A Ruibal[1-3], P Aguiar[1,2], P Menéndez[4], J I Arias[5], A Gonzalez-Sistal[6] and M Herranz[1,7]*

[1]Molecular Imaging Group, Department of Radiology, Faculty of Medicine, Universidade de Santiago Compostela (USC), IDIS, Spain
[2]Nuclear Medicine Service, University Hospital Santiago Compostela (CHUS), Spain
[3]Fundación Tejerina, Madrid, Spain
[4]Pathology Service, University Hospital, Oviedo, Spain
[5]Surgery Service, Hospital Monte del Naranco, Oviedo, Spain
[6]Department of Physiology II, Faculty of Medicine, Universidad de Barcelona, Spain
[7]Galaria, Sergas, IDIS, Santiago de Compostela, Spain

Research Article

Smoking Habit and Clinico-Biological Parameters of Breast Cancer

Abstract

Introduction: The aim of this study was to analyse the possible associations between smoking habit and some clinico-biological parameters of breast infiltrating ductal carcinomas (IDCs).

Material and Methods: The study group included included 291 females with IDC who had undergone no prior treatment. Out of them, 48 were current smoking, 11 quit smoking and 232 never smoking. All were studied at the same Breast Cancer Unit. Age, tumor size, axillary lymph node involvement (N), distant metastasis (M) and histological grade (HG), as well as the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), Ki67, p53, bcl2 and androgen receptor (AR) were analyzed Also, we dose the serum levels of CEA and CA15.3. We can follow up 276 patients during a period of time which ranged between 8 and 240 months (83,3+/-51,8; median 84 months).

Results: IDCs of smoking habit subgroup had lower age than nonsmoking habit subgroup and were more frequently bcl-2+ (p 0,083). There were not differences in the other clinico-biological parameters as well as in the follow-up considering recurrences and deaths due to the tumor. Likewise, age of ex-smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in nonsmoking habit subgroup (p: 0,003). Smokers women showed more frequently history of contraceptives intake (26,4% vs 7,8% ; p<0,001). When we considered the molecular subtypes of IDCs, we not observed any statistically difference; nevertheless, when we analyzed current and quit versus never smoking patients, , we noted than luminal B subtype was less frequent in those (p: 0,068) and the tumors were more frequent bcl-2 positive (p:0,048) than never smoking subgroup.

Conclusion: Our results led us to the following: 1) Women with IDCs and smoking habit had lower age and the tumors were more frequently bcl2 positive than never smoking habit; 2) age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in nonsmoking habit subgroup ; 3) in smoking/ex-smoking women luminal B molecular subtype was less frequent than in nonsmoking women.

Introduction

Breast cancer is the most common tumor in women in the Eastern world. Their different incidence among different geographic areas, suggests that certain environmental or lifestyle factors may be involved in its etiology. Among the known risk factors that can be controlled include the smoking habit, weight, diet, exercise, alcohol consumption, exposure to estrogen, recent oral contraceptive use, stress and anxiety. In this regard, it is believed that modifying certain lifestyle (smoking, body mass index (BMI), alcohol consumption, fruit and vegetable consumption, and physical activity) could reduce a significant number of tumors, highlighting the 6.3% (0.5-12.1%) of postmenopausal breast cancer [1].

The smoking habit has been subject of intense study since its modifiable factor character. We know that there is a higher risk of benign tumors, preferably fibroadenomas, in current smokers, but without statistically significant [2,3] and that tobacco smoke has been implicated in various human disease conditions and the International Agency for Research on Cancer identified tobacco smoking as the cause of cancer at more organ sites that any other human carcinogen [4]. In relation to breast cancer, some groups have denied such a relationship [5-9]; others have shown a positive discreet relationship especially in premenopausal women [10-13], others an inverse relationship [14,15], and finally some suggest an increased risk associated with time, quantity and age of onset of the habit [12-16]. Xue et al. [17] observed that breast cancer incidence was associated with a higher quantity of current and past smoking, younger age at smoking initiation, longer duration of smoking and more pack-years of smoking. Premenopausal smoking was associated with a slightly higher incidence, while

postmenopausal smoking was inversely correlating with the breast cancer risk. Braithwaite et al. [18] noted that women who were current smokers had a two-fold higher rate of dying from breast cancer and an approximately four-fold higher rate of dying from non-breast causes. Daniell et al. [19] have observed that breast tumors with four or more positive nodes were more frequently associated with larger primary tumors, obesity and smoking habit. Likewise, they found little evidence of an association between former smoking and breast cancer mortality.

Illic et al (20) have found that the breast cancer risk was significantly increased in those who quit smoking at ≤50 years of age and in those who quit smoking less than 5 years before diagnosis of the disease. Pierce JP et al. [21] compared with never smokers, former smokers with less than 20 pack-years of exposure had no increased risk of any outcome. However, former smokers with 20 to less than 34,9 pack-years of exposure had a 22% increased risk of breast cancer recurrence. For former smokers with 35 or more pack-years of exposure, the probability of recurrence increased by 37% and breast cancer mortality increased by 54%. Current smoking increased the probability of recurrence by 41% and increased breast cancer mortality by 60%. In meta-analyses, current and former smoking were weakly associated with risk while a stronger association was observed in women who initiated smoking before first birth [22].

Regarding passive smoking, it has been seen that there exist significant interactions between certain types of passive smoke exposure and genetic variants in CYP2E1, NAT2 and UGT1A7 [23], and that those have higher risk, especially if it comes to premenopausal women [24,25].

In last years, prognostic value of preoperative CEA and CA15-3 levels in breast cancer has more important. Study has shown that levels of CEA combined with CA15-3 give us important information for diagnosis and treatment of breast cancer [26]. Accordingly, the European Group on Tumor Markers has recommended the CEA and CA15-3 levels be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer [27].

In this study we aimed to analyze several factors, such as the histological grade (HG), ER status, PgR status, Ki67 (proliferation marker), p53, bcl2 and AR in order to identify biological characteristics of breast carcinomas in smokers, ex-smoker, and non-smoking women. Smokers, ex-smoker, and non-smoking women.

Material and Methods

Our study group included 291 females with Infiltrating Ductal Carcinomas (IDC) who had undergone no prior treatment. Out of them, 48 were current smoking, 11 quit smoking and 232 never smoking. Everyone were studied at the same Breast Cancer Unit. Age, tumor size, axillary lymph node involvement (N), distant metastasis (M) and histological grade (HG), as well the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), Ki67,

p53, bcl2 and androgen receptor (AR) were analyzed. Serum carcinoembryonic antigen (CEA) and CA15.3 were determined by an electrochemiluminescence immunoassay (ECLIA) from Roche (Swiss), and an electro-chemo luminescence assay (ECLIA- Elecsys 170 Roche) respectively. We can follow up 276 patients during a period of time which ranged between 8 and 240 months (83,3+/-51,8; median 84 months).

Immunohistochemical staining on tissue sections of 4-5 microns was done by the EnVision method with a heat-induced antigen retrieval step. Sections were immersed in boiling 10 mmol/l sodium citrate at pH 6.5 for 2 minutes in a pressure cooker. ER and PgR were determined by mAbs ER/PgR phramDx (clones 1D5 (0,1mg/mL) and ER-2123 (0,5 mg/ml) for ER and PgR) 1294 for the PgR. P53 (DO-7, Dako, (335 mg/L) dilution 1/50), Ki67 (MIB-1, Dako, (80mg/L) dilute 1/200), bcl2 (Biogenex (10mg/ml), dilution 1/150) and Androgen receptor (AR441, Dako, dilution 1/150) were used in this study. Dako goat polyclonal biotinylated secondary antibody was used. The ER and PgR were assessed according to the Allred score in negative (scores 0-2) and positive (score 3-8) and the thresholds of positivity for p53, Ki67 were 20% and 15 % respectively. AR were classified as positive or negative without any score, and bcl2 as negative (-: <10% stained cells), weakly positive (+: 10-20%) and strong positive (++: >30%).

Data obtained were evaluated using the SPSS 15.0 software for Windows (SPSS, Chicago, IL. USA). Parameters that did not follow a normal distribution, values were presented as range, 25th percentile, 75th percentile and median. We used the Chi square test with Yates correction, if necessary, for qualitative variables comparison and the *Mann Whitney* test for continuous ones. A p-value ≤ 0.05 was considered as statistically significant.

Results

As shown in Table 1, the IDCs of smoking habit subgroup had lower age than non-smoking habit subgroup (55,0 +/- 6,6 *vs* 60,7 +/- 8,0; p. <0,00001) and were more frequently bcl-2+ (40/45 vs 161/208) near statistical significance (p=0,083). In the other parameters we do not find statistically significant differences, nor in the subsequent outcome considering recurrences and/or deaths from the tumor. Likewise, age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in non-smoking habit subgroup (54,7 +/- 5,3 *vs* 60,7 +/- 8,0; p=0,003). Smokers women showed more frequency background of contraceptives intake (26,4% *vs* 7,8%; p=<0,001. Data not shown). When considering molecular types of IDCs, we found no statistically significant differences between different subtypes; however, by grouping smokers and ex-smokers women, we found that luminal B subtype (ER+ and PgR+/-, HER2-, Ki67 >/=14%) was less frequent in this patient group compared to women who did not smoke (7/50 *vs* 53/202; p= 0,068) (Table 2).

When comparing non-smokers subgroup against active and former smokers, we found, as shown in Table 3, that the different immunohistochemical expression of bcl-2 reached

statistical significance (p=0,048), being lower in non-smoking habit subgroup. We did not observe differences in the other parameters analyzed among smokers and non-smokers women.

Discussion

Tobacco causes approximately 25% of all cancers in men and 4% in women and has been associated with increased mortality following diagnosis of a variety of cancers as prostate, colorectal, leukaemia, malignant melanoma, …etc [18]. Likewise, in women over the age of 50, smoking (>15 cigarettes either currently or in the past) was the only factor associated with cancer (breast 31,9% or colorectal 12,7%) [12]. Several reports have studied smoking habit as a risk factor for breast cancer and the results have been inconclusive. Currently it seems accepted that breast cancer incidence is associated

Table 1: Distribution of different parameters in the subgroups of patients included in the study.

PARAMETER	SH	Non-SH	Ex-SH
Age	41-68 (55.0+/-6,6)	37-88 (60,7+/-8,0)	46-64 (54,7+/-5,3)
Size	0,3-7,0 (1,7+/-1,2)	0,2-8 (1,5+/-0,9)	0,2-3,0 (1,2+/-0,7)
CEA	0,1-8 (1,5)	0,1-13 (0,5)	0,1-1,8 (0,9) ng/ml
CA15.3	1-33,9 (19,3)	0,1-162 (17,2)	9,2-28 (20,1) U/ml
N+	14/48	60/232	02/11
N+>3	4/48	12/232	0/11
N+>10	1/48	2/232	0/11
M+	1/48	5/232	0/11
HG3	12/48	63/232	04/11
ER+	42/48	192/232	08/11
PgR+	33/48	142/232	07/11
AR+	39/45	160/200	08/11
P53+	7/48	47/232	04/11
Bcl-2+	40/45	161/208	10/11
Ki67+	23/48	120/232	03/11

Abbreviations: SH: Smoking Habit; N: axillary lymph Node involvement; M: distant Metastasis; HG: Histological Grade; ER: Estrogen Receptor; PgR: Progesterone Receptor and AR: Androgen Receptor.
Statistically significant differences: Age SH vs Non-SH: p<0,00001; Age Non SH vs Ex-SH: p=0,003; bcl-2 SH vs Non-SH: p= 0,083; CEA SH vs CEA ex-SH; p= 0,004.

Table 2: Molecular subtypes and smoking habit (SH)

TYPE	SH	Non-SH	p
Luminal A	27/39	117/202	ns
Luminal B	6/39	52/202	ns
Triple Negative	3/39	16/202	ns
Her2+	3/39	16/202	ns
TYPE	SH+Ex-SH	11/SH	p
Luminal A	34/50	117/202	ns
Luminal B	7/50	53/202	0,068
Triple Negative	4/50	16/202	ns
Her2+	5/50	16/202	ns

Abbreviation: **SH**: Smoking Habit.

Table 3: Clinical and biological differences between current and ex smoking habit (SH) and non-smoking habit groups.

PARAMETER	SH+Ex SH	Non SH	p
N+	16/59	60/232	ns
N+>3	4/59	12/232	ns
N+>10	1/59	2/232	ns
M+	1/59	5/232	ns
HG3	16/59	63/232	ns
ER+	50/59	192/232	ns
PgR+	40/59	142/232	ns
AR+	47/59	160/200	ns
P53+	11/59	47/232	ns
Bcl-2+	50/56	161/208	0,048
Ki67+	26/59	120/232	ns
CK5/6	2/30	5/57	ns
Recurrences	1/55	6/221	ns
Deaths	0/55	1/220	ns

Abbreviations: SH: Smoking Habit; N: axillary lymph Node involvement; M: distant Metastasis; HG: Histological Grade; ER: Estrogen Receptor; PgR: Progesterone Receptor and AR: Androgen Receptor.

with a higher quantity of current and past smoking, younger age at smoking initiation, longer duration of smoking and more pack-years of smoking [17].

We know that the phenotypical alterations induced by cigarette smoke are accompanied by numerous changes in gene expression that are associated with epithelial to mesenchymal transition and tumorigenesis [28]. Recent studies evaluating the possible modifying role of polymorphisms in genes involved in the metabolism of tobacco products, particularly NAT2, have contributed another dimension to these assessments, although to date that evidence remains equivocal [29-32]. Likewise, other genes related with inflammation, DNA repair, apoptosis, signal transduction, metabolism, cell cycle, cell proliferation and transcription related genes are involved also. Carcinogens in tobacco pass through the alveolar membrane and enter the bloodstream transported to mammary tissue through plasma lipoproteins. Furthermore, because these breast carcinogens are lipophilic, they may be stored in breast adipose tissue and metabolized and activated by mammary epithelial cells. Also, in breast cancer patients with smoking habit there is a significant rise in oxidative stress and low levels of antioxidants. Free radicals facilitate the progression of breast cancer [33]. Smoking affects circulating hormone levels [33]; so, the current smoking and increasing amount of daily smoking is associated with high testosterone levels [34] and postmenopausal current smokers had lower IGF-1 and IGFBP-3 levels [35]. Salem et al. [36], have observed that cigarette smoke exposure is indeed sufficient to drive the onset of the cancer-associated fibroblast phenotype via the induction of DNA damage, autophagy and mitophagy in the tumor stroma. These fibroblasts increased breast cancer tumor growth in vivo up to 4-fold. When comparing smokers and non-smokers women subgroups, we found that those showed breast carcinomas a much younger age (55 vs 60,7 years), confirming highly statistically significant differences.

The same fact was observed among former and non-smokers females, which leads to believe that smoking (current or former) is associated with mammary tumors at earlier ages.

Clinically and biogically, mammary tumors did not differ between the two groups (smokers and non-smokers patients), except in the immunohistochemical expression of bcl-2, which was more frequent in the smokers group near statistical significance. By grouping non-smokers patients compared to active and ex-smokers, we found that the different immunohistochemical expression of bcl-2 reached statistical significance (p=0.048), being lower in non-smoking habit subgroup, all of which support the positive association between the expression of bcl-2 and smoking habit, whether active or not.

The bcl2 gen encodes for a mitochondrial protein which prevents apoptosis and prolongs cell survival, thus antagonizing the effects of the p53 protein [37,38]. This suggest the possibility that increased expression of bcl-2 in mammary tumors from smokers' women determine a worse prognosis by preventing apoptosis [39] or be accompanied by a better prognosis as have been shown in these malignant neoplasms [40]. It could also be that defined a poorer response to certain chemotherapy as demonstrated Samanta et al. [41] in non-small cell lung cancer. Cucima y cols. [42] have observed that nicotine, contained in cigarette smoking, stimulates cell proliferation and suppress physiological apoptosis in colon cancer cells. Nicotine induced a statistically significant increase in the expression of PIK3 and P-Akt/Akt ratio as well as in the expression of PKC, ERK1/2, surviving and P-bcl2 (Ser70; 70 phosphorylated bcl-2) in colon cancer cells. These findings support that we detect higher expression of bcl-2 in smokers' women group.

Many apoptosis related genes are deregulated with smoking habit. One is the bcl2 associated athanogene (BAG1), novel cytoplasmic binding partner membrane form of heparin-binding EGF-like growth factor [43], which is down-regulated. BAG1 is a membrane protein that blocks a step in a pathway leading to apoptosis or programmed cell death. The protein encoded by this gene binds to bcl2 and enhances the anti-apoptotic effects representing a link between growth factor receptors and anti-apoptotic mechanisms. The protein was found at high levels in several types of human tumor cell lines among leukaemia, prostate, breast, etc. Likewise, high levels have been implicated as a prognostic indicator in breast cancer [44]. Also, BAG1 is implicated, among several other functions, in the response to nicotine. Over-expression of BAG1 significantly inhibited p53 induced growth arrest in some tumor cell lines [44] and this is consistent with our findings, since from the 40 cases bcl-2+ in the smoker group, 36 were p53 negative, not appreciating differences in the number of cases with lymph node involvement, distant metastasis, histological grade and cell proliferation (Ki67) between the two subsets of tumors. Some authors shows transformed phenotype in normal breast epithelial cell line (MCF10A) associated with Bcl-xL mRNA increase in a dose-dependent manner whereas mRNA level of Bcl-2 remained unchanged [45], this Bcl-xL increase is regulated by C/EBPbeta in MCF10A cells in response to cigarette smoke condensate (CSC) treatment suggesting this as a potential target for chemotherapy [46].

Considering the molecular types of IDCs [47], we observed that the luminal B subtype was less frequent in the group of smokers (6/39 vs 52/202), near statistical significance (p=0,099), relationship increased when compared non-smoker subgroup with active and former smokers subgroups together (7/50 vs 53/202; p=0,068). Although several groups have described that some of the substances found in cigarette act like estrogens, smoking has been postulated to have anti-estrogenic effect and this would explain our findings also that smoking is associated with an increased occurrence of hormone-receptor negative tumors [48], especially when the patients starting to smoke at an early age of </= 19 years [49,50].

Finally, we observed higher concentrations of serum CEA in smokers (See Table 1), an association known for years [51] and while not in serum CA15.3.

Our results led us to the following: 1) Women with IDCs and smoking habit had lower age and the tumors were more frequently bcl2 positive than never smoking habit; 2) age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in non-smoking habit subgroup; 3) in smoking/ex-smoking women *vs* non-smoking women, luminal B molecular subtype was less frequent than in non-smoking women.

Acknowledgements

References

1. Dartois L, Fagherazzi G, Boutron-Ruault MC, Mesrine S, Clavel-Chapelon F (2014) Association between five lifestyle habits and cancer risk: Results from the E3N cohort. Cancer Prev Res (Phila) 7: 516-525. Link: https://goo.gl/ec37xt

2. Parazzini E, Ferraroni M, La Vecchia C, Baron JA, Levi F, et al. (1991) Smoking habits and risk of benign breast disease. Int J Epidemiol 20: 430-434. Link: https://goo.gl/XRM2ZY

3. Dziewulska-Bokiniec A (1995) Smoking habit and benign breast disease. Neoplasma 42: 285-287. Link: https://goo.gl/o27Az4

4. Sohn SH, Kim IK, Kim HM, Kim HW, Seo SH, Lee SH, et al. (2006) Biological effects of smoking-induced environmental toxicity. Molecular & Cellular Toxicity 2: 202-211. Link: https://goo.gl/9NHoVn

5. Baron JA, Newcomb PA, Longnecker MP, Mittendorf R, Storer BE, et al. (1996) Cigarette smoking and breast cancer. Cancer Epidemiol Biomarkers Prev 5: 399-403. Link: https://goo.gl/BQsNFM

6. Engeland A, Andersen A, Haldorsen T, Tretli S (1996): Smoking habits and risk of cancers other than lung cancer: 28 years' follow-up of 26,000 Norwegian men and women. Cancer Causes Control 7: 497-506. Link: https://goo.gl/R94bxQ

7. Nordlund LA, Cartensen JM, Pershagen G (1997) Cancer incidence in female smokers: a 26 –year follow-up. Int J cancer 27: 625-628. Link: https://goo.gl/5oN7A0

8. Adami HO, Lund E, Bergström R, Meirik O (1988) Cigarette smoking, alcohol consumption and risk of breast cancer in young women. Br J Cancer 58: 832-837. Link: https://goo.gl/9Mpn9r

9. London SJ, Colditz GA, Stampfer MJ, Willett WC, Rosner BA, et al. (1989) Prospective study of smoking and the risk of breast cancer. J Natl Cancer Inst 81; 1625-1631. Link: https://goo.gl/k6PbR5

10. Brownson RC, Blacwell CW, Pearson DK, Reyneold RD, Richens JW, et al. (1988) Risk of breast cancer in relation to cigarette smoking. Arch Intern Med 148: 140-144. Link: https://goo.gl/yfHqZG

11. Lee SM, Park JH, Park HJ (2008) Breast cancer risk factors in Korean women; a literature review. Int Nurs Rev 55: 355-359. Link: https://goo.gl/CCEkDB

12. Baccaro LF, Conde DM, Costa-Paiva L, de Souza Santos Machado V, Pinto-Neto A (2015) Cancer in women over 50 years of age: a focus on smoking. Cancer (Basel) 7: 450-459. Link: https://goo.gl/U2jDRM

13. Kakugawa Y, Kawai M, Nishino Y, Fukamachi K, Ishida T, et al. (2015) Smoking and survival after breast cancer diagnosis in Japanese women: a prospective cohort study. Cancer Sci 106: 1066-1074. Link: https://goo.gl/NDg7yw

14. Hiatt RA, Fireman BH (1986) Smoking, menopause, and breast cancer. J Natl Cancer Inst 76(5): 883-888. Link: https://goo.gl/spQqWl

15. Gannon AM, Stämpfli MR, Foster WG (2013) Cigarette smoke exposure elicits increased autophagy and dysregulation of mitochondrial dynamics in murine granulosa cells. Biol Reprod 88: 63. Link: https://goo.gl/OMDMtJ

16. Reynolds P (2013) Smoking and breast cancer. J Mammary Gland Biol Neoplasia 18: 15-23. Link: https://goo.gl/je27ul

17. Xue F, Willet WC, Risner BA, Hankinson SE, Michels KB (2011) Cigarette smoking and the incidence of breast cancer. Arch Intern Med 171: 125-133. Link: https://goo.gl/HAP96L

18. Briathwaite D, Izano M, Moore DH, Kwan ML, Tammemagi MC, et al. (2012) Smoking and survival after breast cancer diagnosis: a prospective observational study and systematic review. Breast Cancer Res 136: 521-533. Link: https://goo.gl/9D7Lj7

19. Daniell HW (1988) Increased lymph node metastases at mastectomy for breast cancer associated with host obesity, cigarette smoking, age and large tumor size. Cancer 62: 429-435. Link: https://goo.gl/VUZRTt

20. Ilic M, Vlajinac H, Marinkovic J (2014) Cigarette Smoking and Breast Cancer: a Case-control Study in Serbia Asian Pac J Cancer Prev 14 (11): 6643-6647. Link: https://goo.gl/vSq9XS

21. Pierce JF, Patterson RE, Senger CM, Flatt SW, Caan BJ, et al. (2014) Lifetime cigarette smoking and breast cancer prognosis in the After Breast Cancer Pooling Project. J Natl Cancer Inst 106: 359. Link: https://goo.gl/gCTU4H

22. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, at el. (2013) Active smoking and breast cancer risk: original cohort data and meta-analysis. J Natl Cancer Inst 105: 515-525. Link: https://goo.gl/t8rTt7

23. Anderson LN, Cotterchio M, Mirea L, Ozcelik H, Kreiger N (2012) Passive cigarette smoke exposure during various periods of life, genetic variants, and breast cancer risk among never smokers. Am J Epidemiol 175: 289-301 Link: https://goo.gl/iwbMvv

24. Khuder SA, Simon VJ (2000) Is there an association between passive smoking and breast cancer? Eur J Epidemiol 16: 1117-1121. Link: https://goo.gl/Vp5bGm

25. Luo J, Margolis KL, Wactawski-Wende J, Horn K, Messina C, et al. (2011) Association of active and passive smoking with risk of breast cancer among postmenopausal women: a prospective cohort study. BMJ 342:d1016. Link: https://goo.gl/bqVbyL

26. Lee JS, Park S, Park JM, Cho JH, Kim SI, et al. (2013) Elevated levels of preoperative CA 15–3 and CEA serum levels have independently poor prognostic significance in breast cancer. Ann Oncol 24: 1225–1231 Link: https://goo.gl/AC5Q4f

27. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, et al. (2005) Tumor markers in breast cancer- European Group on Tumor Markers recommendations. Tumour Biol 26: 281–293 Link: https://goo.gl/vG5Ja2

28. Di Cello F, Flowers VL, Li H, Vecchio-Pagán B, Gordon B, et al. (2013) Cigarette smoke induces epithelial to mesenchymal transition and increases the metastatic ability of breast cancer cells. Mol Cancer 12: 90. Link: https://goo.gl/2sB3zD

29. Sohn SH, Kim KN, Kim IK, Lee EI, Ryu JJ, et al. (2008) Effects of tobacco compounds on gene expression in fetal lung fibroblasts. Environ Toxicol 23: 423-434. Link: https://goo.gl/X1WSLm

30. Sohn SH, Lee J, Kim KN, Kim IK, Kim MK (2009) Effect of tobacco compounds on gene expression profiles in human epithelial cells. Environ Toxicol Pharmacol 27: 111-119. Link: https://goo.gl/JYBo1m

31. Zheng W, Deitz AC, Campbell DR, Wen WQ, Cerhan JR, et al. (1999) N-acetyltransferase 1 genetic polymorphism, cigarette smoking, well done meat intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8: 233-239. Link: https://goo.gl/cG7tqm

32. Colilla S, Kantoff PW, Neuhausen SL, Godwin AK, Daly MB, et al. (2006) The joint effect of smoking and AIB1 on breast cancer risk in BRCA1 mutation carriers. Carcinogenesis 27: 599-605. Link: https://goo.gl/dEMSU7

33. Nagamma T, Baxi J, Singh PP (2014) Status of oxidative stress and antioxidant levels in smokers with breast cancer from western Nepal. Asian Pac J Cancer Prev 15: 9467-9470. Link: https://goo.gl/pJ6dce

34. Manjer J, Johansson R, Lenner P (2005) Smoking as a determinant for plasma levels of testosterone, androstendione, and DHEAs in postmenopausal women. Eur J Epidemiol 20: 331-337. Link: https://goo.gl/WaBXhm

35. Barnes BB, Chang-Claude J, Flesch-Janys D, Kinscherf R, Schmidt M, et al. (2009) Cancer risk factors associated with insulin-like growth factor (IGF-1) and IGF-binding protein 3 levels in healthy women: effect modification by menopausal status. Cancer Causes Control 20: 1985-1996. Link: https://goo.gl/idZPS2

36. Salem AF, Al-Zoubi MS, Whitaker-Menezes D, Martinez-Outschoorn IE, Lamb R, et al. (2013) Cigarette smoke metabolically promotes cancer, via autophagy and premature aging in the host stromal microenvironment. Cell Cycle 12: 818-825. Link: https://goo.gl/Ar6f82

37. Pacchioni D, Martone T, Ghisolfi G, Bussolatti G, Tizzani A, et al. (1997) Tobacco smoke, recurrences, and p53/bcl2 expression in bladder cancer. Carcinogenesis 18: 1659-1661. Link: https://goo.gl/qnsFpq

38. Gurlek U, Abakay CD, Ozkan L, Saraydaroglu O, Kurt M, et al. (2013) The evaluation of bcl2 expression as a prognostic marker in early stage laryngeal cancer. Tumori 99: 682-688. Link: https://goo.gl/Dux9NT

39. Menor R, Fortunato SJ, Yu J, Milne GL, Sanchez S, et al. (2011) Cigarette smoke induces oxidative stress and apoptosis in normal term fetal membranes. Placenta 32: 317-322. Link: https://goo.gl/EgH9np

40. Callagy GM, Webber MJ, Pharoah PD, Caldas C (2008) Meta-analysis confirms BCL2 is an independent prognostic marker in breast cancer. BMC Cancer 8:153. Link: https://goo.gl/FCVXVP

41. Samanta D, Kaufman J, Carbone DP, Datta PK (2012) Long-term smoking mediated down-regulation of Dmad 3 induces resistance to carboplatin in non-small cell lung cancer. Neoplasia 14: 644-655. Link: https://goo.gl/Df2JNM

42. Cucina A, Diniciola S, Coluccia P, Proietti S, D´Anselmi F, et al. (2012) Nicotine stimulates proliferation and inhibits apoptosis in colon cancer cell lines through activation of survival pathways. J Surg Res 178: 233-241 Link: https://goo.gl/f4oYYE

43. Lin J, Hutchinson L, Gaston SM, Raab G, Freeman MR (2001) BAG-1 is a novel cytoplasmic binding partner of the membrane form of heparin-binding EGF-like growth factor: a unique role for proHB-EGF in cell survival regulation. J Biol Chem 276: 30127-30132. Link: https://goo.gl/Z2eidO

44. Matsuzawa S, Takayama S, Froesch BA, Zapata JM, Reed JC (1998) p53-inducible human homologue of Drosophila seven in absentia (Siah) inhibits cell growth suppression by BAG-11. EMBO J 17: 2736-2747. Link: https://goo.gl/GgwK17

45. Narayan S, Jaiswal AS, Kang D, Srivastava P, Das GM, (2004) Cigarette smoke condensate-induced transformation of normal human breast epithelial cells in vitro. Oncogene 23: 5880-5889. Link: https://goo.gl/KbXgkb

46. Connors SK, Balusu R, Kundu CN, Jaiswal AS, Gairola CG, et al. (2009) C/EBPbeta-mediated transcriptional regulation of bcl-xl gene expression in human breast epithelial cells in response to cigarette smoke condensate. Oncogene 28: 921 –932. Link: https://goo.gl/7t0qXK

47. Ribelles N, Perez Villa L, Jerez JM, Pajares B, Vicioso L, et al. (2013) Pattern of recurrence of early breast cancer is different according to intrinsic subtype and proliferation index. Breast Cancer Res 15: R98. Link: https://goo.gl/fKujle

48. Manjer J, Malina J, Berglund G, Bondeson L, Garne JP, et al. (2001) Smoking associated with hormone receptor negative breast cancer. Int J Cancer 91: 580-584. Link: https://goo.gl/3DAhJL

49. Nishino Y, Minami Y, Kawai M, Fukamachi K, Sato I, et al. (2014) Cigarette smoking and breast cancer risk in relation to joint estrogen and progesterone receptor status: a case-control study in Japan. Springerplus. 3: 65. Link: https://goo.gl/kpzwx4

50. Kawai M, Malone KE, Tang MTC, Li CI (2014) Active smoking and the risk of estrogen receptor-positive and triple-negative breast cancer among women ages 20 to 44 years. Cancer 120: 1026-1034. Link: https://goo.gl/IEigDP

51. Ruibal Morell A (1992) CEA serum levels in non-neoplastic disease. Int J Biol Markers 7: 160-166. Link: https://goo.gl/UMwj8G