Spontaneous regression of low-grade cervical intraepithelial lesions is positively improved by topical bovine colostrum preparations (GINEDIE®). A multicentred, observational, Italian pilot study

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Abstract. – OBJECTIVES: Human papillomavirus (HPV) is the causal agent of cervical cancer. The great majority of abnormal Pap test results – almost 90% – is referable to either atypical squamous intraepithelial lesion or CIN1. For these lesions, worldwide agreement exists concerning the high rate – ranging from 40% to 70% – of spontaneous regression over a period of 1-5 years. Host’s immune response is a key point influencing the natural history of these conditions. Bovine colostrum is a natural agent positively promoting several immune activities against bacterial and viral agents. The aim of this report was to evaluate the potential positive effect of bovine colostrum-containing vaginal tablets administered to CIN1 diagnosed patients in a prospective trial in regards to spontaneous regression rate.

PATIENTS AND METHODS: A series of 256 consecutive patients with histologically proven CIN1 recruited in a multicentre, observational, Italian study. Patients have been enrolled in a 24-weeks protocol of treatment and re-tested at the end of the study. Rates of regression have been recorded.

RESULTS: Overall regression rate to a negative histology at the end of the 6 month follow up was 75.5%.

CONCLUSIONS: Regression to normal histology was observed in a very high rate of cases in a very short period compared to the natural history of these lesions. CIN1 patients could benefit from bovine colostrum topical administration in terms of significantly shortening the regression time.

Key Words: Human Papillomavirus, HPV, Low-grade CIN, CIN1, Regression, Natural history, Bovine colostrum, Immunomodulation.

Introduction

Human papillomavirus (HPV) infection was firstly implicated as an etiologic agent for cervical cancer in 1977, and it actually is the most common viral sexually transmitted disease in the United States. It causes approximately 11,000 cases of invasive cancers per year in the USA but it’s also unequivocally linked with cervical preneoplastic lesions, categorized as squamous intraepithelial neoplasia (CIN) of various degrees. Although the incidence of cervical cancer has dramatically decreased, there has been a steady rise in intraepithelial neoplasia detection worldwide. Approximately 90% of abnormal Pap smears are either atypical squamous intraepithelial lesions or CIN1. Stated the large number of CIN1, many studies about treatment, follow-up and costs of CIN1 management have been performed. A large amount of data agree with a high rate of spontaneous regression showing that the spontaneous resolution of CIN1 is the rule rather than the exception. Studies on the natural history of these abnormalities show that regression to normal is around 50% for CIN1. More, a comprehensive literature review highlighted that spontaneous regression occurs in 57% of patients. Thus, great interest is focused on the timing of CIN1 regression. A cohort study of more than 17,000 women found that spontaneous regression occurred in 44% of patients within 2 years and in 74% after 5 years of follow-up. These data were confirmed in a large group of patients, showing a
52% of CIN1 regression within 1 year of follow-up\textsuperscript{10}. Management of CIN1 lesions follow-up has been largely discussed in multiple studies. Despite low agreement exists on which test better fits for CIN1 follow-up, all recent studies agree with the difficulty in achieving patient’s compliance for such a long follow-up\textsuperscript{11}. Due to this aspect, together with the long-term follow-up costs, great interest is given in obtaining shorter regression time of these lesions; since the host immune response is recognized as a key point influencing natural history of HPV-related cervical intraepithelial lesion, many efforts have been dedicated to immunostimulating agents. Colostrum is the pre-milk fluid produced by female mammary glands immediately after delivery. The bovine and human colostrum contains several immunological factors that confer the first immunization of the newborn, protecting against many microbial pathogens\textsuperscript{12}. Colostrum is a highly complex mixture of various effector molecules; this explains the wide range of pharmacodynamic effects described in in-vitro systems, both in animals and in clinical studies; main actions include an antibacterial effect and a positive modulation of the immune response\textsuperscript{13}. Bovine colostrum provides almost ninety useful components. The most interesting bioactive substances are immune factors (γ-globulins or immunoglobulins or antibodies)\textsuperscript{14,15} and growth factors (EGF, TGF, FGF, IGF-I, IGF-II, TGF-α and TGF-β), but also antimicrobial (lactoferrin), cytokines, immunomodulatory substances (colostrinin or proline-rich-polypeptide) and nutrients (vitamin A, E, B12)\textsuperscript{16}. Colostrum has already been tested in many clinical trials and its therapeutic effect has been largely demonstrated in gastrointestinal disorders, enteric infections, respiratory tract infections and various chronic infections as bacterial, viral, parasitic or fungal\textsuperscript{17}. Furthermore colostrum promotes a positive immune response towards intracellular pathogens such as bacteria and viruses, enhancing IL-12 and IFN-γ production, cytokines involved in the specific Th1 response\textsuperscript{18}.

In this study we investigated the potential positive immunomodulating action of bovine colostrum through vaginal administration in cases of CIN1 followed up according to guidelines of management of these lesions.

**Patients and Methods**

The study has been performed during a 2 year period (2012-2013) with the participation of 6 groups: (1) Department of Obstetrics & Gynecology of San Raffaele Institute, Vita Salute San Raffaele University, Milan; (2) Department of Obstetrics & Gynecology of L. Mangiagalli Institute, University of Milan; (3) Department of Obstetrics & Gynecology of V. Buzzi Hospital, University of Milan; (4) Department of Obstetrics & Gynecology of Careggi Hospital, University of Florence; (5) Obstetrics and Gynecology Unit of Istituti Clinici Zucchi, Monza, and (6) Obstetrics and Gynecology Unit of ASL 4, Prato, Italy. The study design comprised for enrollment of cytologically referred and histologically proven new cases of low-grade cervical intraepithelial lesion (CIN1), that have been topically treated with bovine-colostrum containing vaginal tablets (Ginedie\textsuperscript{®} – Tfarma, Florence – Italy) for a 6 month period, and re-tested. The recruited cases came from screening settings and should not having had a previous episode of cervical intraepithelial neoplasia of any degree in the previous two years prior to the study. At baseline and at study end patients have been tested with cervical cytology (Pap smear), colposcopy and targeted biopsy; in some cases high-risk HPV-DNA testing (hrHPV) was also obtained. In colposcopically negative cases, a random biopsy of the cervical transformation zone (TZ) was obtained. Treatment protocol comprised for administration of vaginal tablets twice/week at bedtime for a total of 24 weeks, without any other medication/detergent topical use for the whole study period. Informed consent was locally obtained for any participant.

**Statistical Analysis**

Results have been statistically analyzed in terms of histological regression vs. persistence vs. progression rate of cervical lesions with the Chi-square and Fisher r-test, assuming a \( \alpha \) value of 0.05 as significant.

**Results**

An overall of 256 cases represents the studied population (mean age 37.7 yrs.). All the cases had a cytological cervical smear that indicated: 207 (80.85%) cases of L-SIL, 43 (16.79%) cases of ASC-US, 4 (1.56%) cases of H-SIL and 2 (0.78%) negative cases (Figure 1). As far as it concerned histology at baseline, 92.96% of cases (n. 238) were diagnosed as CIN1. 1.95% (n. 5) were diagnosed as CIN1 with isolated cells suggestive for
CIN2, and 5.07% (n. 13) were identified as CIN2 (Figure 2). Out of the 256 recruited cases, a subgroup of 89 was also submitted to high-risk HPV-DNA testing both at baseline and at study end; 84.26% of these cases (n. 75) tested positive for hrHPV at baseline (Figure 3). At the end of the treatment protocol, the tests performed were as follows: colposcopic grade 0 of the cervical TZ changed from 19.9% at baseline to 54.2% after treatment, grade 1 changed from 68.7% to 44.1%, and grade 2 changed from 10.9% to 14.0% before and after treatment respectively (Figure 4), with a statistically significant difference ($p < 0.0001$).

When final histology was considered, the following results were obtained: CIN1 cases (n. 238) were found to be histologically negative in 71%, persistently CIN1 in 26.8%, and progressed to CIN2 in 2.1% of cases; cases of CIN1/2 (n. 5) at baseline histology were found to be histologically negative in 80% and purely CIN1 in 20% of cases respectively; cases of CIN2 (n. 13) were found histologically negative in 7.6%, regressed to histological CIN1 in 61.5% and persistently CIN2 in 30.7% of cases respectively (Figure 5); statistical analysis of these results demonstrated a strong significative difference ($p < 0.0001$) comparing baseline to post-treatment. Lastly, as far as it concerned hr-HPV status, CIN1 cases that tested positive for HPV-DNA at baseline (n. 75) were found to be histologically negative in 73.3%, persistently CIN1 in 21.3%, and progressed to histological CIN2 in 5.3% of cases respectively; on the other hand, CIN1 cases that tested negative for HPV-DNA (n. 14) where found to be histologically neg-
and host-correlated factors are the most important and widely investigated. HPV viral load, for example, has been demonstrated to directly correlate with the severity of the lesion and, supported by recently published experiences, can easily be obtained with simple, cost-effective and validated biomolecular HPV-DNA testing assays, without the need to perform hardly affordable laboratory tests\(^{23-25}\); on the other side, the immunological profile of cervical tissues has similarly been studied and very recent results established particular patterns of mucosal immunity having significative prognostic significance in terms of complete regression vs. recurrence of high-grade (CIN2-3) lesions after conservative treatment\(^{26-28}\). In this context, drug-induced immunosuppression (e.g. transplanted recipients) has recently been reported to have less importance\(^{29}\). Bovine colostrum has been demonstrated, either in animal models and in clinical studies in humans, to own a wide range of positive immunomodulating effects against bacterial and viral agents\(^{12-18}\); for this reasons we investigated the potential usefulness of a vaginal preparation (Ginedie® vaginal tablets – Tfarma, Florence – Italy) containing bovine colostrum in cases of histologically proven CIN1. This study represents the first experience in which this preparation has been tested in such cases. In our study, the overall regression rate of lesions to a negative histology in a 6-month period has been 75.5\% (\(p < 0.0001\)). This result, per se, is consistent with what is generally published in literature, where a regression rate of 44-74\% has been reported in follow up ranging from 1 to 5 years\(^{7,10}\). Supporting this result, the colposcopic grade of the lesions at baseline vs. post-treatment evidenced a 34\% increase of grade 0 and a 24\% decrease of grade 1 (\(p < 0.0001\)). Of particular interest, 80\% of complete regression to negative histology was obtained in the group of patients with CIN1/2 (\(p < 0.0001\)). As far as it concerned the hrHPV-DNA positivity of cases at baseline, with the limitation of the small number of patients investigated (89 cases), we did not observed a statistically significative difference in terms of regression to negative histology; this is probably to be correlated with the small sample, as the statistical analysis resulted very close to reach a statistical significance (\(p = 0.05\)). It is noteworthy to underline two important aspects of our results: firstly, the overall regression rate of our cases can be highlighted as one of the highest ever reported in previous experiences; secondly and most relevant,
regression rates around 70% have only been reported in very long follow up periods (5 years), while in our study has been obtained in 6 months. This aspect seems to be particularly interesting in consideration of the known difficulties in obtaining patients’ compliance and acceptance of long lasting observation follow up periods. Moreover, the issue of follow up-related costs can be interestingly and positively influenced. With the clear study limitation of being observational, it is reasonable to hypothesize that the immunomodulating positive effects of bovine colostrum may have increased and improved, at least in terms of time if not of effectiveness, the rate of regression to normality of our cases. This may particularly be correlated with the promotion of a positive immune response towards intracellular pathogens such as bacteria and viruses, enhancing IL-12 and IFN-γ production, cytokines involved in the specific Th1 response; in fact, a positive effect of high levels of IFN-γ production has previously been demonstrated to correlate with favourable outcomes after treatment of high-grade CIN.

Conclusions

Bovine colostrum represents a very interesting natural agent with potentially highly effective implications in clinical practice in a wide range of situations, including the approach to cervical intraepithelial neoplasia (CIN), as our findings suggest. If further results from controlled trials will confirm this first pilot experience, a promising, interesting, safe and cost-effective novel approach to the conservative management of low-grade cervical intraepithelial lesions (CIN1) could be proposed in clinical practice.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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