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DEGREE OF CRYPT ATYPIA CORRELATES WITH PROGRESSION TO HIGH GRADE DYSPLASIA/ ADENOCARCINOMA IN NON-DYSPLASTIC BARRETT'S ESOPHAGUS

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Abstract

Aims: Patients with non-dysplastic Barrett's esophagus (BE) often show a wide range of "atypical" histologic features in the bases of the crypts. However, the significance of crypt atypia has never been evaluated despite prior studies showing the presence of DNA content and other molecular abnormalities in this epithelium. The aim of this study was to evaluate whether the degree of crypt atypia in BE patients without dysplasia correlates with progression to high-grade dysplasia/adenocarcinoma (HGD/EAC).

Methods and results: Baseline biopsies from 114 BE patients without dysplasia, 57 who progressed to HGD/EAC (progressors) and 57 who did not progress (non-progressors), were included in the study. Biopsies were evaluated for the degree of basal crypt atypia on a 3-point scale according to discrete histologic criteria. In non-progressors, 64.9%, 31.6%, and 3.5% of biopsies had a crypt atypia score of 1, 2, and 3 respectively, with a mean score of 1.39 +/− 0.56. The percentage of biopsies with an atypia score of 2 or 3 increased in progressors (42.1%, 42.1%, and 15.8% of biopsies scored 1, 2, or 3 respectively, with a mean score of 1.74 +/− 0.72 (P=0.004)). The odds ratio of grade 3 crypt atypia for progression to HGD/EAC was 5.2 (95% CI 1.1–25.0, P=0.04) and the findings did not change significantly when the data was analyzed according to progression to either HGD or EAC.

Conclusions: This study shows that non dysplastic crypts in BE are biologically abnormal suggesting that neoplastic progression begins prior to the onset of dysplasia. The degree of crypt atypia in BE patients without dysplasia correlates with progression.

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Ethics statement: All archived pathology samples were collected, and studies performed after proper IRB approval.

Keywords

Barrett’s esophagus; dysplasia; esophageal adenocarcinoma; Crypt atypia

Introduction

Barrett’s esophagus (BE) is defined as columnar metaplasia of the esophagus which is usually associated with intestinal metaplasia (IM).(1) BE is the main precursor to esophageal cancer, however, only a minority of patients (approximately 0.5%) actually progress to cancer each year.(2–5) Cancer in BE develops via an inflammation-dysplasia-carcinoma sequence, and is associated with high morbidity and mortality if diagnosed at an advanced stage (SEER database).(6) Thus, patients with BE are advised to undergo regular endoscopic surveillance combined with biopsies in order to detect dysplasia and early cancer that is amenable to minimally invasive endoscopic therapy.(7)

Dysplasia is morphologically graded as either low or high based on the degree of cytologic and architectural atypia of the epithelium. Conventional low (LGD) and high-grade dysplasia (HGD) involves both the crypts and surface epithelium.(8,9) However, as discussed further below, dysplasia in its early stage can involve only the crypts and not the surface epithelium, and this provides evidence that neoplastic transformation begins in the bases of the crypts in BE and likely from basal crypt stem cells. Currently, cancer risk assessment is based entirely on morphologic identification, and grading, of dysplasia since the risk of progression increases according to the presence and grade of dysplasia in the Barrett’s segment.(10) For instance, LGD carries a lower risk of progression to cancer (0.4–13%) compared to HGD (6–19%).(11,12) An important challenge is to determine which patients with non-dysplastic BE are at increased risk for progression. Thus, better methods of cancer risk prediction in BE patients are clearly needed.

Prior studies have documented the presence of a wide variety of differentiation, proliferation, and molecular abnormalities in patients with, but also in those without, dysplasia in their BE.(13–18) These include alterations in cell cycle, DNA content and even tumor suppressor genes, such as *TP53*, among others. Unfortunately, there is, currently, little data regarding whether there are phenotypic alterations that can be seen in the early stages of BE progression, prior to the onset of dysplasia, that can help predict cancer progression. Although it is well known that Barrett’s specialized epithelium is characterized by a variety of atypical changes that render its appearance distinct from normal intestinal epithelium, the spectrum of changes that occur, and their possible correlation with risk of progression, has never been studied. For instance, architecturally, Barrett’s epithelium often shows branching, fission, and fusion of the crypts which is quite distinct from the normally well-aligned and evenly spaced, test tube–like crypts characteristic of normal intestinal epithelium. Cytologically, the crypt epithelium may also exhibit a range of alterations. These include epithelium with little to no atypia, where the crypts are lined by cells with small normo-chromatic nuclei without stratification or mitoses, to epithelium with marked atypia characterized by nuclear hyperchromasia, enlargement, irregularity, loss of polarity, stratification and prominent mitoses. In the extreme, these crypt changes mimic the features

of true dysplasia, and this has been termed crypt dysplasia (CD) in prior studies.(19,20) Several studies have confirmed the presence of p53 and DNA content abnormalities in CD, and in one recent outcome study of brush biopsies, an increased risk of progression to HGD/esophageal adenocarcinoma (EAC).(21–23) Unfortunately, the biological and clinical significance of “baseline” non-dysplastic morphologic changes in BE, as described above, have never been evaluated. Our hypothesis is that these baseline basal crypt changes in BE likely reflect a slow and progressive evolution and proliferation of clones of cells with increasing amounts of atypia, and that these changes may result in a corresponding increased risk of neoplastic progression in BE. Thus, the purpose of this long-term outcome study was to evaluate and categorize the baseline morphologic features of non-dysplastic crypt epithelium in BE, and to correlate these findings with progression to HGD/EAC.

Methods

Patient cohort and processing

After IRB approval and consent where applicable, archived pathology blocks from the Dutch spatial-temporal cohort were utilized for this study.(24) Briefly, this cohort consists of a retrospective collection of patients who presented to four separate tertiary care referral centers within the Netherlands, all with an initial baseline diagnosis of non-dysplastic BE (defined as columnar lined epithelium on endoscopy and intestinal metaplasia on histology). Endoscopic biopsies were originally taken as part of routine patient care, and all samples were retrospectively collected after an extensive search using the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGAdatabase). To be included in the cohort, an endoscopy had to average a minimum of 1 biopsy per cm of BE length (i.e. an endoscopy had to have at least 4 biopsies taken if the patient had 4 cm of BE) and there had to be no visible endoscopic lesions. The patient’s age, sex, BE segment length, and clinical follow-up was recorded. All progressors had HGD or adenocarcinoma (EAC) diagnosed by at least two independent pathologists based on biopsies from two separate endoscopies or a single endoscopic resection specimen. We chose to use both HGD and EAC as progression endpoints since both of these diagnoses are usually treated similarly (endoscopic ablation). To exclude prevalent neoplasia at baseline, progressors were excluded from the study if progression occurred less than two years after the original non-dysplastic BE diagnosis or if the patient was diagnosed with greater than T1 disease at the time of progression. Non-progressors had at least one follow up surveillance endoscopy showing no progression to HGD or EAC greater than 3 years after the baseline endoscopy. Patient demographics and characteristics were blinded to all investigators performing pathologic evaluation. For the study, the follow-up interval was defined as the date of the endoscopy procedure in which the baseline biopsy sample was originally obtained, to the date of the first diagnosis of HGD or EAC for progressors, or to the date of the patients most recent surveillance biopsy for non-progressors. Overall, this study consisted of 57 patients who progressed to HGD or EAC, and 57 patients who did not progress after at least 3 years of follow up.

The patient’s baseline (defined as the patients first endoscopy with biopsies in the study) standard formalin-fixed paraffin-embedded tissue blocks were retrieved and five- micron

thick sections of each biopsy were cut within the laboratory by a trained pathologist. Each tissue section was stained with hematoxylin and eosin (HE). Two HE-stained glass slides of each tissue block with ~50 microns between sections were made for morphology review, unless the tissue in the cell block was exhausted, In which case only a single HE-stained slide was created.

Histologic grading

Each baseline HE-stained glass slide was reviewed by a gastrointestinal pathologist (RO). If the biopsy was confirmed as non-dysplastic, the degree of basal crypt atypia (in the basal one third of the crypts) was then graded for each case according to the grading system outlined in table 1. Overall, grading was performed on a scale of 1–3 as shown in figure 1A–C. For each patient, the highest grade of atypia present in any of the patient's biopsies within a single crypt profile or greater, from the baseline endoscopy, was used as the patient's overall final atypia grade. For the majority of patients one baseline block was scored and there was no difference in the number of baseline blocks scored for progressors (range 1–4, mean 1.12, median 1) vs non-progressors (range 1–3, mean 1.23, median 1). Grading of crypt atypia was performed only in areas void of active inflammation and/or erosion. The pathologist was blinded to the original diagnosis and the progression status of all patients.

Statistics

Atypia scores were correlated with patient outcome (progressor or non-progressor). A Mann-Whitney-U test was utilized to compare atypia grades between progressors and non-progressors. A one-way ANOVA was utilized to compare means of more than two groups. For categorical data, a Fisher's exact test, Chi-squared, or test for one proportion were performed. A P value of 0.05 was considered statistically significant.

Results

Patient characteristics

The demographic features of the study patients are summarized in Table 2. Overall, there were 114 patients in the study. Fifty-seven patients with a baseline non-dysplastic BE diagnosis progressed to HGD or EAC during the follow up period. 94% were male and the mean age was 58 (39–73) years. The mean circumferential BE length was 4.75 (2–11) cm at baseline endoscopy. Fifty-seven non-progression patients (93% male) with a mean age of 58 (30–75) had a mean circumferential BE length of 4.33 (2–12) cm. The mean follow-up time for the progressors and non-progressors was 7.0 (1.6–14.3) and 10.4 (3.7–17.2) years, respectively, and this difference was statistically significant ($p < 0.001$).

Pathology results

Of the 114 patients overall, 61 (53.5%) had grade 1 atypia, 42 (36.8%) had grade 2 atypia, and 11 (9.7%) had grade 3 atypia as the highest grade of crypt atypia in any of their baseline biopsies (Table 3). Patients who progressed to HGD/EAC had a significantly higher mean baseline atypia score than patients who did not progress (1.74 vs 1.39, P=0.007). More specifically, of the patients who progressed, 24 (42.1%) had grade 1 atypia, 24 (42.1%) had grade 2 atypia, and 9 (15.8%) had grade 3 atypia in their baseline biopsies. Grade 2

or 3 atypia was significantly more common in baseline biopsies from progression patients compared to non-progression patients who showed 37 (64.9%) with grade 1 baseline atypia, 18 (31.6%) with grade 2 atypia, and only 2 (3.5%) with grade 3 atypia (P=0.05 for grade 3 vs grade 1+2 in progressors vs non-progressors and P=0.02 for grade 2+3 vs grade 1 in progressors vs non-progressors). Conversely, grade 1 atypia was significantly more common in non-progressors (P=0.02, Table 3). The grade of crypt atypia did not correlate with the mean length of BE in either progressors (P=0.31) or non-progressors (P=0.41). In progressors, the distribution of crypt atypia grades was statistically similar in patients who progressed to HGD vs EAC (P=0.40).

Since advancing grades of crypt atypia was associated with progression to HGD/EAC, we calculated odds ratios for progression to HGD/EAC ((Table 4). In this cohort, baseline grade 3 crypt atypia revealed an odds ratio of 5.2 (95% CI 1.1–25.0, P=0.04) for progression. Grade 3 atypia had a sensitivity for identifying a progression patient of only 15.8%, but it had a specificity of 96.5%. The odds ratio of grade 1 atypia for progression was 0.57 (95% CI 0.28–1.2, P=NS). As expected, the odds ratio of progression for grade 2 atypia was in between grade 1 and grade 3.

To determine if crypt atypia changes over time differently among patients who progressed, versus those who did not progress, to HGD/EAC, we determined the crypt atypia scores in a subset of patients who had at least two separate endoscopies performed prior to the outcome diagnosis of either HGD or EAC in the progressors or prior to the end of the follow-up period in non-progressors (N=54 patients in total) (see supplementary table 1 for further details). Thirty-three non-progression patients and 21 progression patients had biopsies available from multiple successive endoscopies that contained exclusively non-dysplastic BE. For the non-progression patients, 22/33 (66.7%) had the same crypt atypia score in their most recent endoscopy compared to their baseline endoscopy, 6/33 (18.2%) showed a decrease in their atypia score, and only 5/33 (15.2%) revealed an increase in their atypia score, with none increasing to grade 3. In contrast, in the 21 progression patients, although the same crypt atypia score was present in the patient's most recent endoscopy with non-dysplastic BE (prior to any diagnosis of dysplasia or EAC) in 10/21 patients (47.6%), a significantly higher percentage of patients showed an increase in their crypt atypia score to grade 3 (5/21 (23.8%), (P=0.03.). Only 5/21 (23.8%) progression patients showed a decrease in their atypia score with time.

Discussion

Patients with BE are at greatly increased risk of developing EAC, but the factors involved in the progression of neoplasia development are poorly understood.(25,26) Multiple studies have documented the presence of abnormalities of cell differentiation and proliferation, as well as genomic defects including mutations in common genes such as *TP53*, in non-dysplastic BE well prior to the onset of dysplasia.(14,27) In addition, pathologists have long recognized the existence of various degrees of crypt epithelial abnormalities ('atypia') in non-dysplastic BE, but the significance of these changes and their potential association with risk of progression have never been investigated. Interestingly, prior studies have shown that dysplasia in BE normally begins in the crypt bases (where precursor stem cells are

located) and then progresses luminally to the surface epithelium with time, similar to the pathogenesis of neoplasia in the normal and inflamed (ex. IBD) intestinal tract.(28) This is one of several important reasons why the functional epithelial tubular-shaped units in BE mucosa are termed “crypts” rather than “pits”.(20) Early neoplastic clones can expand through a process of gland duplication or fission. It is interesting to note that when there was more than one gland crypt with grade 3 crypt atypia, the crypts were often clustered together, which suggests that whatever genomic or other factors that are responsible for inducing crypt atypia may be ‘inherited’ and spread in a clonal fashion.

Using a unique cohort of 114 BE patients, of which 57 progressed to HGD/EAC, and 57 did not, we have documented for the first time that the level of basal crypt epithelial atypia graded histologically on a three - point scale in BE patients without dysplasia is associated with neoplastic progression. The mean crypt atypia scores, as well as the percentage of patients with grade 2 and 3 crypt atypia, were significantly higher in baseline biopsies of BE patients without dysplasia who progressed to HGD/EAC versus those who did not. The Odds ratio of progression was 2.1 and 5.2 for patients with grade 2 or 3 crypt atypia, respectively. We also showed that patients who progressed to HGD/EAC were statistically more likely to progress from a lower crypt atypia grade to a higher one over the course of time, prior to the ultimate diagnosis of HGD/EAC, indicating a likely histologic pathogenetic sequence in this process. Based on this data, we conclude that non dysplastic crypts in BE are biologically abnormal, and that neoplastic progression likely begins prior to the onset of conventional dysplasia.

This is the first study to evaluate levels of crypt atypia in BE patients without dysplasia and to correlate those results with progression to HGD and/or EAC. Our finding of a strong positive correlation between advancing grades of crypt atypia and neoplastic progression, combined with our finding of a significant progressive increase in crypt atypia with time, prior to HGD/EAC development, is not surprising given the genomic abnormalities found in prior studies of non-dysplastic BE. Although no prior studies have evaluated baseline levels of crypt atypia in BE as in this study, several studies have been published on “crypt dysplasia” (CD), which is a lesion that corresponds to grade 3 crypt atypia as defined in this study. Crypt dysplasia was originally described by Lomo et al in 2006 as dysplasia–like morphologic changes that are limited to the crypt bases without involvement of the upper crypts and surface epithelium, thus, not fulfilling the conventional definition of dysplasia as a lesion that involves both crypt and surface epithelium.(20) In that study, CD showed significantly higher 17p loss of heterozygosity (LOH) and flow cytometric DNA abnormalities compared to background non CD epithelium in BE, and a high association with conventional dysplasia elsewhere in the esophagus from the same patients. In another study by Zhang et al in 2008, DNA content abnormalities similar in quantity to conventional LGD (which by definition shows surface involvement) were identified in the basal aspects of the crypts in CD foci.(21) Furthermore, in one recent outcome study by Shaheen et al of 4545 BE patients either without dysplasia (N=4374), with CD, (N=128) or with LGD (N=43) identified in WATS-3D brush specimens, those with CD progressed to HGD/EAC (confirmed in accompanying forceps biopsies) at a significantly higher rate than non-dysplastic BE patients (1.42% per year vs 0.08% per patient-year).(22) These data, combined with our results in this study, suggests that cancer progression in BE begins early,

prior to the onset of “dysplasia” as currently defined by the World Health organization (WHO), and that these changes can be detected and graded, and may in fact serve as a useful biomarker of neoplastic progression if validated by others in the future.

Since the earliest published morphologic descriptions of BE as consisting of ‘specialized’ epithelium that resembles intestinal crypts, pathologists have long recognized the presence of various degrees of crypt abnormalities in BE patients without dysplasia. Changes such as nuclear enlargement, irregularity and hyperchromaticity, increased mitoses, slight loss of cell polarity and even slight nuclear stratification, have all been interpreted as either “normal” or “reactive” even in the absence of active inflammation (as in this study) because these alterations are not severe enough to fulfill the WHO morphologic criteria of dysplasia. However, as mentioned above, multiple studies have documented a wide variety of genomic alterations in non-dysplastic epithelium in BE, and in fact, several have been suggested as potential biomarkers for progression risk.(13,14,17,27) Based on this data, we speculate that the degree of crypt atypia may be related to a progressive accumulation of genomic alterations as a potential driver of histologic alterations, and subsequent neoplasia development. Future studies with larger number of patients, that also genomically characterize discrete cell populations across the full spectrum of histology using technologies such as laser capture microdissection will be needed to further assess the genetic/morphology correlations more specifically.

Several limitations of our study should be noted. First, since the study was retrospective, potentially important clinical information such as BMI, hiatal hernia, and alcohol or tobacco use could not be assessed. However, none of the patients had a prior history of dysplasia, and all were evaluated at tertiary medical institutes with abundant clinical and research experience in managing patients with BE. Second, only one pathologist (RO) evaluated the biopsy specimens for grade of crypt atypia, so an interobserver variability assessment could not be performed. However, our purpose in this initial discovery study was not to develop a morphologic classification system that can be used clinically, but to evaluate the biological spectrum of changes in the crypts of BE patients and to determine if they have biomarker potential. Furthermore, since this was a case-control study that included equal numbers of progressors and non-progressors, we feel that the results cannot be directly extrapolated to the general BE population where only a small minority of patients will normally progress to HGD or cancer. If our results are validated by other groups, further interobserver studies should be done prior to consideration of clinical applications. Regardless, the pathologist in this study utilized well-defined histologic criteria, and performed the review in a blinded manner without knowledge of the patient’s outcome or the timing of the biopsy prior to HGD/EAC development (baseline vs interval vs outcome). Another limitation was that no attempt was made to quantify crypt atypia, rather patients were assigned a crypt atypia grade based on their maximum value in any one biopsy. Nevertheless, our system more closely followed the method that may be used clinically, where laborious morphologic quantitation is typically avoided and considered cumbersome.

Conversely, there were several strengths of the study that also need to be highlighted. These include the relatively large number of patients who progressed to HGD/EAC, the long follow up period that, in fact, was significantly longer for non-progressed patients, and

the ability to evaluate interval biopsies for evaluation of changes over time. Furthermore, in order to definitively rule out the possibility of including patients who may have had missed prevalent dysplasia at study onset, we specifically excluded those who had dysplasia detected within two years, which is one year more than most prior published outcome studies in BE.

In summary, this study showed that non-dysplastic epithelium in BE patients reveals a range of atypical morphologic changes in the basal aspect of the crypts, and that the severity of basal crypt atypia, as measured on a three-point scale, correlates with risk of future development of HGD/EAC in a progressive, and possibly step wise, manner. This provides evidence that the background non-dysplastic epithelium in BE is biologically abnormal. Further studies utilizing a larger number of patients and designed to evaluate and correlate crypt atypia with genomic abnormalities directly (by crypt or single cell analysis) should be done to better understand the sequence of neoplastic progression in BE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability statement:

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

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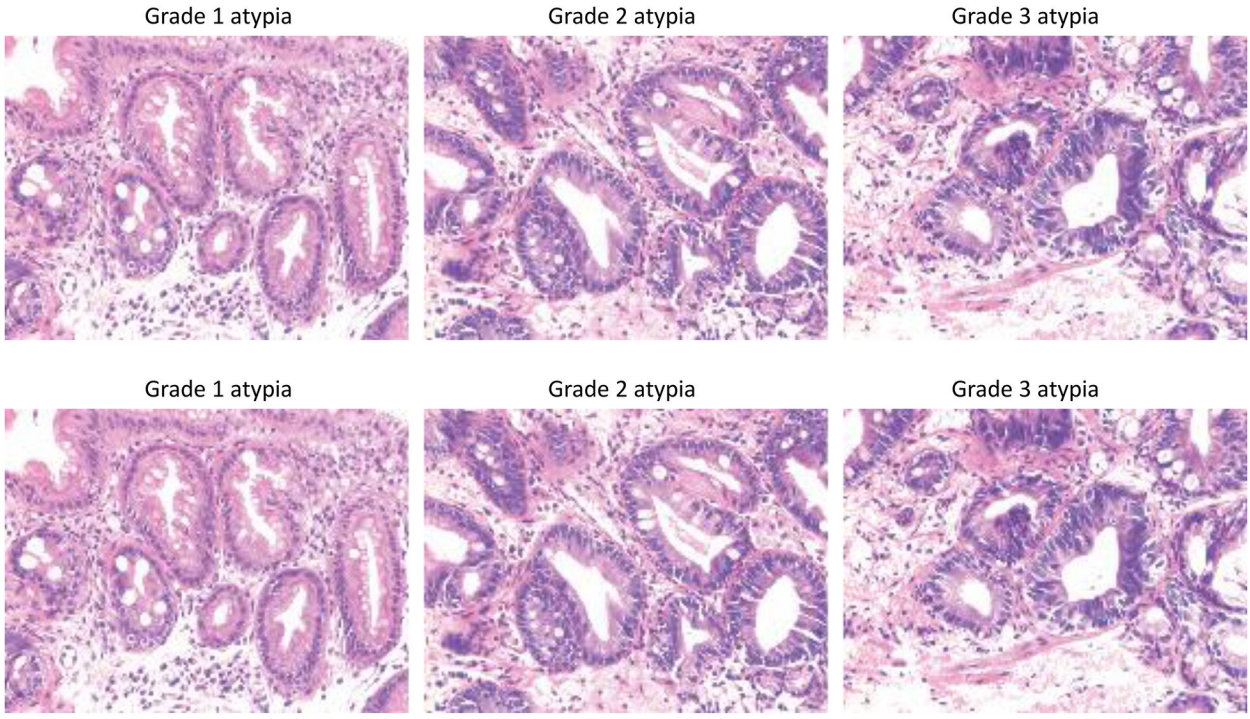

Figure 1.

Representive histologic images of grade 1, grade 2, and grade 3 atypia scores. Grading was performed blinded to outcome status according to the criteria outlined in Table 1.

Table 1:

HISTOLOGIC GRADING CRITERIA

GRADE	Histologic criteria
1	Normal -sized nuclei or only mild nucleomegaly, no or only mild hyperchromasia, no mitoses, no nuclear stratification, no loss of cell polarity.
2	Moderate nucleomegaly and hyperchromasia, occasional mitoses, mild variation in nuclear size and contour, focal nuclear stratification and loss of polarity.
3	Marked nuclear enlargement, elongation and hyperchromaticiy, often with nuclear irregularity, frequent mitoses, diffuse concentric nuclear stratification and loss of polarity.

Table 2:

PATIENT DEMOGRAPHICS

	Progressors	Non-Progressors	Total
# of patients	57	57	114
% Male	94%	94%	90%
Mean age(years)	58 (39–73)	57.5 (30–75)	57.8 (30–75)
BE length(cm)	4.75 (2–11)	4.33 (2–12)	4.54 (2–12)
Mean time to progression or end of follow up(years)	7.0 (1.6–14.3)	10.4 (3.7–17.2)	8.7 (1.6–17.2) *

* P<0.001

Table 3:

SUMMARY OF CRYPT ATYPIA SCORES

Grade	Progressors (N=57)	Non-Progressors (N=57)	Total
GRADE 1	24 (42.1%)	37 (64.9%)	61 (53.5%)
Grade 2	24 (42.1%)	18 (31.6%)	42 (36.8%)
Grade 3	9 (15.8%)	2 (3.5%)	11 (9.6%) *
Grade 2 or 3	33 (57.9%)	20 (35%)	53 (46.5%) **

P value, grade vs other grades in progressors vs non-progressors.

* P=0.05

** P=0.02

Table 4:

SUMMARY OF ODDS RATO'S FOR PROGRESSION

Condition	Odds ratio	Confidence interval
Grade 1	0.57	0.28 – 1.2
Grade 2		
(Vs grade 1)	2.1	0.93 – 4.6
(Vs grade 3)	0.3	0.06 – 1.5
Grade 3	5.2 *	1.1 – 25.0
Grade 2 or 3	2.5 **	1.2 – 5.4

* P=0.04

** P=0.02