Journal of Neurological Sciences [Turkish] **26:(2)**# 19; 198-205, 2009
http://www.jns.dergisi.org/text.php3?id=290

Research Article

Assessment of Proliferative Activity in Rat Brain With AgNOR Following Exposure to Magnetic Field

Hülya ÇETİN SORKUN[1], Nagihan YALÇIN[2], Gülten ERKEN[3], Haydar Ali ERKEN[3], Osman GENÇ[3]

[1]University of Pamukkale, Health Services, Vocational School, Denizli, Turkey [2]University of Pamukkale School of Medicine, Pathology Department, Denizli, Turkey [3]University of Pamukkale School of Medicine, Physiology Department, Denizli, Turkey

Summary

Objective: Nucleolar organizer region (NORs) is directly related to cell cycle and is an indicator of cell proliferation. We explored, in the present study, whether there was a difference in proliferation between brain tissues subjected to magnetic field for different durations.
Study Design: The study comprised 5 groups with six rats each. Groups A and B were exposed to magnetic fields for 2.5 hours/day and 2.5 minutes/day, respectively, everyday for three months. Sham groups for Group A (SA) and Group B (SB) were constructed and switched-off cellular phones were left in the same environment for the same duration as their corresponding experimental groups. Rat's brains were removed, placed in 10% formalin and 3 micron thick sections were obtained following routine histological methods. Sections were stained with AgNOR stain. The number of AgNORs for each 100 cortical neurons, hippocampal neurons, ependymal and choroid plexus cells were counted on every section with an immersion microscope objective.
Results: Statistical analyses revealed that mean number of AgNORs was highest in Group A (3.69±0.54), followed by Group B (3.06±0.48). Even though there was no statistically significant difference between sham groups (SA=1.76±0.56 and SB=1.84±0.65, p=0.990), mean number of AgNORs in sham groups was significantly higher than the control group (1.29±0.42) ($p \leq 0.05$). Groups A and B differed significantly from the sham and control groups in terms of mean number of AgNORs ($p \leq 0.001$).
Conclusion: Increased proliferative activity and protein synthesis in neuronal and glial cells of the brain tissue in response to exposure to magnetic field is demonstrated by AgNORs.

Key words: AgNOR, rat's brain, magnetic field, mobile phone

Manyetik Alana Maruz Kalan Şıçan Beyinlerinin Proliferatif Aktivitesinin Agnor ile Değerlendirilmesi

Özet

Amaç: Nükleolar organizer region (NORs) doğrudan hücre siklusu ile ilişkilidir ve hücre proliferasyonunu yansıtır. Çalışmamızda, farklı sürelerde manyetik alana maruz kalan beyin dokularının proliferasyonları arasında fark olup olmadığını araştırdık.
Materyal ve Metod: Çalışmamız, her birinde 6 sıçan bulunan 5 gruptan oluşmuştur. Deney grupları üç ay süreyle, haftada yedi gün, her gün, A grubu: 2,5 saat, B grubu: 2,5 dakika manyetik alana maruz bırakıldı. A ve B gruplarıyla aynı sürelerde ortamda kapalı olarak cep telefonu bulundurulan sham grupları (SA ve SB) oluşturuldu. Ortamda cep telefonu bulundurulmayan grup kontrol grubu olarak değerlendirildi. Sıçanların beyinleri anestezi

etkisi altında çıkarılarak %10'luk formaldehite konuldu ve doku takibi yapılarak 3 mikronluk kesitler alındı. Kesitler AgNOR boyası ile boyandı. Her kesitte 100'er adet korteks ve hipokampus bölgesi nöronları, ependim ve koroid pleksusa ait hücrelerin AgNOR tanecikleri immersiyon objektifte sayıldı.

Bulgular: İstatistiksel olarak değerlendirildiğinde ortalama tanecik sayısı en fazla grup A'ya aitti (3.69±0.54). B grubu (3.06±0.48) ikinci sırada yer almaktaydı. Sham grupları kendi arasında (SA=1.76±0.56 ve SB=1.84±0.65) istatistiksel olarak anlamlılık olmamakla birlikte ($p=0.990$) ortalama tanecik sayısı bakımından kontrol grubundan (1.29±0.42) daha fazla sayıda taneciğe sahiplerdi ve istatistiksel olarak anlamlıydı ($p\leq0.05$). Ortalama tanecik sayısı bakımından A ve B grupları sham ve kontrol gruplarıyla karşılaştırıldığında istatistiksel olarak ileri düzeyde anlamlı bulundu ($p\leq0.001$).

Sonuç: Manyetik alana maruz kalmaya bağlı olarak beyin dokusundaki nöronal ve glial hücrelerde, proliferatif aktivite artışını AgNOR tanecikleri göstermiştir.

Anahtar Kelimeler: AgNOR, rat beyin, manyetik alan, cep telefonu

INTRODUCTION

Mobile telephones have become one of the most widely used sources of electromagnetic field[14,21]. Having become an indispensible item of our daily lives and been implicated as a source of microwave pollution, it has been argued that mobile phones can cause immune system disorders, damage to the glial cells and neurons and exert carcinogenic effects[14,30,31]. It has been argued that there is an increase in the incidence of cerebral tumors as a result of the increase in mobile phone use and that mobile phones constitute a risk[16,17,22]. Various staining methods such as Ki-67, PCNA, BrdU and AgNOR have been used to assess the proliferation potential of brain tumors histologically[7,29].

NORs can be selectively stained as black dots in the nuclei by a silver staining technique (AgNOR)[15] based on argyrophilia of NOR associated proteins (NOR-Aps) and so-called AgNOR proteins[28]. The amount of silver deposits in a cell indicates the quantity of Nucleolar Organizer Regions (NORs), which are related to protein synthesis. NORs are loops of ribosomal DNA that contain protein synthesis related gene[6]. They are located on the short arms of the chromosomes 13, 14, 15, 21 and 22. the acrocentric chromosomes. With silver staining method, fibrillary non-histone proteins can be demonstrated in the nucleus related to the NOR[24]. This suggests that AgNOR is a marker of cell proliferation[3,9,12,19,20,25,27,35,36]. AgNOR, believed to be an easy and rapid method to calculate proliferation potential,[10] has been widely used in histopathologic evaluation of malignant and benign cerebral tumors[3,4,5,13,18,23,24,26,34]. In the present study, we aimed to investigate, using AgNOR method, whether there was a difference in cell proliferation between different regions of rat brain subjected to magnetic field induced by mobile phones.

MATERIAL AND METHODS

1. Construction of the experimental groups and conducting the experiment

A total of 30 male Wistar albino rats weighing 250-300 g and obtained from Pamukkale University Experimental Research Unit were used in the study. Rats were randomly allocated to one of six groups.

Using a mobile phone on “talk” mode, rats in Group A were exposed to 30 minutes of magnetic field 5 times a day, with 5 minutes of break between exposures, for 7 days a week for 3 months. Therefore, rats in Group A were exposed to magnetic field for 2.5 hours a day.

Rats in Group B were exposed to 0.5 minute of magnetic field 5 times a day while the phone was ringing, with 30 minutes of break between exposures, for 7 days a week for 3 months. Therefore, rats in Group B were exposed to magnetic field for 2.5 minutes a day.

For each experiment group, a sham group was constructed and a mobile phone that was switched off was put within the apparatus the animals were kept. Therefore two sham groups for each experiment group, namely sham A (SA) and sham B (SB) were constructed. Rats kept in the same experimental apparatus with no mobile phone inside comprised the control group.

Rats were housed in plastic cages in a temperature (22ºC) and humidity (50%) controlled environment, had ad libitum access to tap water and standard pellet food and were maintained on a 12:12 light/dark cycle (07.00-19.00 h).

2. Experiment apparatus

Six rats were placed in a plexiglas container with air-vents and containers were placed in a radial manner, equidistant to the center. Handling was carried out initially to help animals adjust to the apparatus. Animals adjusted to containers were then observed to have a tendency to go in the same container each time during the experiment stage.

In this study, we needed to know the electrical characteristics of brain tissue at 900 MHz to calculate the magnitude of induced E_{local} electric field and SAR. In the apparatus used, the external electric field $E_{du}$was 70 V/m, with regard to the localization of the brain tissue. Electric field was measured by Holaday (Holaday Industries, Eden Prairie, Minnesota, USA) electric field probe and electric field meter.

Since $SAR_{local} = \frac{E_{local}^{\ 2} \times \sigma}{\rho}$ W/kg, mean specific absorption ratio generated in the brain tissue under these conditions was calculated as 1.42 W/kg[11,32].

3. Anesthesia and Obtaining tissues

At the end of the experiment stage, anesthesia was induced by ketamin (50 mg/kg) and xylazine (5 mg/kg) combination. Brain tissue was fixed in 10% buffered formalin solution. 2-3 mm thick sagittal sections were obtained from each brain. Following routine histological processing, 2.5-3 µ thick sections from the midbrain were stained by H&E and AgNOR.

Methods described by Crocker and Nar was used for AgNOR staining[8]. On sections obtained from each rat, the AgNORs were counted by the same researcher in 100 neurons each from the cortex and hippocampus, ependymal cells and choroid plexus cells under X1000 magnification using an oil immersion lens. Neurons from the hippocampus and cortex in the right hemisphere were evaluated.

Total number of AgNORs was divided by 100 to obtain mean AgNOR number for each case. Significance of differences between groups was analyzed statistically by t-test and One Way ANOVA.

RESULTS

Statistical comparisons of groups showed that the highest number of AgNORs was in Group A (3.69±0.54) in which rats were exposed to magnetic field for 2.5 hours. Rats in Group B were exposed to magnetic field for 2.5 minutes while the phone was ringing mode had a mean 3.06±0.48 AgNORs. There was no statistically significant difference between the sham groups (SA=1.76±0.56 and SB=1.84±0.65) (p=0.990). The differences between the mean number of AgNORs in the control (1.29±0.42) and sham groups were statistically significant ($p \leq 0.05$). Mean numbers of AgNORs in groups A and B were statistically higher than those of the sham and control groups ($p \leq 0.001$) (Table 1).

Table I. Mean numbers of AgNOR dots in cells of the groups

	Group A mean±SD	Group B mean±SD	Group SA mean±SD	Group SB mean±SD	Control mean±SD
Choroid plexus cells	4.05±0.38*	3.11±0.52*	2.17±0.29	2.23±0.46	1.55±0.39
Ependymal cells	4.05±0.46*	3.38±0.43*	2.35±0.28**	2.49±0.26**	1.69±0.28
Hippocampal neurons	3.59±0.30*	2.94±0.43*	1.39±0.19	1.54±0.42	1.01±0.09
Cortical neurons	3.07±0.33*	2.82±0.44*	1.15±0.22	1.11±0.27	0.89±0.15

*p≤ 0.001 in comparison with control group
** p<0.05 in comparison with control group

In all groups, the highest number of AgNORs was observed in the ependymal cells (2.81±0.91), followed by the cell in the choroid plexus region (2.64±0.97) and hippocampal neurons (2.12±1.05). The least number AgNORs was in the cortical neurons (1.83±1.00).

When we compared the mean number of AgNORs in the ependymal and choroid plexus cells, we did not find a statistically significant difference (p=0.271) whereas the difference between the mean number of AgNORs in the cortical and hippocampal neurons was significant (p=0.013).

Choroid plexus cells (Figure 1)
Group A had the highest number of mean AgNORs. When the Group A was compared with the sham and control groups, statistically significant differences were observed (p<0.001). Group A, in comparison to Group B, contained significantly more granules (p≤0.05). Mean number of AgNORs in Group B was significantly higher than the sham (SA (p=0.010), SB (p=0.011) and control groups (p<0.001).

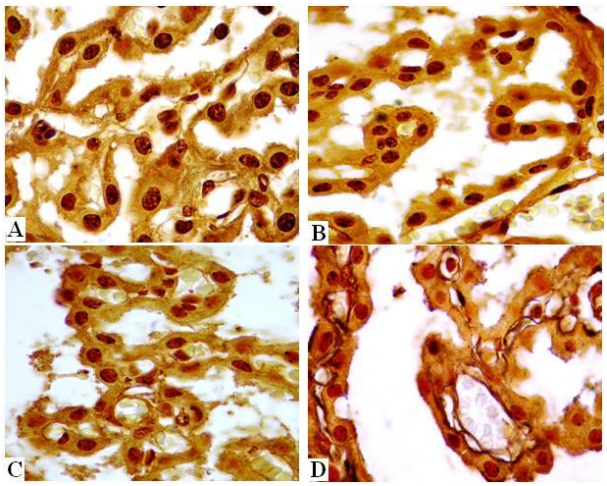

Figure 1: *The appearance of AgNOR dots in Coroidal plexus cells belonging to A) group A, B) group B, C) sham A and D) sham B (X1000).*

Ependymal cells (Figure 2)
Mean number of AgNORs of Group A was significantly higher than that of Group B (p=0.027). Comparisons of Group A, sham groups and the control group yielded significant differences (p≤0.001). Mean number of AgNORs of Group B was significantly different than the SA (p=0.001), SB (p=0.002) and control groups (p<0.001).

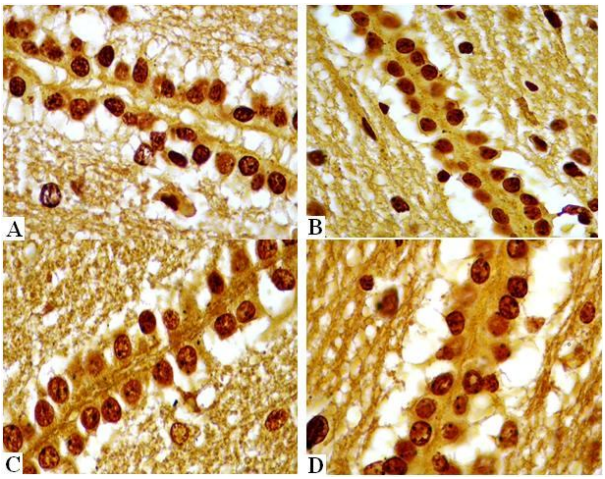

Figure 2: *The appearance of AgNOR dots in ependymal cells belonging toA) group A B) group B, C) Sham A and D) Control groups (X1000).*

Hippocampal neurons (Figure 3)
Mean numbers of AgNORs of Group A and Group B were significantly different than those of the sham and control groups ($p<0.001$). Group A, when compared to group B, had significantly more AgNORs ($p=0.015$). **Cortical neurons** (Figure 4)

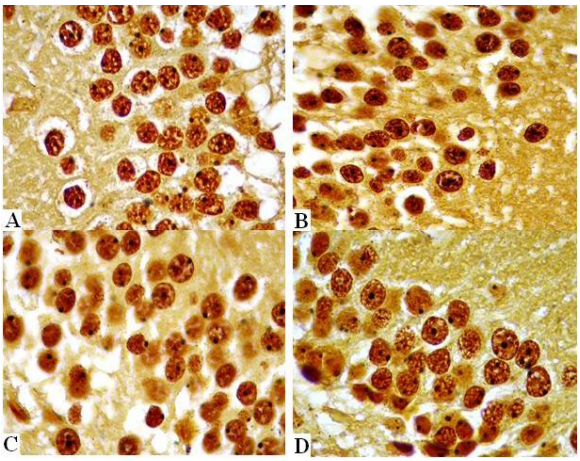

Figure 3: *The appearance of AgNOR dots in Hippocampal neurons A) groupA, B) group B, C) sham B ve D) control group (X1000).*

Mean number of AgNORs was highest in Group A. Comparison of mean AgNORs in Group A and Group B did not yield a statistically significant result ($p=0.6$) whereas mean numbers of AgNORs of Groups A and B were significantly different than those of the sham and control groups ($p<0.001$).

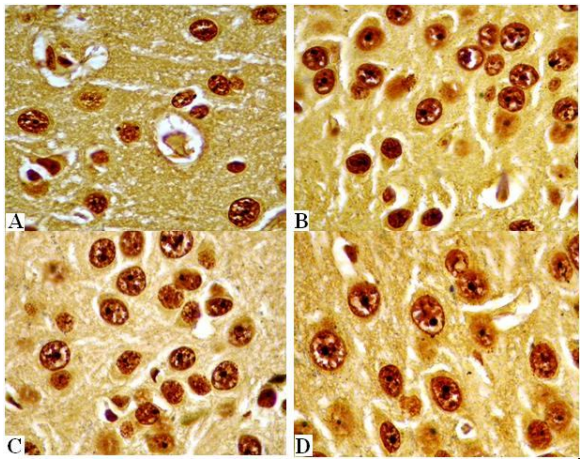

Figure 4: *The appearance of AgNOR dots in Cortical neurons A) group A, B) group B, C) sham B and D) control group (X1000).*

Mean number of AgNORs increased depending on the duration of exposure to magnetic field. Presence of a mobile phone in the vicinity caused an increase in AgNORs. There were also increases in the number of AgNORs in the sham groups in comparison to the control group.

DISCUSSION

AgNORs have long been used as an indicator of proliferative activity. As a result of the advent of immunohistochemical markers such as PCNA and Ki-67, the use of AgNORs as a proliferative marker has declined. As opposed to immunohistochemical markers, AgNORs are preferred due to being less affected by fixation, low cost and ease of use. It has been used safely as an experienced eye can detect an artifact easily. AgNOR has long been used to differentiate normal tissue from tumors.

It has been reported that AgNOR expression was associated with proliferative activity in glial tumors[1,13] and that the number of AgNORs and their sizes increased with the grade of the brain tumor[26,33]. Various studies argued that there is an increase in the frequency of brain tumors in recent years as a result of widespread use of mobile phones[15,17,22]. Changes in brain tissue in response to

exposure to electromagnetic field have been investigated in many studies[2,21,30,31]. Among these, Mausset-Bonnefont and colleagues (2004) reported an increase in reactive fields on day 3 of exposure to electromagnetic field by 140% in striatum and by 20-25% in the hippocampus and cortex of rat brain. In a similar study, researchers showed that, on day 2 of exposure to GSM microwave, there were marked increases in reactive astrogliosis in rat brain tissue, most notably in the frontal cortex, which was closest to the antenna, and cauda and putamen of striatum to the lateral ventricles[2].

Mausset-Bonnefont et al.[21] and Brillaud et al.[2] argued that changes might be related to astrocytic hypertrophy or glial hyperplasia. In the present study, our findings regarding the increased proliferation in the choroid plexus and ependymal cells lend support to these studies.

Studies that utilized AgNOR method reported that the amount of AgNOR in astrocytomas, irrespective of its grade, was significantly higher than the amount of AgNOR in normal brain tissue[1,3,4].

Our finding that very significant statistical difference in the mean number of AgNORs existed between subjects exposed to electromagnetic field and controls was in agreement with the studies of Berny et al.[1] and Bukhari et al.[3,4].

In Salford and colleagues' study injury to the neurons of the cortex, hippocampus and basal ganglia was observed in rats when exposed to non-thermal, 915 MHz GSM electromagnetic fields of various strengths for 2 hours[30]. Increase in the proliferation in cortical and hippocampal neurons with exposure to electromagnetic field observed in the present study is in an agreement with the finding of Salford et al.[30].

It is noteworthy that strong astroglial activation was generally observed in regions adjacent to the ventricles and subarachnoidal regions (cortex of the cerebellum, frontal cortex and caude putamen of striatum). The onset and propagation of this effect in the central nervous system was attributed to the cerebrospinal fluid[2]. We also found that cells neighboring the cerebrospinal fluid (choroid plexus and ependymal cells) had higher number of AgNORs than the cells of other regions (cortex, hippocampus). However, even though choroid plexus and ependymal cells are adjacent to the cerebrospinal fluid, they are actually glial cells. Therefore, it would not be an unexpected finding to observe excessive AgNORs due to their potent proliferative abilities and this supports the hypothesis of Brillaud et al.[2].

CONCLUSION

In the present study, we showed that proliferation and protein synthesis in rat brain markedly increased with prolonged exposure to electromagnetic field. When compared to the control group, increase in the number of AgNORs in the sham groups showed us the adverse effects of mobile phones even if they are not ringing or talk mode.

We detected the increase in proliferation and protein synthesis using the number of AgNORs, a valuable tool though it lost its popularity in recent years. Due to the fact that rat brain is way smaller than human brain, magnetic field generated by mobile phones is expected to affect the rat brain more. Magnetic field generated by mobile phones in human brain is smaller than that generated in rat brain. Nevertheless, we believe that extended use of mobile phones, can increase the proliferation and protein synthesis of glial cell in the brain. These findings need to be supported by further studies.

Correspondence to:
Hülya Çetin Sorkun
E-mail: hcsorkun@pau.edu.tr

Received by: 01 April 2009
Revised by: 20 May 2009
Accepted: 21 May 2009

The Online Journal of Neurological Sciences (Turkish) 1984-2009
This e-journal is run by Ege University Faculty of Medicine,
Dept. of Neurological Surgery, Bornova, Izmir-35100TR
as part of the Ege Neurological Surgery World Wide Web service.
Comments and feedback:
E-mail: editor@jns.dergisi.org
URL: http://www.jns.dergisi.org
Journal of Neurological Sciences (Turkish)
Abbr: J. Neurol. Sci.[Turk]
ISSNe 1302-1664

REFERENCES

1. *Berny W, Weiser A, Markowska-Woyciechowska A, et,al. Analysis of PCNA, Ki67, AgNOR and p53 expression in brain glial tumors. Neurol Neurochir Pol 2004;38:457-63*
2. *Brillaud E, Piotrowski A, de Seze R. Effect of an acute 900 MHz GSM exposure on glia in the rat brain: A time dependent study. Toxicology 2007;238: 23-33*
3. *Bukhari MH, Niazi S, Khan SA, Hashmi I, Perveen S, Qureshi SS, Chaudry NA, Qureshi GR, Hasan M. Modified method of AgNOR staining for tissue and interpretation in histopathology. Int J Exp Pathol 2007;88(1):47-53*
4. *Bukhari MH, Niazi S, Hashmi I, Naeem S, et al. Use of AgNOR index in grading and differantial diagnosis of astrocytic lesions of brain. Pak J Med Sci 2007;23(2):206-210*
5. *Carbajo S, Orfao A, Vicente-Villardon JL, Carbajo-Perez E. Expression of silver-stained nucleolar organizer regions is coupled to cell cycle in rat thymic cells. Cytometry 1993;14:46-52*
6. *Carrillo R, Sneige N, El-Naggar AK. Interphase nucleolar organizer regions in the evalution of serozal cavity effusions. Acta Cytol 1994;38(3):367-372*
7. *Centeno BA, Louis DN, Kupsky WJ, Preffer FI, Sobel RA. The AgNOR technique, PCNA immunohistochemistry, and DNA ploidy in the evaluation of coroid plexus biopsy specimens. Am J Clin Pathol Dec 1993;100(6):690-6*
8. *Crocker J and Nar P. Nucleolar organizer regions in lymphomas. J Pathol 1987;151:111-118*
9. *Crocker J. Nucleolar Organizer Regions. In: Current topics in pathology. Ed. JCE Underwood. Springer Verlag, New York, 1990*
10. *Crocker J. Correlation between NOR sizes and numbers in nonHodgkin's lymphomas. Journal of Clinical Pathology 1996;49:8-11*
11. *Dasdag S, Ketani MA, Akdağ Z et al. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. Urol Res 1999;27(3):219-223*
12. *Derenzini M, Sirri V, Pession A, Trere D, Roussel P, Ochs RL, et al. Quantitive changes of the two major AgNOR proteins, nucleolin and protein B23, related to stimulation of rDNA transcription. Exp Cell Res 1995;219:276-82*
13. *Ferreres JC, Figols J, Val-Bernal JF, Cardesa A, Cruz Sanchez FF. Argyrophilic nucleolar organizer region (AgNOR) counting in astrocytic gliomas: prognostic value. Histol Histopathol 1996;11(1):35-9*
14. *Galeev AL. The effects of microwave radiation from mobile telephones on humans and animals. Neuroscience and Behavioral Physiology 2000;30(2):187-194*
15. *Hara T, Sato T, Mori S, Shirai H, Maruo Y, Minagi S. Argyrophilic nucleolar organizer regions (AgNORs) in mucosal epithelium under experimental denture bases in rats. J Oral Pathol Med 2000; 29: 33-38.*
16. *Hardel L, Carlberg M, Mild KH. Pooled analysis of two case-control studies on the use of cellular and cordless telephones and the risk of benign brain tumours diagnosed during 1997-2003. Int J Oncol Feb 2006;28(2):509-18*
17. *Hardel L, Carlberg M, Mild KH. Case-control study of the association between the use of cellular and cordless telephones and the risk of malignant brain tumours diagnosed during 2000-2003. Environ Res 2006;100(2):232-41*
18. *Khan SA, Chaudry NA, Khalid AW, Akhtar GN, Ibne-Rasa SN. Patterns of argyrophilic nucleolar organiser regions in pleural and peritoneal effusions. J Coll Physicians Surg Pak 2006;16: 412-5*
19. *Li Q, Hacker GW, Danscher G, Sonnleitner-WU, Grimelius L. Argyrophilic nucleolar organizer regions. A revised version of the Ag-NOR-staining technique. Histochem Cell Biol 1995;104:145-50*
20. *Lohr CV, Driemeier D, Teifke JP. Polyethylene glycol-thiosulfate (PEG-Th) staining a modification of the AgNOR method. Zentralbl Pathol 1995;140:465-8*
21. *Mausset-Bonnefont AL, Hirbec H, Bonnefont X, Privat A, Vignon J, de Seze R. Acute exposure to GSM 900-MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol Dis 2004;17:445-454*
22. *Mild KH, Hardel L, Carlberg M. Pooled analysis of two Swedish case-control studies on the use of mobile and cordless telephones and the risk of brain tumours diagnosed during 1997-2003. Int J Occup Saf Ergon 2007;13(1):63-71*
23. *Mourad WA, Erkman-Balis B, Livingstone S, Shoukri M, Cox CE, Nicosia SV, et al. Argyrophilic nucleolar organizer regions in breast carcinoma. Correlation with DNA flow cytometry, histopathology, and lymph node status. Cancer 1992;69:1739-44*
24. *Mourad WA, Connely JH, Sembera DL, Atkinson EN, Brunner JM. The correlation of two*

argyrophilic nucleolar organizer region counting methods with Bromodeoxyuridine-labelling index: A study of metastatic tumors of the brain. Hum Pathol 1993;24:206-210

25. *Ofner D, Riedmann B, Maier H, Hittmair A, Rumer A Totsch M, et al. Standardized staining and analysis of argyrophilic nucleolar organizer region associated proteins (AgNORs) in radically resected colorectal adenocarcinoma, correlation with tumour stage and long-term survival. J Pathol 1995;175:441-8*
26. *Pedal WP, Warzok RW, Hufnagl P, Roth K. Location of nucleolar organizer regions (AgNORs) in the nuclei of astrocytic tumors. Clin Neuropathol 1995;14(1):55-61*
27. *Plate KH, Ruschoff J, Mennel HD. Application of the AgNOR technique to neurooncology. Acta Histochem Suppl 1992;42:171-8*
28. *Ploton D, Menager M, Jeannesson P, Himber G, Pigeon F, Adnet J.J. Improvement in the staining and in the visualization of the argyrophylic proteins of the nucleolar organizer region at the optical level. Histochem J 1986; 18: 5-14.*
29. *Quinones-Hinojosa A, Sanai N, Smith JS, McDermott MW. Techniques to assess the proliferative potential of brain tumors. J Neurooncol Aug 2005;74(1):19-30*
30. *Salford LG, Brun AE, Eberhardt JL, Malmgren L,& Persson BRR. Nevre cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environmental Health Prespectives 2003;111:881-883*
31. *Salford LG, Nittby H, Brun A, Grafström G, Eberhardt JL, Malmgren L, Persson BRR. Non-thermal effects of EMF upon the mammalian brain; the lund experience. Environmentalist 2007; DOI 10.1007/s10669-007-9118-4*
32. *Schönborn F, Pokovic K, Kuster N. Dosimetric analysis of the carousel setup for the exposure of rats at 1.62 GHz. Bioelectromagnetics 2004;25:16-26*
33. *Shiraishi T, Tabuci K, Mineta T, et al. Nuclear organizer regions in various human brain tumors. J Neurosurg 1991;74:979-984*
34. *Suwa T, Kawano N, Oka H, Ito H, Kameya T. Invasive meningioma: a tumor with high proliferating and "recurrence" potential. Acta Neurochir (Wien) 1995;136(3-4):127-31*
35. *Vidal B de C, Mello ML. Re-evaluating the AgNOR staining response in Triton X-100-treated liver cells by image analysis. Anal Cell Pathol 1995;9:39-43*
36. *Yekeler H, Erel O, Yumbul AZ, Doymaz MZ, Dogan O, Ozercan MR, et al. A sensitive staining method for NORs. J Pathol 1995;75:449-52*