar alteraciones estructurales[6].**
  - **Radiografías de cuello. Deben hacerse en las tres proyecciones (AP, lateral y oblicua). Son útiles para descartar lesiones óseas o inestabilidad en caso de emergencia. En estas se pueden encontrar rectificación de la lordosis cervical, perdida de la consistencia o contorno de los cuerpos vertebrales, osteofitos, artrosis o disminución de la altura cervical en caso de patologías degenerativas.**
  - **La tomografía axial computarizada (TAC) posee alto valor en las cervicobraquialgia cuando la causa es ósea. Este estudio nos permite visualizar muy bien la columna cervical. El mielo-TAC es mucho mejor que el TAC simple.**
  - **La Resonancia magnética nuclear (RMN) tiene gran importancia en esta patología como estudio de imagen complementario.**
  - **Electromiografía es una prueba complementaria para la radiculopatía o mielopatía. Esta prueba no se requiere para casos en los que la anamnesis, exploración física y estudios radiográficos evidencian el diagnóstico.**

Tratamiento: El tratamiento va encaminado a reducir el dolor y mejorar la capacidad funcional del paciente.
Conservador

- **Medidas físicas: reposo de 2 a 3 semanas, calor local, ejercicios cuando el dolor empiece a ceder, rehabilitación.**
- **Antiinflamatorios: cualquier tipo de antiinflamatorio no esteroideo como naproxeno, diclofenaco, ibuprofeno. Se recomienda ir disminuyendo la dosis de estos en la segunda semana de tratamiento**
- **Analgésicos**
- **Relajantes musculares**

- **Corticoides para casos severos o que no respondan a AINES o en la fases hiperalgicas.**
- **Bloqueos nerviosos selectivos o infiltraciones epidurales para casos de dolor severo.** [7]

Tratamiento quirúrgico: En caso de que los analgésicos o corticoides no logren controlar el dolor se pensara en la posibilidad de tratamiento quirúrgico dependiendo de la patología.

El tratamiento por radiofrecuencia se ha utilizado en varios síndromes dolorosos, en este caso seria el paciente con dolor cervical radicular, pues actúa bloqueando las vías de conducción de las señales del dolor[7].

Pronóstico: En general estos pacientes tienen buen pronóstico, pues en la mayoría de los casos con una buena historia clínica y estudios complementarios se logra establecer el diagnóstico, sitio preciso de la lesión, con lo anterior se lograría un tratamiento adecuado y oportuno. La cervicobraquialgia suele tener una tendencia natural a la mejoría en un periodo de cuatro a seis semanas, aunque, puede que existan periodos de reincidencias del dolor[6].

REFERENCIAS

1. Vázquez A. Cervico-braquialgia. En: Oliván AS, Pinillos MA, Agorreta J, Rubio T. Guía de Urgencias Hospital de Navarra 1999. Hoechst Marion Roussel; 1999: 267-268.

2. Van Zundert J, Harney D, Joosten EA, Durieux ME, Patijin J, Van Kleef M. Dolor Cervical Radicular Diagnostico, Fisiopatología y Tratamiento. Reg Anesth Pain Med. 2006; 31: 152-167.

3. Allieu Y, Cenac, P. Neurotization via spinal accessory nerve in complete paralisis due to multiple avulsion injuries of the brachial plexus. Clin Orthop 1988, 237: 67-74.

4. Kazemi A, Muñoz-Corsini L, Martín-Barallat J, Pérez-Nicolás L. Henche M. Estudio etiopatogénico de la cervicalgia en la población general basado en la exploración física. Rev Soc Esp Dolor 2000;7(4).

5. Villas C, Collía A, Aquerreta Jd, Aristu J, Torre W, Díaz De Rada P, Gocci S. Cervicobrachialgia And Pancoast Tumor: Value Of Standar Anteroposterior Cervical Radiographs In Early Diagnosis. Orthopedics. 2004;27:1092-1095.

6. Woo-Ram Shin, Hyoung-Ihl Kim, Dong-Gyu Shin, Dong-Ah Shin. Radiofrequency Neurotomy of Cervical Medial Branches for Chronic Cervicobrachialgia. J Korean Med Sci 2006; 21: 119-125.

7. Greenberg MS. Cervical disc herniation. En: Greenberg MS. Handbook of Neurosurgery. 4ª ed. Lakeland, Florida; Greenberg Graphics, Inc; 1997: 198-203.

Luis Rafael Moscote Salazar MD.
Residente de Neurocirugía. Facultad de Medicina. Campus de Zaragocilla.
Universidad de Cartagena. Cartagena de Indias
mail neuromoscote @ gmail.com

Recibido 25 de mayo de 2009.
Publicado: 14 de junio de 2009

Revista Electrónica de Biomedicina
Electronic Journal of Biomedicine

ISSN: 1697-090X

Letters to the Editor / Cartas al Editor

THE CHICK CHORIOALLANTOIC MEMBRANE AS A MODEL TISSUE FOR IRRITATION STUDIES ON MULTIPURPOSE SOLUTION

A. Arora[1], A. Ali[2], M. T. Zzaman[1], S.Chauhan[1]

[1] KIET School of Pharmacy, Ghaziabad.
[2] Jamia Hamdard, Faculty of Pharmacy, Dept of Pharmaceutics. New Delhi. India.

alkamailin @ yahoo.co.in

Rev Electron Biomed / Electron J Biomed 2009;2:79-84

To the Editor:

The chick chorioallantic membrane (CAM), a part of the extraembroynic tissue begins to develop 7 days after initial incubation from the fusion of the chorion and the allantois. Structurally, the outer epithlial layer of the chorion is derived from the trophoblast, which opposes the allantois. This structure forms a supportive matrix for the extensive vascular network that courses through the CAM, analogus to the retina and its vasculture. Overall mature chick CAM (20-100 µm) and human retina (approx 100-300 µm) are of roughly comparable thickness. Mature chick CAM (incubation day 12 and on) can be divided into three anatomically distinct layers. 1.- Primary stratum, 2.- Capillary plexus or blood sinus, 3.- A thin stratum.

The specialized chorionic epithelial cells that have presumably migrated above the capillary plexus are involved in gas exchange and calcium absorption. Immediately above and attached to CAM thin layer is the inner shell membrane[1-3].

The irritation study was performed in order to ascertain the irritating effect if any of multipurpose solution (MPS) used for rinsing and soaking of hydrophilic contact lenses (CL's) before being placed into the eyes. In the present study CAM test was used for performing irritation studies on MPS used for hydrophilic CL's. The CL's are to be rinsed and soaked in multipurpose solution (MPS) before being worn. The composition of MPS coded as MPS-2 is given in table 1. In a fertile egg, the CAM test has been used extensively in the past for ocular angiogenesis studies. The tissue is a useful tool for ocular irritation studies including hyperaemia, hemorrhage and coagulation.

Table-I Composition of MPS-2

Ingridents	Qt.
Polyhexanide hydrochloride (20%)	0.0002%
Poloxamer 407	1.25%
Sodium citrate	0.1%
Disodium edetate	0.1%
HPMC 2906 grade	0.1%
PEG 400	0.1%
Isotonic simulated tear fluid of pH 7.4...Q.S… (Prepared as per U.S.P.)	100 ml

The CAM test is a convenient model as it involves low cost and hence considered less expensive than the living animal. The eggs are maintained at adequate humidity at 37 + 0.5oC temperature condition and rotated after every few hours. These conditions can be maintained in an incubator and the eggs of 14 days old are fit for experimental work. The CAM experiment can be performed between 12-17 days of incubation. Before 12 days, the CAM vasculture is not adequately matured and after 17 days the embryo is large enough and hence its size and movement underneath the CAM could disturb the experimental maneuvers on the CAM hence 12-14 days of incubation is the ideal time for experimental work[4-8].

The test is based on the scoring scheme for hyperaemia, hemorrhage and coagulation parameters. Any increase in red color of CAM (hyperaemia) can be given a score number from 0 to 5. Similarly the haemorrhage (leaking of blood) can be given a score from 0 to 7 i.e. no haemorrhage at 0 and strong haemorrhage at 7. Scores from 0 to 9 is used for coagulation parameter. A detailed account is given in table 2.

Table-II: Scoring scheme for hyperaemia, haemorrhage and coagulation.

Effect	Scores	
Hyperaemia (Any increase in the red colour of CAM)	0	- No hyperaemia
	1	- Very slight hyperaemia
	2	- Slight hyperaemia
	3	- Moderate hyperaemia
	4	- In between moderate and strong hyperaemia
	5	- Strong hyperaemia
Haemorrhage (Place filter paper on CAM, remove and see any blood content)	0	- No haemorrhage
	1	- Very slight haemorrhage
	2	- Slight haemorrhage
	3	- Slightly moderate haemorrhage
	4	- Moderate haemorrhage
	5	- Slightly strong haemorrhage
	6	- Moderately haemorrhage
	7	- Strong haemorrhage
Coagulation (Any change in colour of CAM rising depression in the membrance, granular or shaggy appearance of membrane or opaqueness of membrane)	0	- No coagulation
	1	- Obscure coagulation
	2	- Very light coagulation
	3	- Slight coagulation
	4	- Slightly moderate coagulation
	5	- Moderate coagulation
	6	- In between slightly strong and moderate coagulation
	7	- Slightly strong
	8	- Moderately strong
	9	- Strong

The cumulative scores were used for assessment of extent of irritation and taking hyperaemia, haemorrhage and coagulation into consideration as given below[9-13]. Cumulative scores and irritation assessment: 0 - 0.9 practically none; 1 - 4.9 slight; 5 - 8.9 moderate; 9 - 21.0 strong.

Multipurpose solution (MPS-2) prepared on pilot scale at Gaymed labs Delhi, India. Propylene glycol and Sodium lauryl sulphate from E Merck, Mumbai, India. Baby shampoo from Johnson & Johnson Mumbai. All other materials were used as received.

Fertilled chick eggs are easily ordered and procured from poultry farm and do not require an extensive animal protocol (as long as they are used and disposed before 19 days). These are considerably less expensive than the living animals currently used in ophthalmic and contact lens experimentation (e.g. rabbits, rats, cats, minipigs, dogs). Maintenance of eggs with 75% of relative humidity, a 37°C environment and rotation after 2 hours, can be performed in an inexpensive incubator.

Immediately after procurement the fertilized chick eggs (12 days old) were washed with water and kept on cotton surface already placed in a tray. The tray was kept in an incubator for 2 days at 37 ± 0.5 °C. The eggs were rotated after every 2 hours (figure 1).

Figure 1.- One of the egg in the tray

To prepare CAM for typical experiment, the eggs were examined under candle light and only healthy and fertile eggs were selected. The egg shell is cracked and peeled away from the region over the air space that exists between the shell and the inner shell membrane (ISM) at one pole of the egg (figure 2).

This air space can be visualized before the egg is cracked by holding the egg under intense light. The eggs were cracked. The white part of the egg was removed carefully with the help of a pointed needle and a sharp blade in order to expose, once the opaque CAM-ISM dual layer is exposed, the irrigation with saline 0.9% will cause the dual layer to become transluscent, allowing for visualization of the CAM vasculture as shown in (figure 3).

Figure 2.- The cracked egg shell.

Figure 3.- The CAM of Hen's fertile egg

The exposed CAM was divided into four parts 1, 2, 3 and 4. These were written on the four sides.The preparations were applied on these spots as a drop in the following manner: Area 1: Multipurpose solution coded as MPS-2. Area 2: Sodium lauryl sulphate (SLS). Area 3: Baby shampoo (Johnson). Area 4: Propylene glycol (PG)

Immediately, these were kept in the incubator and after 0.5, 2 and 5 minutes, the area were examined for hyperaemia, haemorrhage and coagulation. The resultant scores obtained were shown in table 3

Table-III: Scores of Haemorrhage, Hyperaemia and Coagulation on CAM for MPS-2, Propylene glycol, SLS and Baby shampoo

	Egg	Scores									Cumulative score for 5 min.
		Haemorrhage (A)			Hyperaemia (B)			Coagulation (C)			
		Time (in mins)			Time (in mins)			Time (in mins)			
		0.5	2	5	0.5	2	5	0.5	2	5	
MPS – 2	Egg 1	0	0	0	0	0	0	0	0	1	
	Egg 2	0	0	0	0	0	0	0	0	0	
	Egg 3	0	0	0	0	0	0	0	0	1	
	Egg 4	0	0	0	0	0	0	0	0	1	A + B + C = 0.50
	Egg 5	0	0	0	0	0	0	0	0	0	
	Egg 6	0	0	0	0	0	0	0	0	0	
	Mean	0	0	0	0	0	0	0	0	0.50	

	Egg	Scores									Cumulative score for 5 min.
		Haemorrhage (A)			Hyperaemia (B)			Coagulation (C)			
		Time (in mins)			Time (in mins)			Time (in mins)			
		0.5	2	5	0.5	2	5	0.5	2	5	
Propylene glyhcol	Egg 1	0	0	0	0	0	0	0	0	0	
	Egg 2	0	0	0	0	0	0	0	0	0	
	Egg 3	0	0	0	0	0	0	0	0	1	A + B + C = 0.33
	Egg 4	0	0	0	0	0	0	0	0	1	
	Egg 5	0	0	0	0	0	0	0	0	0	
	Egg 6	0	0	0	0	0	0	0	0	0	
	Mean	0	0	0	0	0	0	0	0	0.33	

	Egg	Scores									Cumulative score for 5 min.
		Haemorrhage (A)			Hyperaemia (B)			Coagulation (C)			
		Time (in mins)			Time (in mins)			Time (in mins)			
		0.5	2	5	0.5	2	5	0.5	2	5	
Baby shampoo	Egg 1	0	0	0	0	0	1	0	0	1	
	Egg 2	0	0	0	0	0	1	0	0	1	
	Egg 3	0	0	0	0	0	0	0	0	1	A + B + C = 1.49
	Egg 4	0	0	0	0	0	1	0	0	0	
	Egg 5	0	0	0	0	0	1	0	0	1	
	Egg 6	0	0	0	0	0	1	0	0	0	
	Mean	0	0	0	0	0	0.83	0	0	0.66	

	Egg	Scores									Cumulative score for 5 min.
		Haemorrhage (A)			Hyperaemia (B)			Coagulation (C)			
		Time (in mins)			Time (in mins)			Time (in mins)			
		0.5	2	5	0.5	2	5	0.5	2	5	
Sodium lauryl sulphate	Egg 1	0	0	0	0	1	2	0	2	3	
	Egg 2	0	0	1	0	1	2	0	2	3	
	Egg 3	0	0	1	0	1	2	0	3	5	A + B + C = 10.99
	Egg 4	0	0	1	0	1	1	0	4	6	
	Egg 5	0	0	1	0	1	2	0	2	6	
	Egg 6	0	0	1	0	1	2	0	2	6	
	Mean	0	0	0.83	0	1.00	1.83	0	2.50	4.83	

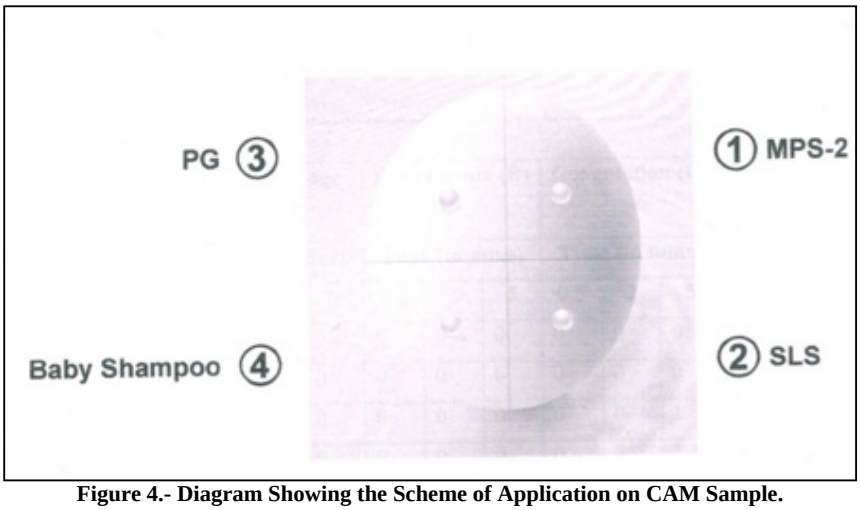

Figure 4.- Diagram Showing the Scheme of Application on CAM Sample.

The multipurpose solution coded as MPS-2 was formulated on pilot scale. The irritation studies on the preparation MPS-2 used as rinsing and soaking solution for hydrophilic contact lenses were performed using chorio allantoic membrane (CAM) test of fertile hen's egg. The preparation was applied as a drop on the CAM and scores for haemorrhage, hyperaemia and coagulation were observed and were recorded. The mean score was calculated and cumulative score was obtained. This was obtained as 0.50.As per the interpretation of standard parameter a cumulative score in between 0-0.9 is practically non-irritant. Therefore multipurpose solution MPS-2 was found to be practically non-irritant. For comparative purpose the test was also performed for propylene glycol, baby shampoo and sodium lauryl sulphate. For propylene glycol the cumulative score was 0.33 hence it is also nonirritant. This was practically observ.ed on CAM. A substance is slightly irritant if the mean score is in the range of 1-4.9. For sodium lauryl sulphate, the cumulative score was 10.99 hence it indicated a strong irritation effect as it was in the cumulative score range of 9-21. The three materials that were used. Propylene glycol, baby shampoo and SLS as controls in the study.

Irritation studies should be carried out on preparation like multipurpose solution because it has to be used for rinsing and soaking of CL's (hydrophilic contact lenses). The CAM is very much similar to retina of the eye hence can be used for irritation studies on eye preparations. Such experiments are less expensive and do not require extensive animal protocol. Maintenance of eggs requires adequate humidity and a 37°C environment and rotation after every few hours, which can be performed in an inexpensive incubator.

The type of CAM experiments described herein can optimally be performed between incubation days 12 and 18. Before day 12, the CAM vasculture has not adequately matured. After day 18, the embryo is large enough that its size and movements underneath the CAM can disrupt experimental maneuvers on the CAM, Additionally, standard animal protocols for chick embryos past 18 require more complicated euthanasia techniques.

Conclusion: The optimized product MPS-2 and marketed by the name of Multisol is practically non irritant and can be used for rinsing, soaking, disinfecting, lubricating and deproteinising hydrophilic contact lenses.

REFERENCES

1.- Ribatti D, Nico B, Vacca A, Roncali L, Burri PH, Djonov V. Chorioallantoic membrane capillary bed: a useful target for studying angiogenesis and anti-angiogenesis in vivo. Anat Rec. 2001;264:317-324.

2.- Oncel M, Peyman GA, Khoobehi B. Tissue plasminogen activator in the treatment of experimental retinal vein occlusion. Retina. 1989;9:1-7.

3.- Leng T, Miller JM, Bilbao KV, Palanker DV, Huie P, Blumenkranz MS. The chick chorioallantoic membrane as a model tissue for surgical retinal research and simulation. Retina. 2004;24:427-434.

4.- Thompson WD, Reid A. Quantitative assay for the chick chorioallantoic membrane. Adv. Exp Med Biol 2000; 476:225-236

5.- Ponce ML, Kleinmann HK. The Chick chorioallantoic membrane as an in vivo angiogenesis model. Curr Protoc Cell Biol 2003;Chapter 19:Unit 19.5

6.- Liao Y, Wang X, Zhang L, Li G. Study on using the hen's egg test-chorioallantoic membrane as an alternative method of draize eye irritation test. Wei Sheng Yan Jiu. 2004;33:279-283.

7.- Debbasch C, Ebenhahn C, Dami N, Pericoi M, Van den Berghe C, Cottin M, Nohynek GJ. Eye irritation of low-

irritant cosmetic formulations: correlation of in vitro results with clinical data and product composition. Food Chem Toxicol. 2005;43:155-165.

8.- Tufan AC, Satiroglu-Tufan NL. The chick embryo chorioallantoic membrane as a model system for the study of tumor angiogenesis, invasion and development of anti-angiogenic agents. Curr Cancer Drug Targets. 2005;5:249-266

9.- Tavaszi J, Budai P. Toxicity study of agrochemicals on chorioallantoic membrane of the egg. Commun Agric Appl Biol Sci. 2006;71(2 Pt A):101-5.

10.- Tavaszi J, Budai P. The use of HET-CAM test in detecting the ocular irritation. Commun Agric Appl Biol Sci. 2007;72:137-141.

11.- Deryugina EI, Quigley JP. Chapter 2. Chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. Methods Enzymol. 2008;444:21-41.

12.- Slodownik D, Grinberg I, Spira RM, Skornik Y, Goldstein RS. The human skin/chick chorioallantoic membrane model accurately predicts the potency of cosmetic allergens. Exp Dermatol. 2009;18:409-413.

13.- Deryugina EI, Quigley JP. Chick embryo chorioallantoic membrane model systems to study and visualize human tumor cell metastasis. Histochem Cell Biol. 2008;130:1119-1130

Prof. Alka Arora PhD
3- B-264 Derawal Nagar, opp. Model Town-2 Delhi-110009, India.
mail alkamailin @ yahoo.co.in

Received reviewed, July 1, 2009.
Published, July 19, 2009

ANNEXES

INFLUENCE OF ARYLPIPERAZINES AROMATIC STRUCTURE OVER DIFFERENTIAL AFFINITY FOR 5-HT1A AND 5-HT2A RECEPTORS