Am J Transl Res 2020;12(7):4043-4050
www.ajtr.org /ISSN:1943-8141/AJTR0106543

Original Article

Effects of alkaline water intake on gastritis and miRNA expression (miR-7, miR-155, miR-135b and miR-29c)

Juliana R Chaves[1,5,6], Carolina R T de Souza[4], Antonio A C Modesto[2,3], Fabiano C Moreira[1,2,3], Eliel B Teixeira[1], Jonathan S Sarraf[3,5], Thaís S R Allen[5,7], Taíssa M T Araújo[2], André S Khayat[1,2,3,4]

[1]Post Graduation in Oncology and Medical Sciences-Federal University of Pará (UFPA), Brazil; [2]Oncology Research Center-Federal University of Pará (UFPA), Brazil; [3]Post Graduation in Genetics and Molecular Biology-Federal University of Pará (UFPA), Brazil; [4]Institute of Biological Sciences-Federal University of Pará (UFPA), Brazil; [5]Oncológica do Brasil-Learning and Research, Brazil; [6]Brazilian Navy, Brazil; [7]State University of Pará (UEPA), Brazil

Received December 17, 2019; Accepted June 27, 2020; Epub July 15, 2020; Published July 30, 2020

Abstract: It is known that abnormal expression of miRNAs in the gastric cancer (GC) contributes to its carcinogenesis. Therefore, ingestion of commercial (usual) water on a daily basis may be a contributing factor for the occurrence of alterations in the gastric mucosal. In this study, it was evaluated the expression of the miRNAs miR-29c, miR-7, miR-155, and miR-135b in the gastric tissue of patients with gastritis before and after the consumption of alkaline water (pH range from 8.0 to 10.0), as well as the clinic pathological characteristics. Methods: 50 subjects from the Amazon region, diagnosed with gastritis that routinely used commercial (usual) water with a pH lower than 5.0, were enrolled to change the consume water to a pH of 8.5 to 10.0 for 5 months. Results: Endoscopic findings of gastritis were such different (less severe disease), P = 0.024; in 43% diagnosed with moderate gastritis upfront esophagogastroduodenoscopy (EGD) presented mild gastritis after the consumption of alkaline water, according to study methods; there were no worsening gastritis and there were a significant increase in the expression of miR-135b (P = 0.039) and miR-29c (P = 0.039). Conclusion: Modified pH range water (from 8.0 to 10.0) ingested for 5 months was able lead to a less severe gastritis according to the Sidney classification system, suggesting that this lifestyle change represented a clinical benefit in patients with gastritis on the Amazon region. In addition, higher expression of miR-135b and miR-29c was observed after the consumption of alkaline water for 5 months.

Keywords: Gastritis, water alkalinity, epigenetics, microRNAs, Amazon population

Introduction

Gastric cancer (GC) is the fifth most common malignant neoplasm worldwide and accounts for 8.2% of all cancer deaths [1, 2]. In northern Brazil, it is the second most common cancer in men and fifth in women and is a public health problem in the state of Pará [3].

Gastric carcinoma is the most common cancer type in the stomach, and the intestinal subtype is the most frequent [4]. It develops in multiple stages: normal mucosa, chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia and malignant neoplasia [4]. These conditions are usually sequential and occur over a period of many years as a result of the interaction between a variety of endogenous and exogenous factors [4].

The association between GC and diet is well established in the literature [5]. The characteristic diet of Pará, e.g., high intake of salty foods (jerky, fish, and shrimp), low intake of greens and vegetables and high consumption of glucose (flour), has contributed to the high incidence of GC in the region [6].

The cellular transformation characteristics of gastric carcinogenesis may take time to present; however, epigenetic transformations, such as DNA methylation, histone changes and miRNA expression changes [7], can be identified early and are more likely to be influenced by environmental factors (smoking, sun exposure, inflammation and pollutants, and diet) [8].

Previous studies have shown that the abnormal expression of several miRNAs contribute to the

Table 1. Composition and chemical characteristics of the alkaline water used in the study

Parameters	Results
Electric Conductivity	243.40 µS/cm
pH	10.01
Total Alkalinity	34.19 mg/L $CaCO_3$
Alkalinity to Hydroxides	< 0.5 mg/L $CaCO_3$
Alkalinity to Carbonates	25.87 mg/L $CaCO_3$
Alkalinity to Bicarbonates	8.32 mg/L $CaCO_3$
Ammoniac Nitrogen	0.14 mg/L NH_3
Nitrate	7.38 mg/L N
Nitrite	< 0.10 mg/L N
Calcium	2.57 mg/L Ca^{2+}
Magnesium	13.92 mg/L Mg^{2+}
Sodium	21.80 mg/L Na^{2+}
Potassium	4.70 mg/L K^+
Iron	< 0.05 mg/L Fe^{3+}
Carbonate	15.52 mg/L CO_3^{2-}
Bicarbonate	10.15 mg/L HCO_3^-
Sulfate	4.68 mg/L SO_4^{2-}
Chloride	33.76 mg/L Cl^-

Certificate of analysis No. 0456/2017 provided by the filter manufacturer.

carcinogenesis and progression of neoplasms, including GC, and may also be useful as predictors of drug resistance and as prognostic factors [8-10]. The epigenetic targets chosen in the present study (miR-29c, miR-7, miR-155, miR-135b) are dysregulated in GC and altered in tissues with inflammation, as occurs in gastritis, and are thus potential biomarkers for the carcinogenesis of GC [10-13].

In the North region of Brazil, the waters sold have a low pH, ranging from 3.0 to 5.0, and are therefore outside the standards required by the World Health Organization and the Brazilian Ministry of Health [14, 15]. In addition, in this region, there is also a high incidence of gastritis and GC, above the national average [3]. Thus, water pH may be a contributing factor for the occurrence of these gastric issues, and therefore, its evaluation is extremely important for understanding the high incidence rates of this cancer in the region. Mousa [16]. Furthermore, it was observed that electrolyzed alkaline water has anti-inflammatory properties because of its ability to reduce the expression of TNF-α in the gastric mucosa Naito [17].

In addition, a study showed the ability of water with a pH of 8.8 to inactivate pepsin. This irreversible inactivation of pepsin in this alkaline medium can generate a possible benefit of an alkalizing diet for the treatment of acid reflux because this enzyme is related to protein lysis and is activated when pepsinogen is in acidic medium [18].

Thus, the objective of this study was to evaluate the expression of the miRNAs miR-29c, miR-7, miR-155, and miR-135b in the gastric tissue of patients with gastritis before and after the consumption of alkaline water to understand how changes in consumption may affect the modulation of the expression of these microRNAs as well as the clinicopathological characteristics of the patients.

Materials and methods

A total of 50 individuals, positive and negative for *Helicobacter pylori*, were recruited by non-probabilistic and convenience sampling. This study was approved by a research ethics committee under protocol 2.033.180.

Individuals older than 35 years, diagnosed with gastritis by anatomopathological examination, were included. The patients excluded were those with a prior or familial history of GC, those who were using alkaline water and/or antacids, those who had endoscopic criteria for immediate eradication of *H. pylori* (past and present gastric and/or duodenal ulcers with or without complications), or those who had gastric MALT lymphoma or atrophic gastritis.

The individuals recruited routinely used water with a pH lower than 5.0 and began to consume water with a pH of 8.5 to 10.0 voluntarily for a median of 5 months. These filters provided pretreated, filtered, purified, ionized and alkaline water with negative potential renal acid load (PRAL), obtained using a water ionizer. **Table 1** shows the composition of the alkaline water provided to the participants.

After this period, all the individuals underwent a new esophagogastroduodenoscopy (EGD) during which the anatomopathological parameters and the proposed outcomes were again evaluated.

For all participants, miR-155, miR-7, miR-29c and miR-135b expression was evaluated before

Table 2. Descriptive analysis of the qualitative variables for patients with gastritis before the consumption of alkaline water in Belém do Pará

Variable	N	%
Sex		
Male	5	17.86
Female	23	82.14
H. pylori 1st test		
Negative	14	50.00
Positive	14	50.00
Sydney classification 1st test		
Mild	2	7.14
Moderate	26	92.86
Amount of liquid ingested per day		
1 liter or less	7	25.00
1 liter-1.5 liters	10	35.71
2 liters or more	8	28.57
Did not answer	3	10.72
Type of water ingested per day		
Filtered public water	6	21.43
Carbonated mineral water	1	3.57
Noncarbonated mineral water	14	50.00
Filtered water from artesian well	4	14.28
Did not answer	3	10.72
Alcohol consumption		
No	11	39.28
Yes	14	50.00
Did not answer	3	10.72
Frequency of alcohol consumption		
Once a week	1	3.57
Weekend	13	46.43
Did not answer	14	50.00
Volume of alcohol (weekly)		
Up to 500 milliliters	11	39.29
Up to 2 liters	2	7.14
Did not answer	15	53.57
Type of alcohol		
Beer	8	28.57
Wine and spirits	6	21.43
Did not answer	14	50.00
Physical activity		
No	7	25.01
Yes, sometimes	9	32.14
Yes, regularly	8	28.57
Did not answer	4	14.28
Smoking		
No	28	100.00
Did not answer	0	0.00
Use of controlled medication		
No	16	57.14
Yes	9	32.14
Did not answer	3	10.72
Prior treatment for gastritis		
Never received	11	39.29
Received	13	46.43
Did not answer	4	14.28

and after the study period (median of 5 months). Total RNA extraction was performed using an mirVana miRNA isolation kit (Ambion, Texas, USA) according to the manufacturer's instructions. RNA concentrations were determined using a SpectraMax i3 spectrophotometer (Molecular Devices, Sunnyvale, California, USA) with a concentration of 10 ng/μL as the standard, and RNA integrity was visualized in an agarose gel.

To detect miRNA expression, reverse transcription was performed using total RNA samples (TaqMan MicroRNA Reverse Transcription Kit, Life Technologies, Foster City, CA), followed by real-time PCR with commercially available primers and probes (TaqMan MicroRNA Assays, Life Technologies, Foster City, CA). The endogenous miRNAs used for standardization were RNU6B, miR-1403p and miR-101, which were selected after analysis of data available in the literature [19-21]. After analyzing the results in NormFinder software [22], RNU6B and miR-1403p were the endogens selected to calculate differential expression.

All assays followed the manufacturer's recommendations and were performed in quadruplicate, according to the following thermocycling program (95°C for 10' and 40 cycles of 95°C for 15" and 60°C for 1'). Gene expression was evaluated using the comparative CT method ($-2^{\Delta\Delta CT}$).

Identification of *H. pylori* bacteria was performed both by histological analysis and by real-time PCR through amplification of the bacterial 16SRNA region [23, 24]. The sample was considered infected when positive in any of the methodologies.

For the statistical analysis, clinical-epidemiological qualitative variables are presented as absolute and relative frequencies. The quanti-

Table 3. Descriptive analysis of the qualitative variables of patients with gastritis after the consumption of alkaline water in Belém do Pará

Variable	N	%
Sydney classification 2nd test (return)		
Mild	12	42.30
Moderate	16	57.70
H. Pylori 2nd test (return)		
Negative	11	39.28
Positive	17	60.71

Table 4. Evaluation of the Sidney classification between endoscopies in patients with gastritis in Belém do Pará

Sydney classification 1st EGD	Sydney classification 2nd EGD (return)		P*
	Mild N (%)	Moderate N (%)	
Mild	2 (7%)	0	0.024
Moderate	12 (43%)	14 (50%)	

*Fisher's exact test.

tative variable "age" (Shapiro-Wilks < 0.05) is presented as the median and 25%-75% percentiles. Fisher's exact test was used to assess the correlation between categorical variables.

The difference in microRNA expression between the 2 groups (before and after alkaline water intake) was determined by the paired t-test for all samples (Stata 11.0 and R-studio). Two-tailed P < 0.05 was considered statistically significant.

Results

A total of 50 individuals, positive and negative for *H. pylori*, were recruited between May 2017 and February 2018 after confirmation of gastritis by EGD to evaluate the effects of changing the type of water consumed from water with an acidic pH (pH less than 5) to water with an alkaline pH (pH between 8.5 and 10). Of these, 28 completed all steps of the study with the use of alkaline water for the period established and then performed a new EGD.

The absolute and relative frequencies of qualitative variables for the data collected before and after the consumption of alkaline water are shown in **Tables 2** and **3**, respectively.

The median age was 44.5 years (IQR 38.75-52.25). The results showed endoscopic improvement of gastritiS (P = 0.024) because 12 patients (43%) diagnosed with moderate gastritis in the first EGD presented mild gastritis (**Table 4**) after the consumption of alkaline water. In addition, there were no patients with worsening gastritis.

Figure 1 shows a box plot comparing the expression levels of miR-135b (P = 0.039) and miR-29c (P = 0.039) before and after the median period of 5 months of alkaline water consumption. The expression levels were higher after the consumption of alkaline water. There was no significant increase in miR-155 and miR-7 expression (**Figure 1**).

The FoldChange analysis of the differences between $2^{-\Delta\Delta CT}$, before and after the consumption of alkaline water for a median of 5 months, of the target microRNAs showed values greater than 1 for every miRNA (**Figure 2**).

Discussion

Currently, there is a wide discussion about the effects of the pH of drinking water and its consequences on human health, such as its relationship with inflammation and cancer [25]. However, there is little scientific evidence supporting this association.

In the present study, we found a significant increase in the expression of 2 microRNAs, miR-29c and miR-135b, after the consumption of alkaline water for 5 consecutive months, as well as an improvement in gastritis as evaluated by a second EGD; i.e., 42% of the patients who had moderate gastritis in the first EGD had mild gastritis in the second EGD, according to the Sydney classification system. It is also worth noting that the patients did not undergo pharmacological treatment for gastritis during the intervention period and were not instructed to change their diet during the period.

Gastritis is considered a preneoplastic condition, as it participates in gastric carcinogenesis

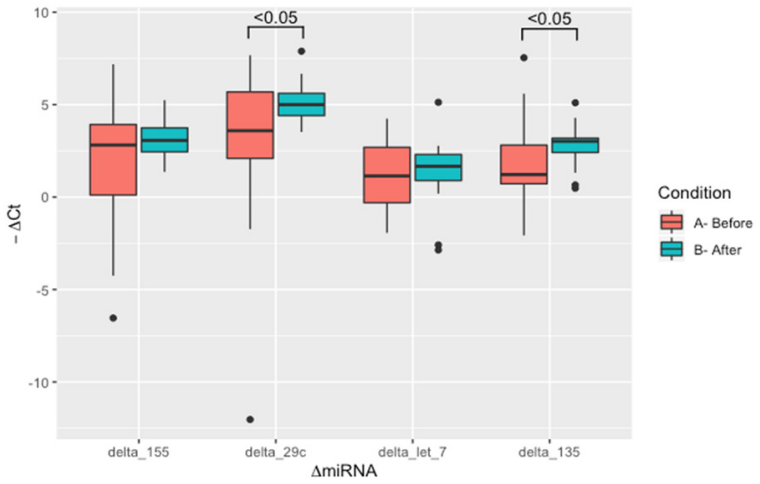

Figure 1. Expression of miRNAs miR-155, miR135, miR29c and miR7 before and after the consumption of alkaline water for a median of 5 months (first and second EGD).

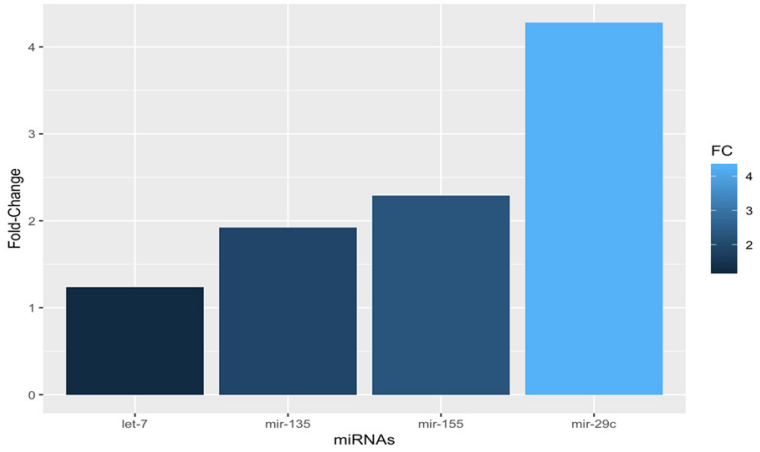

Figure 2. Fold change of the differences between the $2^{-\Delta\Delta CT}$ for the target micro-RNAs before and after the consumption of alkaline water for 5 months.

in the early stages of the process, followed by gastric atrophy, ulcerations, intestinal metaplasia, dysplasia and, finally, malignant neoplasia [4, 26, 27]. Thus, the improvement in gastritis observed in the present study with the use of alkaline water may represent a protective factor of inflammation in the gastric mucosa. Thus, this simple lifestyle change may act positively in the early stages of the carcinogenesis cascade of GC.

Proton pump inhibitors (PPIs) are among the most commonly prescribed drugs in the world; however, although they are generally considered safe, they have several adverse effects associated with prolonged use [28, 29]. These medications are associated with an increased risk of the development of premalignant lesions (fundic gland polyps, worsening of gastric atrophy and metaplasia) and GC, particularly in individuals infected with *H. pylori*, especially when PPIs are used for over one year [28-30]. The use of alkaline water does not compare with the mechanism of action of PPIs because there is no inhibition of acid secretion but, rather, a reduction in exposure to acidic substances of a mucosa that is damaged by gastritis [30].

miR-29c is commonly described as a tumor suppressor because it inhibits the proliferation, invasion and migration of malignant cells. Decreased expression of this microRNA has been reported in several human malignancies, such as pancreatic cancer [31], breast cancer [32], prostate cancer [33], hepatocellular cancer [34], nasopharyngeal cancer [35] and GC [10, 36].

The miR-29c expression levels were significantly decreased ($P < 0.001$) in GC tissues compared to normal tissue [37]. In addition, it has been demonstrated by *in vitro* and *in vivo* assays that the increased expression of this miRNA suppressed tumor growth through the downregulation of *ITGB1* (β1 integrin, also known as CD29) [37].

As previously described, miR-29c expression levels gradually decrease as gastric carcinogenesis progresses at different stages of mucosal aggression, demonstrating a direct correlation between the loss of mir-29c expression and the tumorigenesis of this cancer [10]. Thus, in our study, the increased expression of this miRNA after the consumption of alkaline water may suggest that its frequent use may function as a modifying factor of the epigenetic environment of these tissues, providing an

environment with lower chances of carcinogenic progression. Transfection of the miR-7 precursor into AZ521 and Kato III GC cells significantly inhibited cell proliferation capacity [13].

miR-135b has been described in the literature as an oncomiR in most tumor tissues, such as colon cancer [38], lung cancer [39], oral cancer [40] and breast cancer [41], but few studies have evaluated its role in gastric tissue. Vidal, et al. [10] observed that miR-135b expression was increased in gastric lesions when compared with normal gastric mucosa but was decreased when gastritis tissue was compared with cancer tissue; thus, its role in the various stages of gastric carcinogenesis remains uncertain.

miR-135b has been described as a tumor suppressor in osteosarcoma, and the *MYC* gene, which is related to the progression of several tumors, is its direct target [42]. That role is similar to that observed for prostate cancer, for which Wang, et al. [43] observed a decrease in miR-135b expression in prostate cancer tissues compared to normal tissues, acting as a tumor suppressor by inactivating the STAT6 pathway, known as oncogenic in several tumors. Thus, as the signaling pathways and activity of this miRNA are not yet certain, we cannot affirm or suggest protective or deleterious effects generated by increased miR-135b expression after alkaline water consumption.

Conclusion

The present study evaluated the effect of a patient lifestyle intervention on the first abnormal stage of the gastric carcinogenesis cascade, gastritis, and it was observed that the pH change in the water ingested for 5 months was able to improve gastritis according to the Sidney classification system, suggesting that this lifestyle change represented a clinical benefit in patients with gastritis. In addition, higher expression of miR-135b ($P = 0.039$) and miR-29c ($P = 0.039$) was observed after the consumption of alkaline water for 5 months.

Thus, the present study generates preliminary hypotheses and provides initial information about the ideal pH for regularly consumed beverages, such as water, and can serve as a basis for larger, randomized studies.

Acknowledgements

We acknowledge University of Pará, the company Acqualive, and Oncological Clinic of Brazil for technical support, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for fellowship support and doctor Otávio da Cunha Bembom, who performed endoscopies (in memoriam).

Disclosure of conflict of interest

None.

Address correspondence to: Juliana R Chaves, Post Graduation in Oncology and Medical Sciences-Federal University of Pará (UFPA), Brazil. E-mail: julianaramoschaves@gmail.com

References

[1] Bernard W. Stewart and Christopher P. Wild. World cancer report, ISBN 2014; 978-92-832-0429-9.
[2] Organization WH. 2019.
[3] INCA. Estomago Brasil, 2018 [cited 2018 02/09/2018]. Available from: http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home/estomago/definicao.
[4] Cotran R, Kumar V and Collins T. Red cells and bleeding disorders. Robbins pathologic basis of disease. 6th edition. Philadelphia: WB Saunders Company; 1999. pp. 638-640.
[5] Tsugane S and Sasazuki S. Diet and the risk of gastric cancer: review of epidemiological evidence. Gastric Cancer 2007; 10: 75-83.
[6] Resende ALDS, Mattos IE and Koifman S. Dieta e câncer gástrico: aspectos históricos associados ao padrão de consumo alimentar no Estado do Pará. Rev Nutr 2006; 511-519.
[7] Kiec-Wilk B, Razny U, Mathers J and Dembinska-Kiec A. DNA methylation, induced by beta-carotene and arachidonic acid, plays a regulatory role in the pro-angiogenic VEGF-receptor (KDR) gene expression in endothelial cells. J Physiol Pharmacol 2009; 60: 49-53.
[8] Kanherkar RR, Bhatia-Dey N and Csoka AB. Epigenetics across the human lifespan. Front Cell Dev Biol 2014; 2: 49.
[9] Krützfeldt J, Poy MN and Stoffel M. Strategies to determine the biological function of microRNAs. Nat Genet 2006; 38: S14-S19.
[10] Vidal AF, Cruz AM, Magalhães L, Pereira AL, Anaissi AK, Alves NC, Albuquerque PJ, Burbano RM, Demachki S and Ribeiro-dos-Santos Â. hsa-miR-29c and hsa-miR-135b differential expression as potential biomarker of gastric

carcinogenesis. World J Gastroenterol 2016; 22: 2060-70.
[11] Xiao B, Liu Z, Li BS, Tang B, Li W, Guo G, Shi Y, Wang F, Wu Y and Tong WD. Induction of microRNA-155 during Helicobacter pylori infection and its negative regulatory role in the inflammatory response. J Infect Dis 2009; 200: 916-925.
[12] Zabaglia LM. Expressão de MicroRNAs e dos genes da interleucina 2 e fator de necrose tumoral e suas correlações com o H. pylori. 2017.
[13] Kong D, Piao YS, Yamashita S, Oshima H, Oguma K, Fushida S, Fujimura T, Minamoto T, Seno H and Yamada Y. Inflammation-induced repression of tumor suppressor miR-7 in gastric tumor cells. Oncogene 2012; 31: 3949-60.
[14] Edition F. Guidelines for drinking-water quality. WHO Chronicle 2011; 38: 104-108.
[15] EdSF R. Águas envasadas: características físico-química, processo de produção E comercialização no nordeste Paraense. 2012.
[16] Mousa HA. Health effects of alkaline diet and water, reduction of digestive-tract bacterial load, and earthing. Altern Ther Health Med 2016; 22 Suppl 1: 24-33.
[17] Naito Y, Takagi T, Uchiyama K, Tomatsuri N, Matsuyama K, Fujii T, Yagi N, Yoshida N and Yoshikawa T. Chronic administration with electrolyzed alkaline water inhibits aspirin-induced gastric mucosal injury in rats through the inhibition of tumor necrosis factor-α expression. J Clin Biochem Nutr 2002; 32: 69-81.
[18] Koufman JA and Johnston N. Potential benefits of pH 8.8 alkaline drinking water as an adjunct in the treatment of reflux disease. Ann Otol Rhinol Laryngol 2012; 121: 431-434.
[19] Ribeiro-dos-Santos Â, Khayat AS, Silva A, Alencar DO, Lobato J, Luz L, Pinheiro DG, Varuzza L, Assumpção M, Assumpção P, Santos S, Zanette DL, Silva WA Jr, Burbano R and Darnet S. Ultra-deep sequencing reveals the microRNA expression pattern of the human stomach. PLoS One 2010; 5: e13205.
[20] Wu X, Tan X and Fu SW. May circulating microRNAs be gastric cancer diagnostic biomarkers? J Cancer 2015; 6: 1206-13.
[21] Anauate AC, Leal MF, Wisnieski F, Santos LC, Gigek CO, Chen ES, Geraldis JC, Calcagno DQ, Assumpção PP and Demachki S. Identification of suitable reference genes for miRNA expression normalization in gastric cancer. Gene 2017; 621: 59-68.
[22] Andersen CL, Jensen JL and Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 2004; 64: 5245-5250.
[23] Ho SA, Hoyle JA, Lewis FA, Secker A, Cross D, Mapstone N, Dixon M, Wyatt J, Tompkins D and Taylor G. Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. J Clin Microbiol 1991; 29: 2543-2549.
[24] Saeidi E and Sheikhshahrokh A. VacA genotype status of Helicobacter pylori isolated from foods with animal origin. Biomed Res Int 2016; 2016: 8701067.
[25] Fenton TR and Huang T. Systematic review of the association between dietary acid load, alkaline water and cancer. BMJ Open 2016; 6: e010438.
[26] Kishino M, Nakamura S and Shiratori K. Clinical and endoscopic features of undifferentiated gastric cancer in patients with severe atrophic gastritis. Intern Med 2016; 55: 857-862.
[27] Yakirevich E and Resnick MB. Pathology of gastric cancer and its precursor lesions. Gastroenterol Clin North Am 2013; 42: 261-284.
[28] Brusselaers N, Wahlin K, Engstrand L and Lagergren J. Maintenance therapy with proton pump inhibitors and risk of gastric cancer: a nationwide population-based cohort study in Sweden. BMJ Open 2017; 7: e017739.
[29] Cheung KS, Chan EW, Wong AY, Chen L, Wong IC and Leung WK. Long-term proton pump inhibitors and risk of gastric cancer development after treatment for Helicobacter pylori: a population-based study. Gut 2018; 67: 28-35.
[30] Tran-Duy A, Spaetgens B, Hoes AW, de Wit NJ and Stehouwer CD. Use of proton pump inhibitors and risks of fundic gland polyps and gastric cancer: systematic review and meta-analysis. Clin Gastroenterol Hepatol 2016; 14: 1706-1719, e5.
[31] Lu Y, Hu J, Sun W, Li S, Deng S and Li M. MiR-29c inhibits cell growth, invasion, and migration of pancreatic cancer by targeting ITGB1. Onco Targets Ther 2016; 9: 99-109.
[32] Li W, Yi J, Zheng X, Liu S, Fu W, Ren L, Li L, Hoon DS, Wang J and Du G. miR-29c plays a suppressive role in breast cancer by targeting the TIMP3/STAT1/FOXO1 pathway. Clin Epigenetics 2018; 10: 64.
[33] Li J, Fu F, Wan X, Huang S, Wu D and Li Y. Upregulated miR-29c inhibits cell proliferation and glycolysis by inhibiting SLC2A3 expression in prostate cancer. Gene 2018; 665: 26-34.
[34] Dong C, Wang Y, Du F, Ding W and Hu S. Low miR-29c expression is a prognostic marker in hepatocellular carcinoma. Genet Mol Res 2016; 15.
[35] Niu M, Gao D, Wen Q, Wei P, Pan S, Shuai C, Ma H, Xiang J, Li Z and Fan S. MiR-29c regulates the expression of miR-34c and miR-449a by targeting DNA methyltransferase 3a and 3b in nasopharyngeal carcinoma. BMC Cancer 2016; 16: 218.

[36] Matsuo M, Nakada C, Tsukamoto Y, Noguchi T, Uchida T, Hijiya N, Matsuura K and Moriyama M. MiR-29c is downregulated in gastric carcinomas and regulates cell proliferation by targeting RCC2. Mol Cancer 2013; 12: 15.

[37] Han TS, Hur K, Xu G, Choi B, Okugawa Y, Toiyama Y, Oshima H, Oshima M, Lee HJ, Kim VN, Chang AN, Goel A and Yang HK. MicroRNA-29c mediates initiation of gastric carcinogenesis by directly targeting ITGB1. Gut 2015; 64: 203-214.

[38] Valeri N, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, Hart JR, Ueno L, Grivennikov SI and Lovat F. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. Cancer Cell 2014; 25: 469-483.

[39] Li H, Xie S, Liu M, Chen Z, Liu X, Wang L, Li D and Zhou Y. The clinical significance of downregulation of mir-124-3p, mir-146a-5p, mir-155-5p and mir-335-5p in gastric cancer tumorigenesis. Int J Oncol 2014; 45: 197-208.

[40] Lopes CB, Magalhães LL, Teófilo CR, Alves APN, Montenegro RC, Negrini M and Ribeiro-dos-Santos Â. Differential expression of hsa-miR-221, hsa-miR-21, hsa-miR-135b, and hsa-miR-29c suggests a field effect in oral cancer. BMC Cancer 2018; 18: 721.

[41] Arigoni M, Barutello G, Riccardo F, Ercole E, Cantarella D, Orso F, Conti L, Lanzardo S, Taverna D and Merighi I. miR-135b coordinates progression of ErbB2-driven mammary carcinomas through suppression of MID1 and MTCH2. Am J Pathol 2013; 182: 2058-2070.

[42] Liu Z, Zhang G, Li J, Liu J and Lv P. The tumor-suppressive microRNA-135b targets c-myc in osteoscarcoma. PLoS One 2014; 9: e102621.

[43] Wang N, Tao L, Zhong H, Zhao S, Yu Y, Yu B, Chen X, Gao J and Wang R. miR-135b inhibits tumour metastasis in prostate cancer by targeting STAT6. Oncol Lett 2016; 11: 543-550.