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Epigenetic biomarkers in progression from non-dysplastic Barrett's Oesophagus to Oesophageal Adenocarcinoma: A Systematic Review protocol

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Epigenetic biomarkers in progression from non-dysplastic Barrett's oesophagus to oesophageal adenocarcinoma: A systematic review protocol

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Key-words

Epigenetics, Barrett's, Adenocarcinoma, Systematic Review

1886 words

ABSTRACT

Introduction

Barrett's Oesophagus (BO), a metaplastic condition affecting the lower oesophagus due to long standing gastro-oesophageal reflux and chronic inflammation, is a precursor lesion for oesophageal adenocarcinoma (OADC). There is no clinical test to predict which patients with BO will progress to OADC. The British Society of Gastroenterology recommend endoscopic surveillance of patients with BO. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis to colonic carcinoma, another gastrointestinal cancer associated with chronic inflammation. This systematic review protocol aims to identify and evaluate studies which examine epigenetic biomarkers in BO and their association with progression to OADC.

Methods and analysis

All prospective and retrospective primary studies, and existing systematic reviews investigating epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types are eligible for inclusion. Eligible patients are those over the age of 18 with BO, BO with dysplasia, OADC or unspecified oesophageal cancer. A comprehensive search of bibliographic databases using combinations of text and index words relating to the population, exposure and outcome will be undertaken with no language restrictions. Results will be screened by 2 independent reviewers and data extracted using a standardised proforma. The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool. A narrative synthesis of all evidence will be performed with key findings tabulated. Findings will be interpreted in the context of the quality of included studies. The systematic review will be reported according to PRISMA guidelines.

Ethics and dissemination

This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

Trial registration number International Prospective Register for Systematic Reviews (PROSPERO) number CRD42016038654

Strengths and limitations of this study

Study Strengths:

- Systematic review protocol following PRISMA-P guideline structure.
- Original research, need for systematic review identified.
- Exhaustive search of published literature on epigenetic markers for progression of Barrett's Oesophagus to oesophageal adenocarcinoma.
- Systematic review methodology detailed

Study Limitations:

- Heterogeneity of published research anticipated (differing epigenetic biomarkers studied, variation of study design, sampling methods and follow up length).
- Above may limit certain epigenetic markers to narrative evidence synthesis

INTRODUCTION

The incidence of oesophageal adenocarcinoma (OADC) has dramatically increased in recent years to 5.7 per 100,000 for females and 14.1 per 100,000 for males in the UK[1, 2]. Unfortunately the majority of patients present with advanced unresectable disease with an overall 5 year survival of less than 13%[3] . Five year survival rates improve considerably to 39% with localised disease[4]. Barrett's Oesophagus (BO), a metaplastic condition affecting the lower oesophagus due to long standing gastro-oesophageal reflux disease (GORD) and chronic inflammation, is a precursor lesion for OADC with progression through the metaplasia-dysplasia-carcinoma sequence[5]. The likelihood of developing OADC is increased 1.7 times in patients with gastro-oesophageal reflux disease, increasing to 10.6 times with BO[6]. The incidence of OADC has risen in parallel with increasing obesity and GORD in Western populations[1]. With rising rates of obesity the incidence of OADC is predicted to further increase[7]. Currently there is no robust way of predicting which patients with BO will progress to OADC. As a result, the British Society of Gastroenterology recommend endoscopic surveillance of patients with BO and the American College of Gastroenterology endorse screening of high-risk patients for BO[8, 9]. Endoscopic surveillance is invasive, expensive and despite rigorous biopsy protocols, dysplasia and early cancers can be missed. Importantly a recent meta-analysis published in 2012 demonstrated lower risk for progression of non-dysplastic BO than previously reported with a pooled 0.33% (95% CI 0.28–0.38%) annual incidence of OADC[10]. The annual incidence rate of OADC with HGD is 7-19%[11-13].

Epigenetics is an emerging field which describes mechanisms of alteration of gene regulation and expression without changing the genetic code[14]. These regulatory mechanisms are important in normal human development, for example silencing of the X-chromosome in females[15]. Epigenetic changes may be inherited but can also be acquired through environmental factors such as cigarette smoking[16]. Epigenetic change can occur through various methods. The most recognised are covalent modifications including DNA methylation, histone modification and altered gene expression by non-coding RNAs[14]. DNA methylation occurs when DNA methyltransferase adds a methyl group (CH_3) to a DNA base. In humans this is most commonly a cytosine base creating 5-methylcytosine[14]. Methylation which occurs at gene promoter (CpG) sites causes downregulation of these

genes. It is thought that the mechanism responsible is the projection of a methyl group into the DNA groove which physically blocks transcription[17]. Histone modification is a post translational alteration to histone proteins which package DNA into nucleosomes and eventually chromosomes by winding DNA around them. If the histone structure is altered, the DNA cannot be correctly unravelled and cannot be correctly transcribed. The above modifications are carried over when a cell divides and can be inherited[18]. Many different types of non-coding RNAs have been discovered to alter gene expression by targeting coding messenger RNA (mRNA) after its transcription from DNA. Both micro RNAs (miRNA) and long non-coding RNAs have been implicated in gene regulation. These bind to mRNA molecules and cause them to be denatured and halt protein translation and cause genetic silencing[19].

Abnormal silencing of a tumour suppressor or a DNA repair gene through hypermethylation of their CpG promoter sites may cause cells to grow uncontrollably and lead to tumorigenesis. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis (UC)[20-23], another tumour arising as a result of chronic inflammation progressing through dysplasia and resulting in colonic carcinoma[24]. Intriguingly epigenetic change has been shown to occur early in this process before neoplasia has developed[25]. The Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C) trial is investigating whether a panel of methylated biomarkers detected in endoscopic biopsy samples can be used as a tool in conjunction with screening colonoscopy to help risk stratify patients who are at higher risk of progressing to carcinoma[26]. With the latest next generation sequencing and methylation microchip arrays it is possible to detect epigenetic changes accurately and reproducibly even in archival tissue samples. In light of this there is a need to consolidate the literature on epigenetic changes in Barrett's carcinogenesis to determine if such changes provide a method of risk stratifying patients who are at risk of progression to OADC.

A scoping search was performed using MEDLINE, the Cochrane Library and internet sources to identify any systematic reviews or meta-analyses on epigenetic biomarkers in BO and oesophageal cancer (OC). It revealed that there have been no systematic reviews which draw together all aspects of epigenetic change within the field of Barrett's carcinogenesis.

Nine systematic reviews and meta-analyses were identified[27-35] which included mixed patient populations with OADC and oesophageal squamous cell carcinoma (OSCC) with only 3 reviews incorporating patients with BO[30, 31, 35]. These studies concentrated on a single type of epigenetic alteration with 4 investigating DNA methylation[27-29] and 3 looking at miRNA expression[30-33]. The remaining 2 studies investigated genetic alterations in progression of BO to OADC[34, 35].

Research aims

This systematic review will identify and summarise studies which examine epigenetic biomarkers in BO and their association with progression to OADC with the aim of consolidating the literature and informing future laboratory work.

METHODS AND ANALYSIS

This systematic review protocol has been reported in accordance with PRISMA-P guidelines.

Selection criteria

Population All patients over the age of 18 with BO, BO with dysplasia, OADC or unspecified oesophageal cancer will be included. Oesophageal squamous cell carcinoma (OSCC) and established oesophageal cancers with no evidence of a pre-existing BO diagnosis will be excluded.

Study design All prospective and retrospective primary studies, and systematic reviews investigating epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types will be included. Case reports, narrative reviews, in vitro, studies of genetic mutations, studies using biomarkers to predict a response to treatment (eg. chemotherapy), cell line and animal studies will be excluded.

Publication type Abstract and full texts will be included with exclusion of letters and editorials.

Outcome Progression to dysplastic BO or OADC from non-dysplastic BO.

Search strategy

The following electronic bibliographic databases will be searched from inception: EMBASE, MEDLINE, MEDLINE in Process, DARE, CDSR, Cochrane Central. Conference (Conference Proceeding Citation Index, Zetoc) and grey literature databases (OpenGrey, Oaister) and registers of clinical trials (ClinicalTrials.gov and ICTRP) will also be searched. Reference lists of identified studies and systematic reviews will be screened for any relevant primary studies that were not retrieved from the database searches. Date or language restrictions will not be placed on searches. A search strategy will be developed using combinations of text and index words relating to the population, exposure and outcome, such as: "Barrett's Oesophagus", "epigenetic", "DNA methylation", "marker", "oesophageal adenocarcinoma". A sample search strategy for MEDLINE is shown in Appendix 1.

Study selection

This will be a two-step process. Titles and abstracts identified in our literature search will be screened independently by two reviewers using pre-specified screening criteria. These are broadly based on whether the studies firstly include measuring epigenetic markers in patients with OADC and secondly whether these patients have progressed from BO to OADC. Full texts of any potentially relevant articles will be obtained and subjected to the full inclusion criteria. Any discrepancies found will be referred to a third reviewer. The study selection process will be documented using the PRISMA flow diagram. Endnote X7 will be used as reference management software and decisions on inclusion or exclusion will be recorded.

Data extraction

Data will be extracted by two independent reviewers using an agreed, standard data extraction form. Any disagreements which cannot be resolved by discussion will be referred to a third reviewer who will act as arbitrator.

Data will be extracted on the following study characteristics

1. Study design characteristics – for example, prospective, case control, length of follow up, risk of bias and power calculations.
2. Population – for example, tissue samples from patients with BO or patients with OADC looking retrospectively at BO samples, patient demographics.
3. Exposure – Epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types.
4. Outcomes – Progression to OADC or BO with high grade dysplasia from non-dysplastic BO.

Assessment of study quality

The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool. This tool will review each individual study in six criteria: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding factors, and statistical analysis and reporting. We anticipate that due to the difficulty obtaining samples and the length of follow up required to assess progression from BO to OADC there may be significant sample selection bias. There is also likely to be large variation in the population demographics and comorbidity of patients which could act as confounding factors. Published guidelines recommend confirmation of high grade dysplasia by two independent pathologists[8]. These factors need to be assessed carefully for each study so that a judgement can be made on whether epigenetic changes seen in these studies are truly reflective of Barrett's carcinogenesis on a population level and whether they can be reproduced easily and accurately for screening purposes. We do not anticipate finding any studies that test models predicting progression based on patient factors and panels of epigenetic markers.

Evidence synthesis

A narrative synthesis of all evidence will be performed with key findings tabulated. An assessment of clinical and methodological heterogeneity will be undertaken in order to determine the feasibility of meta-analysis. The main sources of heterogeneity are likely to be sub-type of biomarker, study design, length of follow up, sampling interval and experimental technique and equipment used to demonstrate epigenetic change. Meta-analysis may be performed if there are multiple studies reporting on individual biomarker

types such as DNA methylation, histone methylation, histone acetylation, micro RNA and non-coding RNA providing the same outcomes (and outcome statistic) are reported. Results will most likely be presented as different risks of progression, e.g. relative risk (RR) of progression with and without the prognostic marker. Where studies have reported time to progression, hazard ratios will be extracted where possible.

Studies of different study design and those reporting adjusted or unadjusted results will be analysed separately. Relative risk of progression will be examined for non-dysplastic BO, BO with low grade dysplasia and BO with high grade dysplasia populations. Adjusted results, e.g. from multivariate analyses, are likely to be more informative in terms of the prognostic ability of a given marker in the context of other potential prognostic factors (such as clinical and lifestyle factors). Where meta-analyses are performed a random effects model will be more appropriate to account for between-study heterogeneity. Heterogeneity will also be measured statistically using the I^2 statistics and the χ^2 test. Publication bias will be assessed (by generating Funnel plots) only if greater than 10 studies are present in each meta-analysis. The strength of the overall body of evidence generated by the systematic review will be assessed using the GRADE approach (Grades of Recommendation, Assessment, Development and Evaluation Working Group) [36].

DISCUSSION

This systematic review will aim to comprehensively identify studies reporting on epigenetic changes in progressive BO. The results will help to inform future research on risk stratification and a personalised approach to endoscopic surveillance in patients with BO. The findings may inform future research into the optimisation of the Barrett's surveillance programmes using epigenetic markers as part of a multimodal screening tool.

ETHICS AND DISSEMINATION

This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

AUTHORS' CONTRIBUTIONS

TN, CLT, JD and OT conceived the systematic review protocol. TN, CLT, SB and JD undertook and reviewed scoping searches and contributed to the methodological development of the protocol with input from OT. TN drafted the initial manuscript and all authors (TN, CLT, JD, SB, MD, ADB, OT) were involved in its critical revision. All authors have given approval of the final version to be published. Review guarantor, OT.

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COMPETING INTERESTS

The authors do not have any competing or conflicts of interest.

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APPENDIX 1

Sample search strategy

Database: Ovid MEDLINE(R) <1946 to March Week 1 2016>

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1 Barrett Esophagus/

2 ((esophageal or oesophageal) adj3 (cancer or adenocarcinoma or carcinoma or neoplasm$)).ti,ab.

3 (intestinal adj (metaplas$ or dysplas$ or adenocarcinoma$)).ti,ab.

4 (Barrett$ adj1 (esophagus or oesophagus)).ti,ab.

5 (Barrett$ adj3 (adenocarcinoma$ or epithelium or dysplasia or carcinoma$ or cancer or neoplasm$)).ti,ab.

6 or/1-5

7 (methylation or hypermethylation).mp. or hypomethylation.ti,ab. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]

8 DNA methylation/

9 (histone adj2 (methylation or modification or acetylation)).ti,ab.

10 chromatin remodel$.ti,ab.

11 ((non-coding or untranslated) adj1 RNA).ti,ab.

12 (microRNA or siRNA or piRNA or sncRNA or lncRNA).ti,ab.

13 ((biomarker$ or marker$) adj2 (progress$ or predict$ or prognos$)).ti,ab.

14 (epigen$ adj2 (alteration$ or change or changes or marker$ or biomarker$)).ti,ab.

15 or/7-14

16 6 and 15

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Current manuscript
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1, line 2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 2, line 63
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1, line 4-9
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 10, line 284-288
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 10, line 291
Sponsor	5b	Provide name for the review funder and/or sponsor	Page 10, line 291
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Page 10, line 291
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Page 4-5, line 96-164
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 5, line 167-169
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 6, line 176-191
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 6, line 194-203
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Page 7, line 200-203, Appendix 1

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 7, line 206-219
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 7, line 206-214
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 7, line 217-219
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 7, line 221-229
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 7, line 191 Page 8, line 228-229
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 8, line 232-239
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 8-9, line 252-268
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Page 8-9, line 248-268
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 8-9, line 251-257
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 8, line 248
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 9, line 266-268
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 9, line 267-269

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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Keywords

Epigenetics, Barrett's, Adenocarcinoma, Systematic Review

2104 words

ABSTRACT

Introduction Barrett's Oesophagus (BO), a metaplastic condition affecting the lower oesophagus due to long standing gastro-oesophageal reflux and chronic inflammation, is a precursor lesion for oesophageal adenocarcinoma (OADC). There is no clinical test to predict which patients with BO will progress to OADC. The British Society of Gastroenterology recommend endoscopic surveillance of patients with BO. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis to colonic carcinoma, another gastrointestinal cancer associated with chronic inflammation. This systematic review protocol aims to identify and evaluate studies which examine epigenetic biomarkers in BO and their association with progression to OADC.

Methods and analysis All prospective and retrospective primary studies, and existing systematic reviews investigating epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types will be eligible for inclusion. Eligible patients are those over the age of 18 with BO, BO with dysplasia, OADC or unspecified oesophageal cancer. A comprehensive search of bibliographic databases using combinations of text and index words relating to the population, prognostic markers and outcome will be undertaken with no language restrictions. Results will be screened by 2 independent reviewers and data extracted using a standardised proforma. The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool. A narrative synthesis of all evidence will be performed with key findings tabulated. Meta-analysis will be considered where studies and reported outcomes are considered sufficiently homogenous, both clinically and methodologically. Findings will be interpreted in the context of the quality of included studies. The systematic review will be reported according to PRISMA guidelines.

Ethics and dissemination This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

Registration number International Prospective Register for Systematic Reviews (PROSPERO) number CRD42016038654

Strengths and limitations of this systematic review protocol

Strengths:

- Systematic review protocol following PRISMA-P guidelines, including description of key methodological steps
- Rationale for a new systematic review in this area based on scoping searches.
- Exhaustive search strategy likely to capture all relevant published literature on epigenetic markers for progression of BO and OADC.

Limitations:

- Heterogeneity of published research anticipated (differing epigenetic biomarkers studied, variation of study design, sampling methods and follow up length).
- Above may limit certain epigenetic markers to narrative evidence synthesis

INTRODUCTION

The incidence of oesophageal adenocarcinoma (OADC) has dramatically increased in recent years to 5.7 per 100,000 for females and 14.1 per 100,000 for males in the UK[1, 2]. Unfortunately the majority of patients present with advanced unresectable disease with an overall 5 year survival of less than 13%[3] . Five year survival rates improve considerably to 39% with localised disease[4]. Barrett's Oesophagus (BO), is defined as an oesophagus in

which any portion of the normal distal squamous epithelial lining is replaced by metaplastic columnar epithelium which is clearly visible endoscopically (≥1 cm) above the GOJ and confirmed histopathologically from oesophageal biopsies [5]. Barretts Oesophagus arises due to long standing gastro-oesophageal reflux disease (GORD) and chronic inflammation and is a precursor lesion for OADC with progression through the metaplasia-dysplasia-carcinoma sequence[6]. The likelihood of developing OADC is increased 1.7 times in patients with GORD, increasing to 10.6 times with BO[7]. The incidence of OADC has risen in parallel with increasing obesity and GORD in Western populations[1]. With rising rates of obesity the incidence of OADC is predicted to further increase[8]. Currently there is no robust way of predicting which patients with BO will progress to OADC. The current clinical biomarker for progression of BO is the presence of worsening cellular dysplasia, also known as intraepithelial neoplasia (IEN), on histological examination of serial oesophageal biopsies[5]. The presence of high grade dysplasia (HGD), and more recently low grade dysplasia (LGD), triggers intervention[9]. As a result, the British Society of Gastroenterology recommend endoscopic surveillance of patients with BO and the American College of Gastroenterology endorse screening of high-risk patients for BO[5, 10]. Endoscopic surveillance is invasive, expensive and despite rigorous biopsy protocols, dysplasia and early cancers can be missed. Importantly a recent meta-analysis published in 2012 demonstrated lower risk for progression of non-dysplastic BO than previously reported with a pooled 0.33% (95% CI 0.28–0.38%) annual incidence of OADC[11]. The annual incidence rate of OADC for patients with BO with HGD is 7-19%[12-14].

Epigenetics is an emerging field which describes mechanisms of alteration of gene regulation and expression without changing the genetic code[15]. These regulatory mechanisms are important in normal human development, for example silencing of the X-chromosome in females[16]. Epigenetic changes may be inherited but can also be acquired through environmental factors such as cigarette smoking[17]. Epigenetic change can occur through various methods. The most recognised are covalent modifications including DNA methylation, histone modification and altered gene expression by non-coding RNAs[15]. DNA methylation occurs when DNA methyltransferase adds a methyl group (CH_3) to a DNA base. In humans this is most commonly a cytosine base creating 5-methylcytosine[15].

Methylation which occurs at gene promoter (CpG) sites causes downregulation of these genes. It is thought that the mechanism responsible is the projection of a methyl group into the DNA groove which physically blocks transcription[18]. Histone modification is a post translational alteration to histone proteins which package DNA into nucleosomes and eventually chromosomes by winding DNA around them. If the histone structure is altered, the DNA cannot be correctly unravelled and cannot be correctly transcribed. The above modifications are carried over when a cell divides and can be inherited[19]. Many different types of non-coding RNAs have been discovered to alter gene expression by targeting coding messenger RNA (mRNA) after its transcription from DNA. Both micro RNAs (miRNA) and long non-coding RNAs have been implicated in gene regulation. These bind to mRNA molecules and cause them to be denatured and halt protein translation and cause genetic silencing[20].

Abnormal silencing of a tumour suppressor or a DNA repair gene through hypermethylation of their CpG promoter sites may cause cells to grow uncontrollably and lead to tumorigenesis. Epigenetic changes have been well characterised in the neoplastic progression of ulcerative colitis (UC)[21-24], another tumour arising as a result of chronic inflammation progressing through dysplasia and resulting in colonic carcinoma[25]. Intriguingly epigenetic change has been shown to occur early in this process before neoplasia has developed[26]. The Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C) trial is investigating whether a panel of methylated biomarkers detected in endoscopic biopsy samples can be used as a tool in conjunction with screening colonoscopy to help risk stratify patients who are at higher risk of progressing to carcinoma[27]. With the latest next generation sequencing and methylation microchip arrays it is possible to detect epigenetic changes accurately and reproducibly even in archival tissue samples. In light of this there is a need to consolidate the literature on epigenetic changes in Barrett's carcinogenesis to determine if such changes provide a method of risk stratifying patients who are at risk of progression to OADC.

A scoping search was performed using MEDLINE, the Cochrane Library and internet sources to identify any systematic reviews or meta-analyses on epigenetic biomarkers in BO and oesophageal cancer (OC). It revealed in excess of 2000 primary studies which are relevant

for inclusion into the proposed systematic review. No systematic reviews which draw together all aspects of epigenetic change within the field of Barrett's carcinogenesis were identified. Nine systematic reviews and meta-analyses were identified[28-36] which included mixed patient populations with OADC and oesophageal squamous cell carcinoma (OSCC) with only 3 reviews incorporating patients with BO[31, 32, 36]. These studies concentrated on a single type of epigenetic alteration with 4 investigating DNA methylation[28-30] and 3 looking at miRNA expression[31-34]. The remaining 2 studies investigated genetic alterations in progression of BO to OADC[35, 36]. Based on these results we believe that a systematic review on this topic is both timely and required.

Research aims

This systematic review will identify and summarise studies which examine epigenetic biomarkers in BO and their association with progression to OADC with the aim of consolidating the literature and informing future laboratory work.

METHODS AND ANALYSIS

This systematic review protocol has been reported in accordance with PRISMA-P guidelines.

Selection criteria

Population All patients over the age of 18 with BO, BO with dysplasia, OADC or unspecified oesophageal cancer will be included.

Prognostic markers Epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types will be included.

Outcome Progression from non-dysplastic BO with or without intestinal metaplasia to BO with LGD, HGD or OADC.

Study design All prospective and retrospective primary studies, and systematic reviews will be included.

Publication type Abstract and full texts will be included with exclusion of letters and editorials

Exclusion criteria: Oesophageal squamous cell carcinoma (OSCC) and established oesophageal cancers with no evidence of a pre-existing BO diagnosis will be excluded. Case reports, narrative reviews, in vitro studies (e.g. cell lines), studies of genetic mutations, studies using biomarkers to predict a response to treatment (e.g. chemotherapy) will be excluded. A decision was made to exclude animal studies, as scoping searches indicated that there were comparatively few (compared to human studies), and therefore were likely to add heterogeneity to an already heterogeneous evidence base. In addition we concluded that issues relating to transferability of experimental findings from animal models to a clinical setting would occur.

Search strategy

The following electronic bibliographic databases will be searched from inception: EMBASE, MEDLINE, MEDLINE in Process, DARE, CDSR, Cochrane Central. Conference (Conference Proceeding Citation Index, Zetoc) and registers of clinical trials (ClinicalTrials.gov and ICTRP) will also be searched. Reference lists of identified studies and systematic reviews will be screened for any relevant primary studies that were not retrieved from the database searches. Date or language restrictions will not be placed on searches. A search strategy will be developed using combinations of text and index words relating to the population, exposure and outcome, such as: “Barrett’s Oesophagus”, “epigenetic”, “DNA methylation”, “marker” and “oesophageal adenocarcinoma”. A sample search strategy for MEDLINE is shown in Appendix 1.

Study selection

This will be a two-step process. Titles and abstracts identified in our literature search will be screened independently by two reviewers using pre-specified screening criteria. These are broadly based on whether the studies firstly include measuring epigenetic markers in patients with OADC and secondly whether these patients have progressed from BO to OADC. Full texts of any potentially relevant articles will be obtained and subjected to the full inclusion criteria. Any discrepancies found will be referred to a third reviewer. The study selection process will be documented using the PRISMA flow diagram. Endnote X7 will be

used as reference management software and decisions on inclusion or exclusion will be recorded.

Data extraction

Data will be extracted by two independent reviewers using an agreed, standard data extraction form. Any disagreements which cannot be resolved by discussion will be referred to a third reviewer who will act as arbitrator.

Data will be extracted on the following study characteristics

1. Study design characteristics – for example, prospective or retrospective and length of follow up
2. Population – for example, tissue samples from patients with BO or patients with OADC looking retrospectively at BO samples, patient demographics.
3. Prognostic markers – Epigenetic markers including DNA methylation, histone modification, chromatin remodelling, micro and non-coding RNAs of all types.
4. Outcomes – Progression from non-dysplastic BO with or without intestinal metaplasia to BO with LGD, HGD or OADC.

Assessment of study quality

The quality and risk of bias of individual studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool[37]. This tool will review each individual study in six criteria: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding factors, and statistical analysis and reporting. We anticipate that due to the difficulty obtaining samples and the length of follow up required to assess progression from BO to OADC there may be significant sample selection bias. Eligible studies are likely to be subject to confounding, with main confounding factors relating to age, obesity, smoking and alcohol intake. The risk of bias assessment will therefore include an assessment of which confounding factors (if any) have been measured and whether they were adjusted for in the design or analysis of the study. There may be differences in how robust the methods are for measuring the prognostic markers and the outcome; for example, published guidelines recommend confirmation of high grade dysplasia by two independent pathologists[5]. These factors need to be assessed carefully for each study so that a

judgement can be made on whether epigenetic changes seen in these studies are truly reflective of Barrett's carcinogenesis on a population level and whether they can be reproduced easily and accurately for screening purposes. We do not anticipate finding any studies that test models predicting progression based on patient factors and panels of epigenetic markers.

Evidence synthesis

A narrative synthesis of all evidence will be performed with key findings tabulated. An assessment of clinical and methodological heterogeneity will be undertaken in order to determine the feasibility of meta-analysis. The main sources of heterogeneity are likely to be sub-type of biomarker, study design, length of follow up, sampling interval and experimental technique and equipment used to demonstrate epigenetic change. Meta-analysis may be performed if there are multiple studies reporting on individual biomarker types such as DNA methylation, histone methylation, histone acetylation, micro RNA and non-coding RNA providing the same outcomes (and outcome statistic) are reported. Results will most likely be presented as different risks of progression, e.g. relative risk (RR) of progression with and without the prognostic marker. Where studies have reported time to progression, hazard ratios will be extracted where possible.

Studies of different study design and those reporting adjusted or unadjusted results will be analysed separately. Relative risk of progression from non-dysplastic BO to BO with LGD, HGD or OADC will be calculated where possible. Adjusted results, e.g. from multivariate analyses, are likely to be more informative in terms of the prognostic ability of a given marker in the context of other potential prognostic factors (such as clinical and lifestyle factors). Where meta-analyses are performed a random effects model will be more appropriate to account for between-study heterogeneity. Heterogeneity will also be measured statistically using the I^2 statistics and the χ^2 test. Publication bias will be assessed (by generating Funnel plots) only if greater than 10 studies are present in each meta-analysis. The strength of the overall body of evidence generated by the systematic review will be assessed using the GRADE approach (Grades of Recommendation, Assessment, Development and Evaluation Working Group) [38]. The full systematic review will be reported according to PRISMA guidelines[39].

DISCUSSION

This systematic review will aim to comprehensively identify studies reporting on epigenetic changes in progressive BO. The results will help to inform future research on risk stratification and a personalised approach to endoscopic surveillance in patients with BO. The findings may inform future research into the optimisation of the Barrett's surveillance programmes using epigenetic markers as part of a multimodal screening tool.

ETHICS AND DISSEMINATION

This is a systematic review of completed studies and no ethical approval is required. Findings from the full systematic review will be submitted for publication and presentation at national and international conferences which will inform future research on risk stratification in patients with BO.

AUTHORS' CONTRIBUTIONS

TN, CLT, JD and OT conceived the systematic review protocol. TN, CLT, SB and JD undertook and reviewed scoping searches and contributed to the methodological development of the protocol with input from OT. TN drafted the initial manuscript and all authors (TN, CLT, JD, SB, MD, ADB, OT) were involved in its critical revision. All authors have given approval of the final version to be published. Review guarantor, OT.

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COMPETING INTERESTS

The authors do not have any competing or conflicts of interest.

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APPENDIX 1

Sample search strategy

Database: Ovid MEDLINE(R) <1946 to March Week 1 2016>

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1 Barrett Esophagus/

2 ((esophageal or oesophageal) adj3 (cancer or adenocarcinoma or carcinoma or neoplasm$)).ti,ab.

3 (metaplas$ adj (intestinal or columnar or dysplas$ or adenocarcinoma$)).ti,ab.

4 (Barrett$ adj1 (esophagus or oesophagus)).ti,ab.

5 (Barrett$ adj3 (adenocarcinoma$ or intra-epitheli$ or epitheli$ or dysplasia or carcinoma$ or cancer or neoplas$)).ti,ab.

6 or/1-5

7 (methylation or hypermethylation).mp. or hypomethylation.ti,ab. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]

8 DNA methylation/

9 (histone adj2 (methylation or modification or acetylation)).ti,ab.

10 chromatin remodel$.ti,ab.

11 ((non-coding or untranslated) adj1 RNA).ti,ab.

12 (microRNA or siRNA or piRNA or sncRNA or lncRNA).ti,ab.

13 ((biomarker$ or marker$) adj2 (progress$ or predict$ or prognos$)).ti,ab.

14 (epigen$ adj2 (alteration$ or change or changes or marker$ or biomarker$)).ti,ab.

16 or/7-14

17 6 and 14

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Current manuscript
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1, line 2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 2, line 63
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1, line 4-9
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 10, line 284-288
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 10, line 291
Sponsor	5b	Provide name for the review funder and/or sponsor	Page 10, line 291
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Page 10, line 291
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Page 4-5, line 96-164
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 5, line 167-169
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 6, line 176-191
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 6, line 194-203
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Page 7, line 200-203, Appendix 1

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 7, line 206-219
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 7, line 206-214
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 7, line 217-219
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 7, line 221-229
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 7, line 191 Page 8, line 228-229
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 8, line 232-239
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 8-9, line 252-268
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Page 8-9, line 248-268
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 8-9, line 251-257
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 8, line 248
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 9, line 266-268
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 9, line 267-269

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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