რეზიუმე

ვირთაგვების ხერხემლის წელის მალების მიდამოებში ნახშირბად/ნახშირბადის კომპოზიტის იმპლანტაციის შემდეგ ძვლის რეგენერაციის ჰისტომორფოლოგიური შეფასება

[1]ა. პოპოვი, [1]ნ.აშუკინა, [1]ვ.მალცევა, [2]ი.გურინი, [1]გ.ივანოვი

[1]სახელმწიფო დაწესებულება "უკრაინის სამედიცინო მეცნიერებათა ეროვნული აკადემიის პროფ. მ. სიტენკოს სახ. ხერხემლისა და სახსრების პათოლოგიის ინსტიტუტი", ხარკოვი; [2]ეროვნული სამეცნიერო ცენტრი "ხარკოვის ფიზიკისა და ტექნიკის ინსტიტუტი", უკრაინა

კვლევის მიზანს წარმოადგენდა ძვლის რეგენერაციის თავისებურების შესწავლა ვირთაგვების მალებში ნახშირბად-ნახშირბადის კომპოზიტური მასალის პირნახშირბადის დანაფარით და დანაფარის გარეშე იმპლანტაციის შემდეგ.

9 თეთრი ლაბორატორიული ვირთაგვების (6-7 თვე, წონა 250-350 გ) L_{III} მალების დეფექტებში იმპლანტირებული იყო ნახშირბად-ნახშირბადის კომპოზიტური მასალა (ნნკმ) და ნნკმ პირონახშირბადის დანაფარით (n=9). მასალა დამზადებული იყო ცილინდრის ფორმით (დიამეტრი 2 მმ, სიმაღლე 2 მმ). იმპლანტაციიდან 15, 30 და 90 დღე-ღამის შემდეგ ჩატარდა ჰისტოლოგიური და ჰისტომორფომეტრიული გამოკვლევები.

15 დღეში ორივე მასალის პერიმეტრზე აღმოჩნდა ძვლოვანი და გრანულაციური ქსოვილი შეფარდებით ½. 30 დღე-ღამის შემდეგ იმპლანტანტების ირგვლივ ჩამოყალიბდა უხეშბოჭკოვანი ძვლოვანი და სხვადასხვა ხარისხის სიმწიფის შემაერთებელი ქსოვილები. ძვლოვანი ქსოვილის ფარდობითი შემცველობა ნნკმ-ს სიახლოვეში 30 და 90 დღე-ღამის შემდეგ იყო 1,4-ჯერ ნაკლები დანაფარით ნნკმ-თან შედარებით ($p<0.001$).

ჩატარებული კვლევის შედეგად გამოვლინდა, რომ მასალები ბიოშეთავსებადია, არ არღვევენ ძვლის რეგენერაციის პროცესს, არ იწვევენ ანთებით რეაქციებს. ოსტეოინტეგრაციული თვისებები უფრო მაღალი აღმოაჩნდა ნნკმ-ს დანაფარით.

INTERNAL STRUCTURE OF THE LYMPHOID NODULES OF THE PEYER'S PATCHES OF SMALL INTESTINE IN ALBINO RATS

Hryn V.

Ukrainian Medical Stomatological Academy, Department of Human Anatomy, Poltava, Ukraine

An individual Peyer's patch, regardless of its localization, shape and size, is a group combination of several homogeneous lymphoid formations, currently called as nodules (although their former name, follicles, is sometimes found in the literature). Consequently, the study of Peyer's patches can be reduced to the analysis of the microscopic structure of a single lymphoid nodule.

It has long been considered that a lymphoid nodule of Peyer's patches is a locally concentrated mass of immunocompetent cells functionally associated with a single-layered intestinal epithelium (follicle-associated epithelium) [4,12,19], consisting of the base (embedded into the submucous layer of the small intestine wall), the mantle, in the middle of which a germinal (reactive) core is located, and the apical part or dome (its top is covered with intestinal epithelium). At the same time, separate T- and B-dependent zones are distinguished [3,8,14,17,20,21]. The first of them occupies its marginal, i.e., peripheral, position in the nodule, while the second one directly adjoins the germinal core. Consequently, in the Peyer's patch, all its nodules (with the exception of those ones located on its periphery) border each other with their T-zones. Moreover, along these zones, within the Peyer's patch, the lymphoid nodules are embordered by the intestinal villi of various shapes, between which (at their base) intestinal crypts (glands of Lieberkühn) are opened with their orifices [2,7,13]. Obviously, the major depth of the lymphoid nodules may seem like a simple accumulation of lymphoid tissue, devoid of signs of any structural architecture. In fact, a careful examination of microimages, provided by many authors in their publications, reveals that the depth of the lymphoid nodules is a complex honeycombed network woven from sinuous fine stripes of lymphoid tissue. However, in the literature this structural feature is not given any significance, which prompted a study to clarify this issue.

Purpose - the paper is aimed at the study of the internal structure of the lymphoid nodules of the Peyer's patches of albino rats' small intestine.

Material and methods. 30 mature albino male rats weighted 200,0±20,0 g were involved into the study. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at the Ukrainian Medical Stomatological Academy in compliance with the regulations on keeping experimental animals, adopted by the European Parliament and Council Directive (2010/63 / EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013), (Minutes No. 155 as of 26.04.2017 of meeting the Commission on Biomedical Ethics at Ukrainian Medical Stomatological Academy) [10,11,15].

After vivisection, which was carried out by an overdose of thiopental anesthesia (75 mg/kg of animal body weight intramuscularly in the upper third of the thigh of the hind paw) [1]

in compliance with the requirements for dissection of the abdominal cavity, the entire complex of the gastrointestinal tract was removed, which was fixed in 10% formalin solution for two days. Subsequently, short sections of the small intestine, containing Peyer's patches, were selectively excised. Finding the latter was not difficult due to their clear visualization on the external (non-mesenteric) surface of the small intestine in the form of whitish spots.

The specimens, after washing from formalin and dehydration in alcohol of increasing concentration, were embedded into paraffin blocks, from which serial sections of 4 μm thick (Microm HM 325) were obtained with subsequent staining with hematoxylin-eosin and Van Gieson. Their study and documentation was carried out using the "Konus" light microscope equipped with the Sigeta DCM-900 9.0MP digital microphoto attachment and the Biorex 3 program (serial number 5604) adapted for these studies. The morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system of visual analysis of histological specimens, as well as using the Sigeta X1 mm/100 Div.x0.01mm stage micrometer, the scale of which (equal to 1 mm, where a small step corresponds to 10 μm) was applied to the corresponding microimage obtained in the same magnification.

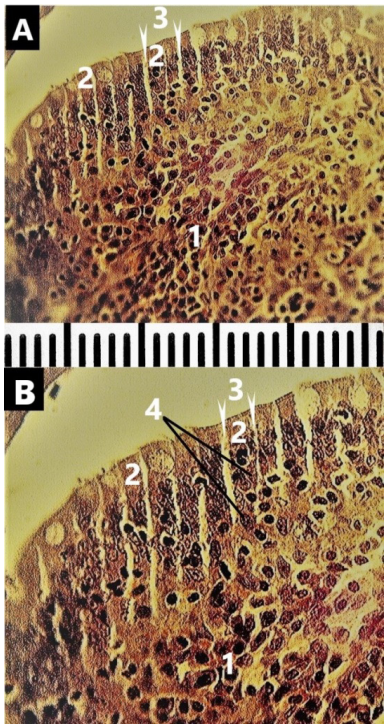

Fig. 1 (A, B). Apical part of the lymphoid nodule of the Peyer's patch of the small intestine. Paraffin section; H&E stain. A – Lens: 40 ×magnification (one step of the scale is equal 10 μm), B – Lens: 100×magnification. 1 – lymphoid elements; 2 – lymphoepithelial columns and 3 – intercellular fissures, separating them; 4 – dendritic cells

Results and their discussion. The findings of the study have shown that the lymphoid nodules of Peyer's patches of the small intestine of albino rats are not a simple massive aggregation of lymphocytic elements. In any plane of section, histological sections clearly revealed, that the lymphoid tissue was distributed in the form of twisted strands, aligned mainly from the apical part of the dome of the lymphoid nodule to its base, adjacent to the muscular tunic of the small intestine. For better understanding the basic principle of the lymphoid strands' architecture, it is necessary to refer to one of the forms of organization of the follicle-associated (dome) epithelium that was identified during the study, which was described in the previous publication. On histological sections it had a columnar pattern of organization. Notably, in its description attention was drawn to the fact that these lymphoepithelial columns were separated by relatively wide intercellular fissures, which were closed by tight contacts from the apical surface of enterocytes, whereas in the basal part they were linked with clear gaps separating the sinuous strands of lymphoid tissue (Fig. 1 A, B).

The clear gaps are interstitial fissures, filled with an amorphous substance, which is a colloidal fluid. Throughout the entire thickness of the lymphoid nodules, these interstitial fissures form a dense, looped network. At the same time, as they approach the base of the nodule, they expand, and in some cases take the form of extensive cisterns, the longitudinal axis of which are oriented at right angles to the muscular tunic of the small intestine. It is in their looped coverage, in the form of fine strands of various shapes, that aggregations of lymphocytic elements are located (Fig. 2). Notably, they are interrelated with the layers of loose fine-fibrous connective tissue, which has an eosinophilic color, against which lymphocytic elements are clearly distinguished by the dense basophilia of their nuclei.

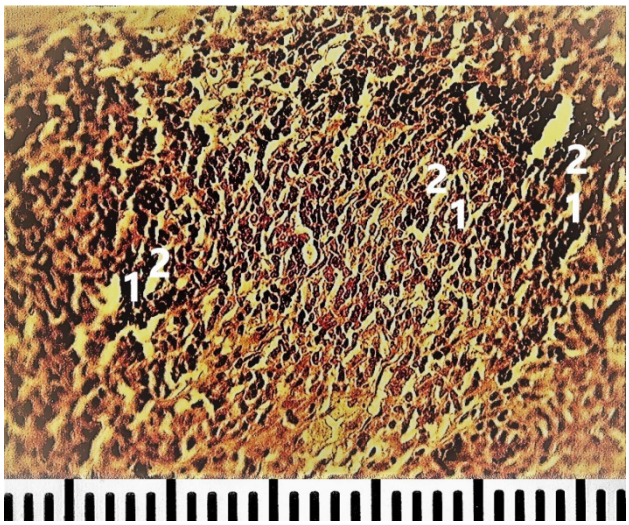

Fig. 2. Basal part of the lymphoid nodule of the Peyer's patch of the small intestine. Paraffin section; H&E stain; Lens: 40×magnification. The smallest step of the scale is equal 10 μm. 1 – lymphocytic strands and 2 – interstitial layers, separating them

Obviously, all this is clearly not sufficient to understand the constructive principle of the microscopic organization of lymphoid nodules. Clarification of the nature of their looped interstitial network is required with regard of the fundamental principles of microangiology, as well as publications on the organization of lymphatic vessels in Peyer's patches [6,9,16]. Thus, microangiology shows that the interstitial space, consisting of separate compartments of amorphous substance of loose connective tissue, is considered as a mediating medium between the blood and the working cells of the organ. At the same time, it is drained by blindly originating lymph capillaries, through the endothelial wall of which the filtering process of the interstitial fluid is carried out together with the antigenic substances contained in it, which ultimately flow through the afferent lymphatic

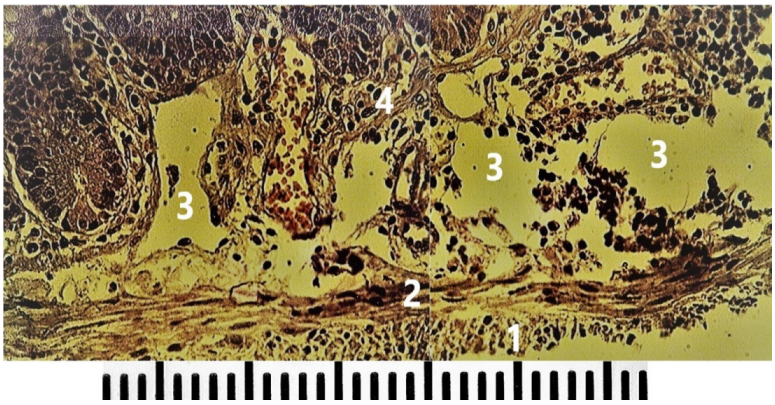

Fig. 3. Basal part of the small intestine at the Peyer's patch. Paraffin section; Van Gieson stain; Lens: 40×magnification. One step of the scale is equal 10 μm. 1 – serous membrane; 2 – muscular tunic; 3 – lymphatic sinuses of the submucous layer; 4 – muscle plate of the mucous membrane

vessels into the regional lymph nodes. Naturally, such general principle of the conversion of interstitial fluid to lymph also occurs in Peyer's patches of the small intestine [9,18]. Moreover, some guidelines on the lymphatic system contain schematic representations of the lymphatic flow of Peyer's patches [5]. They indicate that the lymph capillaries of intestinal villi located around the circumference of lymphoid nodules are the sources of lymph in the Peyer's patch. Further conjoining, they transfer into the lymphatic vessels, which are localized in the depth of the inernodular zones. Passing along them, the lymph is directed to the general lymphatic network located beneath the bases of the lymphoid nodules, i.e., in the gap between them and the muscular tunic of the small intestine, which is visualized on histological sections (Fig. 3). Apparently, this process requires a link between the interstitial network of lymphoid nodules, described above, and the lymphatic vessels.

Therefore, this link is to be the lymphatic capillaries, which are blindly originated in the compartments of the interstitial network of nodules. Unfortunately, they cannot be detected on conventional histological preparations.

Conclusion. Consistent with this scheme, we can presumably indicate the direction of the processes of extravascular fluid microcirculation and lymph outflow pathways in the lymphoid nodules of Peyer's patches. Notably, one of the origins of these pathways could be intercellular fissures of the lymphatic epithelial columns, providing paracellular transport of fluid from the contents of the small intestine to the interstitial network of lymphoid nodules. If this leaking fluid contains antigenic substances, then in the course of their movement they will inevitably come into contact with the macrophages of these lymphoepithelial columns, after which information about this antigen will be presented to T-lymphocytes through the dendritic cells, initiating the development of the immune response of the mucous membranes.

In this case, a variant of the initiation of immune responses in Peyer's patches is presented, without the participation of specific enterocytes, called the M-cells. In the literature, this variant of events is not excluded, as well as the fact that other types of enterocytes also possess phagocytic properties in the intestinal epithelium associated with lymphoid nodules. However, it is currently not known whether these mechanisms are alternative or whether each of them is intended for the selective perception of antigenic substances. Moreover, when conducting experimental studies, it should be taken into account that pronounced functional polymorphism is characteristic of Peyer's patches.

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SUMMARY

INTERNAL STRUCTURE OF THE LYMPHOID NODULES OF THE PEYER'S PATCHES OF SMALL INTESTINE IN ALBINO RATS

Hryn V.

Ukrainian Medical Stomatological Academy, Department of Human Anatomy, Poltava, Ukraine

An individual Peyer's patch, regardless of its localization, shape and size, is a group combination of several homogeneous lymphoid formations, currently known as nodules. The internal structure of the lymphoid nodules of Peyer's patches of the small intestine of albino rats has been studied. 30 mature albino male rats weighing 200.0±20.0 g were involved into the study. Areas of the small intestine with Peyer's patches have been studied. Serial paraffin sections have been analyzed using the "Konus" light microscope. Morphometric characteristics of the tissue structures were obtained using the Sigeta X 1 mm / 100 Div.x0.01 mm stage micrometer. Apparently, intercellular fissures of the lymphatic epithelial columns, providing paracellular transport of fluid from the contents of the small intestine to the interstitial network of lymphoid nodules can initiate the processes of extravascular fluid microcirculation and lymph outflow pathways in the lymphoid nodules of Peyer's patches. In this case, a variant of the initiation of immune responses in Peyer's patches is presented. A pronounced functional polymorphism is characteristic of Peyer's patches, which must be taken into account when conducting experimental studies.

Keywords: Peyer's patch, small intestine, albino rats, lymphoid

РЕЗЮМЕ

ВНУТРЕННЕЕ СТРОЕНИЕ ЛИМФОИДНЫХ УЗЕЛКОВ ПЕЙЕРОВЫХ БЛЯШЕК ТОНКОЙ КИШКИ БЕЛЫХ КРЫС

Гринь В.Г.

Украинская медицинская стоматологическая академия, департамент анатомии человека, Полтава, Украина

Отдельная пейерова бляшка, независимо от ее локализации, формы и размера, представляет собой групповое объединение нескольких однотипных лимфоидных образований, именуемых в настоящее время узелками. Изучено внутреннее строение лимфоидных узелков пейеровых бляшек тонкой кишки белых крыс. Исследование осуществлено на 30 белых крысах-самцах репродуктивного возраста, массой 200,0±20,0 грамм. Материалом для изучения служили участки тонкой кишки с пейеровыми бляшками. Изучали серийные парафиновые срезы под световым микроскопом «Konus». Морфометрические характеристики тканевых структур получали с помощью объект-микрометра Sigeta X1 мм/100 Div.x0.01 мм. Одним из признаков начала процессов внесосудистой микроциркуляции жидкости и путей лимфооттока в лимфоидных узелках пейеровых бляшек могут являться межклеточные щели лимфоэпителиальных колонок, по которым возможен парацеллюлярный транспорт жидкости из содержимого тонкой кишки в интерстициальную сеть лимфоидных узелков. В данном случае имеет место вариант инициации иммунных реакций в пейеровых бляшках. Для пейеровых бляшек свойственен выраженный функциональный полиморфизм, что необходимо учитывать при проведении экксперименталных исследований.

რეზიუმე

თეთრი ვირთაგვების წვრილი ნაწლავის პეიერის ფოლაქების ლიმფოიდური კვანძების შიდა სტრუქტურა

ვ. გრინი

უკრაინის სამედიცინო სტომატოლოგიური აკადემია, ადამიანის ანატომიის კათედრა, პოლტავა, უკრაინა

ცალკეული პეიერის ფოლაქები, მიუხედავად მათი ადგილმდებარეობის, ფორმისა და ზომის, წარმოადგენენ რამდენიმე ერთგვაროვანი ლიმფოიდური წარმონაქმნის ჯგუფურ კომბინაციას, რომელსაც ამჟამად უწოდებენ კვანძებს.

შესწავლილია თეთრი ვირთაგვების წვრილი ნაწლავის პეიერის ფოლაქების ლიმფოიდური კვანძების შიდა სტრუქტურა. კვლევა ჩატარდა რეპროდუქციული ასაკის 30 თეთრ მამრ ვირთაგვაზე, წონით 200.0±20.0 გრ. შესწავლილია წვრილი ნაწლავის ნაწილები პეიერის ფოლაქებით. პარაფინის სერიული ანათლები შესწავლილია «Konus»-ის სხივური მიკროსკოპის მეშვეობით. ქსოვილის სტრუქტურების მორფომეტრული მახასიათებლები მიღებულია Sigeta X 1 mm/100 Div.x0.01 mm მიკრომეტრის ობიექტის გამოყენებით. პეიერის ფოლაქების ლიმფოიდურ კვანძებში ექსტრავასკულური სითხის მიკროცირკულაციის და ლიმფური დრენაჟის დასაწყისს წარმოადგენენ უჯრედ-

შორისი ნაპრალები, რომელთა მეშვეობით შესაძლებელია პარაცელულური სითხის ტრანსპორტირება წვრილი ნაწლავიდან ლიმფოიდური კვანძების ინტერსტიციულ ქსელში. ამ შემთხვევაში აღინიშნება პეიერის ფოლაქებში იმუნური რეაქციების დასაწყისი. პეიერის ფოლაქებისთვის დამახასიათებელია გამოხატული ფუნქციური პოლიმორფიზმი, რომელიც გასათვალისწინებელია ექსპერიმენტული კვლევების ჩატარებისას.

TLR9 EXPRESSION, LANGERHANS CELL DENSITY AND LYMPHOCYTIC INFILTRATION IN PROGRESSING CERVICAL INTRAEPITHELIAL NEOPLASIA

Manjgaladze K., Tevdorashvili G., Muzashvili T., Gachechiladze M., Burkadze G.

Tbilisi State Medical University, Georgia

Toll-like receptors (TLRs) are a family of receptors, which are present in phagocytic cells. They are homologs to the Drosophila receptor called Toll [1]. To date there are 12 members of TLR family identified in mammals [2]. They represent the crucial component of the innate immune system to help host orgasm from infectious diseases [1]. TLRs are expressed in a number of various immune cells including: macrophages, dendritic cells, B cells, T cells and some non-immune cells including fibroblasts and epithelial cells. Expression of TLRs are regulated by pathogen dependent manner, by a variety of cytokines and environmental stresses[1].

TLR9 recognizes and is activated by unmethylated cytosine-phosphate-guanine (CpG) dinucleotides, which are common in bacterial and viral DNA but are suppressed and methylated in vertebrate DNA [3]. Binding of DNA containing unmethylated CpG motifs to TLR9 causes a conformational shift in the receptor, which is suggested to result in recruitment of the adapter protein MyD88, activation of signalling pathways with the phosphorylation of mitogen-activated protein kinases and activation of nuclear factor-κB [4]. At a cellular level, activation of TLR9 initiates a cascade of innate and adaptive immune responses. The immune role of TLR9 has been studied in plasmacytoid dendritic cells (pDCs) and B cells [5], which might represent the only human immune cells which constitutively express TLR9. Cellular activation is reported to induce TLR9 expression in additional cell types, including human neutrophils, monocytes and monocyte-derived cells and CD4 T cells, but the biologic role for this is less well understood. TLR9 expression has also been reported in some nonimmune cells, including pulmonary epithelial cells and lung cancers, keratinocytes and intestinal epithelium [6], in addition some studies report the increased expression of TLR9 in cervical squamous intraepithelial neoplasia [7].

CD1a presents lipid antigens with defined alkyl chain length to activate T cells [8] and it is used as a marker of Langerhans cells. It has been shown that in cervical epithelium Langerhans cells represent the first line of the immune defence, which are responsible for antigen recognition, activation of T lymphocytes and virus eradication [9]. Some studies show that the distribution of Langerhans cells are altered in different kinds of cervical epithelial lesions. The aim of our study was to analyse the expression of TLR9 and CD1a in cervical squamous intraepithelial neoplasia, together with NK cell and lymphocytic infiltration, such as CD3, CD8 and CD4 T cells and proliferation and apoptosis markers, including Ki67 and BCL2.

Material and methods. Archival formalin-fixed and paraffin-embedded (FFPE) tissue samples, diagnosed as CIN or in situ CA, between 2015-2018 years, were obtained from the department of pathological anatomy, N. Kipshidze central university clinic, Tbilisi, Georgia. Study cohort included 20 cases with normal cervical tissue, 31 cases of CIN1, 24 cases of CINII, 26 cases of CINIII and 42 cases of in situ carcinoma (CA), and 35 cases of invasive cervical carcinoma (CA), altogether 178 cases. Specimens of lesions were obtained from cervical biopsies, cone biopsies, loop electrosurgical excision procedure and radical hysterectomy. Normal cervical samples were obtained from hysterectomy, due to benign conditions, without a history of CIN or abnormal Pap smears. Standard Haematoxylin and Eosin (H&E) stained sections were revised by two independent pathologists (T.M. and G.B.). From 31 CIN1 cases, 8 cases were further progressed in CIN2 and from original 24 CIN2 cases, 12 cases were progressed into CIN3 or in situ CA. The age of patients varied from 30 to 50 years.

4μ FFPE tissue sections were deparaffinized in xylene and rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Antibodies against the following antigens were used: Ki67, BCL2, TLR9, CD1a, CD56, CD3, CD4 and CD8. Lymphocyte counts were analysed in 20 high power fields (HPF) per case. We have calculated the NK cell epithelial index meaning the ratio between 100 epithelial cells and CD56 positive NK cells and Langerhans cell epithelial index meaning the ratio between 100 epithelial cell and CD1a positive Langerhans cells. The ratio 0.1-0.2 was considered as low, the ratio 0.3-0.5 was considered as moderate and the ratio >0.5 was considered as high. In addition, immune cell proliferation apoptotic index was calculated as the proportion of Ki67 and BCL2 positive cells as following: the number of Ki67 positive immune cells were divided into the number of BCL2 positive cells and the index was recorded.

Comparisons between different groups has been performed by the use of Kruskall-Wallis test and non-parametric correlations have been estimated by Spearman's rank test. In all tests, p values < 0.05 considered as significant. Statistical analysis of data has been performed using SPSS 19 statistical program.

Results and their discussion. TLR9 expression in cervical tissues was characterised with marked variability from negative to weak to moderate and strong expression. In normal cervix negative TLR9 expression was seen in 8/20 (40%) and weak expression was seen in 12/20 (60%) cases. In CINI negative TLR9 expression was seen in 6/31 (19.4%), weak TLR9 expression was seen in 20/31 (64.5%) and moderate TLR9 expression was seen in 5/31 (16.1%) cases. In CINII negative TLR9 expression was seen in 1/24 (4.2%), weak expression was seen in 11/24