Nucleolar Organizer Regions In Cervical Intraepithelial Neoplasia

Thesis for Partial Fulfilment
of Master in Pathology

BY

Dr. Reem Abdel Azeem Hussein Abdel Salam
(M. B, B. ch)

Under Supervision of

Dr. Lila Elsayed Omar El Shabrawy
Professor of Pathology Ain Shams University

Dr. Mahmoud Abdel Aleem Abdel Salam El Kholy
Assistant Professor Ain Shams University

Dr. Amal Aziz Metry
Assistant Professor Ain Shams University

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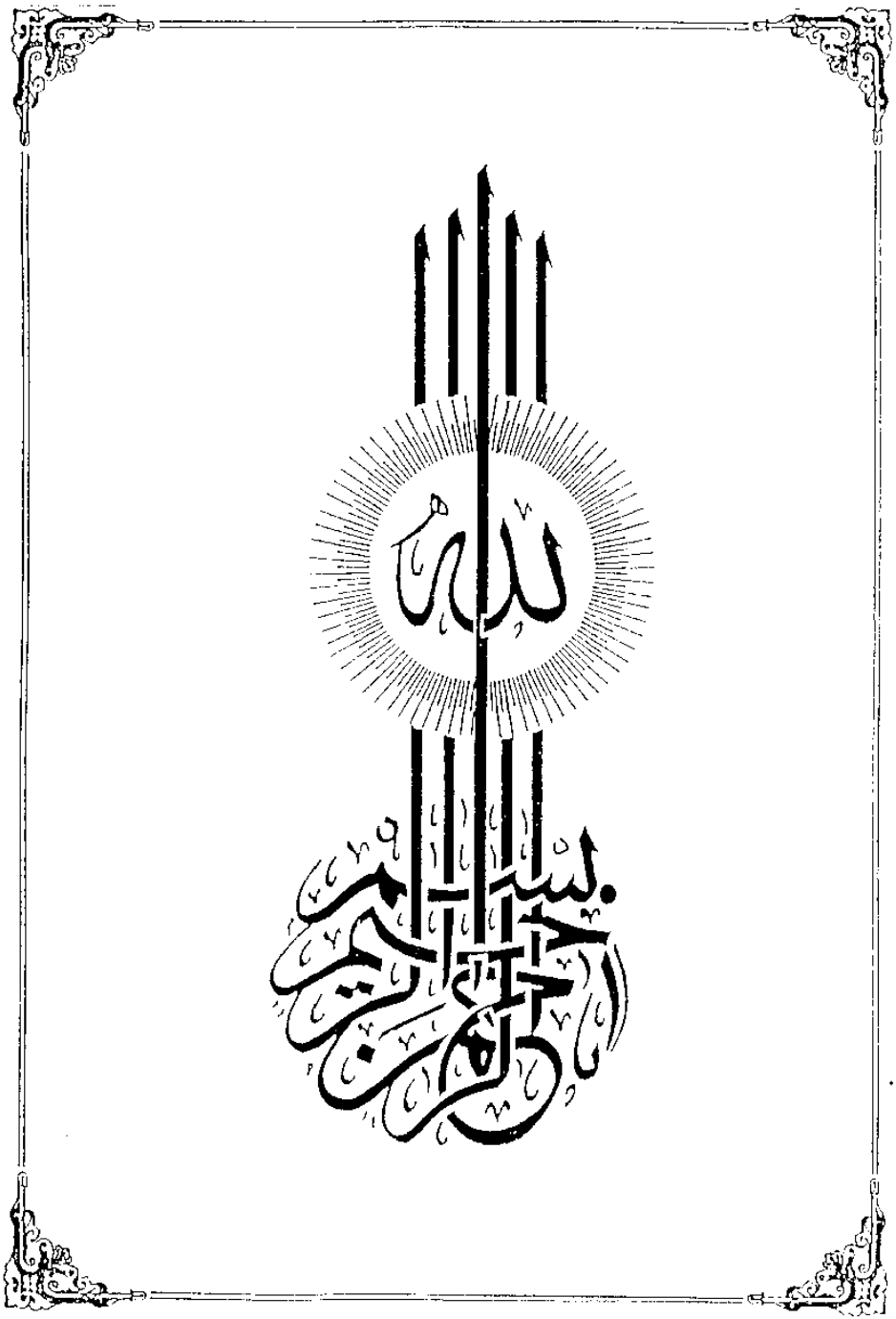

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INTRODUCTION

As the cervix uteri is specially accessible to cell and tissue study, and to direct physical examination, this has permitted intensive investigations on the nature of malignant lesions of the cervix. Although our knowledge is incomplete, investigations have shown that most of those lesions have a gradual, rather than an explosive onset. Preinvasive precursors may exist in a reversible phase of surface, or in-situ disease for some years, although this may be changing, at least in some patients.

Although early phases of the disease may be asymptomatic, they are detectable by currently available methods. This concept of development of cervical malignancy have convinced many that the control of this disease is well within of grasp of the foreseeable future. It is possible to eradicate most deaths resulting from cervical cancer by proper dealing with preinvasive lesions of the cervix (**Disaia, Cresman 1993**). Most of the evidence available supports the concept that CIN is a significant precursor to invasive carcinoma (**Giuntoli et al, 1987**).

Recent studies have revealed the importance of colloid silver staining of the nucleolar organizer regions (AgNORs) in different benign, border-line and malignant lesions of different tissues of the body (**Crocker, 1990, Morad et al, 1992, Crocker and Egan, 1992**). This technique which is relatively simple has been applied in the study of different lesions of the uterine cervix. It was found to have a considerable clinicopathological potential in cervical pathology and cytology (**Egan et al, 1988; Tricet et al 1989; Cardillo, 1992**).

AIM OF WORK

The aim of this work was to study AgNORs both quantitatively and qualitatively in different grades of CIN, in a trial to evaluate its role in grading the malignant potentialities of these lesions, and to compare the results with that of invasive carcinoma of the cervix.

Embryology of the cervix:

The uterine cervix is of mesodermal origin. It arizes from the fusion of the middle portions of mullarian ducts, the adjacent mesenchyme gives rize to the connective tissue stroma and muscle fibres. Epithelium of the ectocervix originates from the epithelium of the uterine canal. The glandular epithelium becomes mucus-producing by metaplasia form this same endometrial epithelium. During the late fetal and neonatal period, the junction between the two epithelia is located on ectocervix; this ectropion may be the result of maternal hormonal stimulation. The cervical stroma is invaded by straight glands arizing from the surface epithelium; these glands rami by secondarily (**Forsberg, 1973, Duncan, 1992**).

Anatomy of the cervix

Although the cervix is part of the uterus, its structure and function differ from the body of the uterus.

It forms the lower third of uterus and is the area below the isthmus which includes the external and internal os. It enters the vagina at right angle and is sometimes called the "neck" of the uterus (**Verralls, 1993**).

The cervical canal is fusiform, and the cervix as a whole tends to be barrel-shaped. In adult life the cervix is 2.5 cm long and forms one third of the whole length of the uterus. During intrauterine life however, it forms the greater part of the uterus and then in the last weeks of pregnancy there is an accelerated growth of the

uterine body brought about by the high levels of maternal oestrogenic hormones. At the time of birth, the cervix and body of the uterus are approximately equal in size. When the ovarian hormones are activated at puberty, there is further acceleration of growth of the uterine body until it is approximately twice the length of the cervix.

The supra vaginal cervix, is that portion of the cervix which lies outside and above the vagina. Superiorly, it meets the border of the uterus at the isthmus (**Verralls, 1993**). The intravaginal cervix (portio-vaginalis) is the portion which projects into the vagina. The internal os opens into the cavity of the uterus. Although it is not a sphincter in the true sense of the word, it dilates during labour (**Vellacott, 1992, Verralls, 1993**) Fig (1).

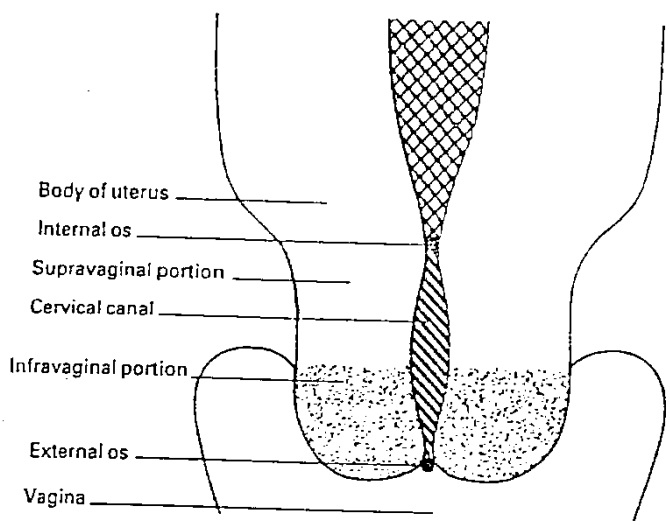


Figure 1. Gross structure of the cervix

Histology of the cervix:

The cervix comprises two distinct parts; ectocervix and endocervix. The ectocervix is covered by stratified squamous epithelium that overlies a dense fibrous stroma. The endocervix is 2-3 mm thick, covered by unstratified columnar mucosa resting on stroma rich in mucus secreting glands (racemose glands). These glands are actually clefts arizing from the surface mucosa (**Fluhman and Dickmann 1958, Novak 1981**). The endocervix is arranged in folds, arbor vitae, allowing dilatation of the cervix to occur without trauma (**Verralls, 1993**).

The squamous ectocervical epithelium is composed of basal, intermediate and superficial cell layers identical to those of the vaginal mucosa. Five layers are recognizable:-

1. The basal cell layer (C-1) which is in contact with the basement membrane, this is the reserve cell layer. The cells are oriented perpendicular to basal lamina and normally exhibit mitotic figures because they constitute the reproductive cells of the epithelium. The enzymes phosphorylase and amyl-6-glycosidase, required for glycogen synthesis, are localized in these cells.

2. layer C-2, consists of two or three rows of parabasal cells. This is the most rapidly proliferative layer.

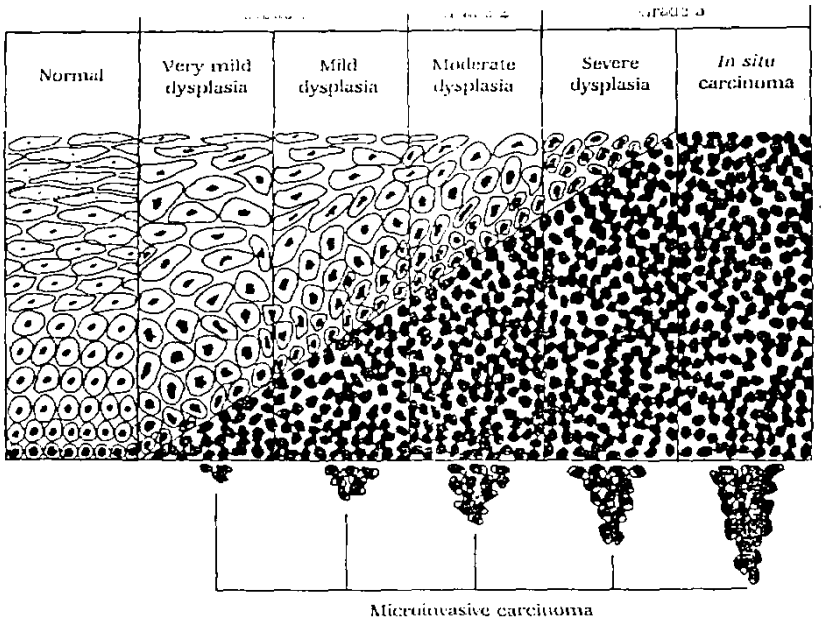
3. layer C-3, is characterized by the presence of glycogen (manifested by clear cytoplasm) and the development of intercellular bridges (desmosomes).

4. Layer C-4 is characterized by the presence of glycogen in greater amounts and the intercellular bridges begin to disappear.

5. Layer C-5 is composed of a superficial layer of large cells with pyknotic nuclei.

Eosinophilia of the cytoplasm is related to the presence of keratin microfilaments. The endocervical muciparous columnar epithelium is composed of tall cells with elongated basal nuclei and occasional ciliated cells. This mucosa penetrates into the stroma, where it gives rise to glands lined by columnar cells with abundant clear cytoplasm and basal nuclei. The stroma is dense and comprises vesicles of fusiform connective tissue cells, and collagenous and elastic fibers, as well as scattered smooth muscle fibres. The stroma does not show cycle histologic modifications such as those of the endometrial stroma (**Fanger and Barker 1963**) (**Gompel and Silverberg 1985**).

The junction of the two epithelia occurs normally at the level of the external os by the transformation zone of metaplastic squamous epithelia (**Frenczy and Richard, 1973**). During the period of genital activity, the endocervical mucosa has a tendency to extend toward the external cervical orifice (ectopy or ectropion). Before puberty and after ovarian activity ceases, the glandular epithelium is pushed back into the endocervical canal by the squamous ectocervical and epithelium (entropion).



CIN

Figure 2. Schematic representation of premalignant lesions. This diagram compares the premalignant lesions of dysplasia/CIS and CIN. (Blaustein A: Pathology of the Female Genital Tact, 2nd ed, p158. New York, Springer-Verlag, 1982)

The squamous mucosa presents the same hormonal modification as the vaginal mucosa and undergoes cyclical keratinization in relation to oestrogenic activity. It contains glycogen particularly in the intermediate cell layer (**Gompel and Silverberg 1985**).

The Glands of the cervix secrete a viscid mucous secretion during reproductive years. These secretions are influenced by the cyclic changes of ovarian hormones. The alkaline secretion is increased in amount and decreased in viscosity during the oestrogen phase. After ovulation the mucus becomes thick under the influence of progesterone (**Masayoshi 1981**).

The Transformation Zone (T.Z):

Definition:-

The T.Z. is defined as the area of the cervix, or cervix and vagina, that was initially covered by columnar epithelium and that, through a process called metaplasia, has been replaced all or part by squamous epithelium (**Townsend, 1988**). The complete transformation from columnar to squamous epithelium requires many years.

Origin:

In most women during the reproductive period, the mucus secreting columnar epithelium of the endocervix extends into portio vaginalis forming the so called endocervical erosion (ectropion or ectopy). The development of endocervical eversion is not clear; characteristically, it develops during fetal life, at the time of puberty and during the reproductive years especially during pregnancy and is absent in post menopausal women. Under the action of increased hormonal stimuli, particularly estradiol, the volume of the portio vaginalis increases and the lips of the portio vaginalis protrudes (**Fluhman 1961**).

During the post menopausal period when estrogenic influence is inconspiciuos, the volume of the portio vaginalis decreases considerably, consequently the lips are retracted and the endocervical mucosa is brought into the endocervical canal. So the squamocolumnar junction is virtually always located above the external os and often within the cervical canal. The surface columnar